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THE DIAGNOSTIC ACCURACY OF GENE XPERT MTB/RIF (CBNAAT) FOR PREDICTION OF PULMONARY AND EXTRA PULMONARY TUBERCULOSIS SAMPLES AT A TERTIARY CARE CENTRE.

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ABSTRACT **Background:** India is a country with one fourth of the Global Tuberculosis (TB) burden. According to the latest World Health Organisation (WHO) guidelines, there were estimated 10 million new TB cases worldwide, of which 5.7 million were men, 3.2 million were women and 1.1 million were children. Globally, the mortality reported was reported as 1.5 million, including 0.25 million with HIV co-infection.**Aims and objective:** To evaluate the role of Gene Xpert MTB/RIF assay in diagnosing Mycobacterium tuberculosis (MTB) in PULMONARY and EXTRA PULMONARY samples.**Methods:** A cross-sectional study was conducted on consecutive presumptive pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB) cases from July 2018 to June 2019 at a tertiary care centre in West India. Socio-demographic characteristics and other variables were collected using a structured questionnaire. Two hundred clinical specimens were collected, 100 each for PTB and EPTB, using all aseptic precautions. All specimens were subjected to fluorescent microscopy and Gene Xpert MTB/RIF assay. Statistical analysis was done using Chi-square test and logistic regression analysis.**Results:** Fluorescent microscopy yielded 41 PTB and 6 EPTB positive results. Sixty-four sputum samples and 20 EPTB (pus-11, pleural fluid-4, CSF-1 and ascetic fluid-1) were detected positive for MTB by Gene Xpert MTB/RIF assay. Rifampicin resistance was detected in 4 sputum and 2 pus samples amongst all positive cases, using CBNAAT assay.**Conclusion:** The diagnostic accuracy of Gene Xpert assay in predicting TB was more than fluorescent smear. Also, PTB was readily diagnosed as compared to EPTB. Among all EPTB samples, pus has highest Gene Xpert positivity and ascetic fluid has least positivity.

KEYWORDS : pulmonary tuberculosis, extrapulmonary tuberculosis, Gene Xpert MTB/RF, Rifampicin resistance

INTRODUCTION

India is a country with one fourth of the Global Tuberculosis (TB) burden. According to the latest World Health Organisation (WHO) guidelines, there were estimated 10 million new TB cases worldwide, of which 5.7 million were men, 3.2 million were women and 1.1 million were children. Globally, the mortality reported was reported as 1.5 million, including 0.25 million with HIV co-infection[1]

The estimated incidence of TB in India is approximately 2.7 million. HIV co-infection among TB was nearly fifty thousand cases amounting to TB HIV co-infection rate of 3.4%. [2]

One of the health targets of sustainable development goals by WHO is to end tuberculosis by 2030.[2] Indian government has targeted to end TB by 2025[2]. TB can manifest as pulmonary TB (PTB) or extra pulmonary TB (EPTB). Globally, EPTB accounts for 25% of all TB cases.In immune compromised states, like HIV infection and children, the percentage increases to even 50%. In India, EPTB incidence is about 20% of all cases.[3]. Amongst all EPTB cases, pleural involvement is maximum (30%) [4]

Conventional technique for detecting tubercular bacilli using smear microscopy has low sensitivity for EPTB samples. [5,6] Culture of *Mycobacterium tuberculosis* (MTB) is the gold standard technique, but requires trained laboratory personnel, and the absence of MTB in the sample can only be confirmed after 8 weeks. This delay in diagnosis can be more disastrous for the patients, so the treatment is often started empirically. [7,8]

The WHO recently implemented the light emitting diode (LED) fluorescent microscopy and the Gene Xpert MTB/RIF assay in the National Tuberculosis Programme for diagnosis of TB in developing countries. Compared to the conventional fluorescence microscopy, LED fluorescent microscopy is less expensive and has a sensitivity of 84% and specificity of 98% against culture as reference standard. [9]

The purpose of the current study is to evaluate the performance of Gene Xpert MTB/RIF assay for detection of MTB in PTB and EPTB samples, and to compare it with other conventional methods.

MATERIALS AND METHODS

The study was conducted according to the tenets of the Declaration of Helsinki after approval from the Institutional Review Board. It was a cross sectional study, conducted at a tertiary care centre. The study was conducted during one year period, from June 2018 to September 2019. Data was obtained from lab records of patients, who visited the Department of Respiratory Medicine, New Medical College Hospital, Kota, Rajasthan, for seeking medical advice. The sample size was calculated as 200, which included patients with both, pulmonary and extra pulmonary presumptive TB. 100 pulmonary samples [sputum] and 100 extra pulmonary samples [25 samples each from pleural fluid, ascitic fluid, CSF and pus] were collected using all aseptic precautions. All pulmonary and extra pulmonary samples (sputum, pleural fluid, ascetic fluid, CSF and pus) were taken from patients who were clinically suspected of EPTB, and who would be able to give their clinical history. The sample volume of at least 3 ml for expectorated samples and any kind of body fluid was taken.Patients with age less than 10 years, patients presenting with haemoptysis or any other serious critical illness like congestive heart failure, chronic renal failure, liver failure etc. were excluded from the study. Insufficient sample volume (less than 3ml) and patients not willing to participate were also excluded from the study.

Initially, for all samples, (both PTB and EPTB) smears were made, stained and looked for the presence or absence of MTB (acid fast bacilli) under fluorescent microscope. All samples were simultaneously loaded in the Gene Xpert and the results were compared with fluorescent smear microscopy.

RESULTS

Out of total of 200 pulmonary and extra pulmonary samples, 62% samples were obtained from males while remaining 38% were from females [Table-1]. Maximum number of patients were in the age group 31-50 years i.e.79 patients (39.5%) followed by 59 patients (29.5%)

between 11-30 years, 49 patients (24.5%) between 51-70 years and 13 patients (6.5%) above 70 years.

Among the total 200 samples collected, there were 100 samples from sputum i.e. pulmonary (50%), and 100 samples were extrapulmonary (50%). Among extra pulmonary samples, 12.5 % samples (25/100) were collected each from pleural fluid, pus, ascitic fluid and CSF. Pus samples included lymph node aspirates and empyema. Among the total 200 samples, 47 were smear positive for acid fast bacilli. Amongst EPTB samples maximum positivity was seen in pus sample by fluorescent microscopy. Eighty four samples were detected positive by Gene Xpert MTB/RIF out of which, there were 64 samples of sputum, 11 pus samples, 4 samples of pleural fluid, 4 samples of CSF and 1 sample of ascetic fluid [Table-2, Figure 1]. Among all extrapulmonary samples, pus samples had highest Gene Xpert positivity and ascetic fluid had least positivity. In all CBNAAT positive cases, 4 sputum samples and 2 samples of pus were found rifampicin resistant.

Pooled Sensitivity of fluorescent sear microscopy against Gene Xpert MTB/RIF in PTB was 60.93%. Pooled Specificity of fluorescent sear microscopy against Gene Xpert MTB/RIF in PTB was 94.44%. The Positive predictive value of the test was 95.12% while Negative predictive value was 57.62% [Table-4].

Pooled Sensitivity of fluorescent sear microscopy against Gene Xpert MTB/RIF in EPTB was 30%. Pooled Specificity of fluorescent sear microscopy against Gene Xpert MTB/RIF in EPTB was 98.75%. The Positive predictive value of the test was 85.71% while Negative predictive value was 84.94% [Table-4].

Using CBNAAT, the prevalence of multi drug resistant tuberculosis (MDR-TB) in total samples (PTB and EPTB) was detected as 7.14% (4% PTB and 2% EPTB).

DISCUSSION

Tuberculosis is an important cause of mortality and morbidity in high prevalence country like India, therefore there is a need for prompt diagnosis, mainly to reduce tuberculosis burden. Rapid diagnosis of MTB in pulmonary and extra pulmonary samples is essential for effective treatment and to reduce the emergence and spread of MDR-TB. Mortality and morbidity due to TB is higher in developing countries.The incidence of EPTB is higher in patients with HIV co-infection. [10]

The diagnosis of EPTB is difficult due to its paucibacillary nature and its non- specific clinical signs and symptoms. As fluorescent smear microscopy is less sensitive due to the requirement of large bacillary load and conventional culture method requires time, trained persons and biosafety cabin, Expert MTB/RIF assay emerges as a rapid diagnostic tool that offers accurate results in less than 2 hours.[10]

In our study, majority of samples were from age group 41-50 years. Higher prevalence of EPTB was noted in younger age, *i.e.* 21- 30 years, which is similar to a study by Arora et al, which showed higher prevalence in the same age group.[10]

In the present study, out of 200 PTB and EPTB samples, 47 samples (23.5%) were AFB positive by Fluorescent smear microscopy and 84 samples (42%) were positive by Expert MTB/RIF assay.

The Gene Expert MTB/RIF assay (Cepheid USA) cartridge based nucleic acid amplification test is a newly developed, WHO recommended, automated diagnostic molecular test based on nested real time PCR and molecular beacon technology with a sensitivity of detecting 131 cu /ml of *Mycobacterium tuberculosis* in the specimen. *Mycobacterium tuberculosis* by Xpert MTB/RIF was detected in 64% in sputum samples, 16% of CSF samples, 16% of pleural fluid, 44% of pus samples and 4% in ascetic fluid samples. Out of 84 positive extra pulmonary samples 6 showed rifampicin resistance.

Conventional technique of smear microscopy had low sensitivity of around 0-40% on extra pulmonary samples and lead to higher false negative rates. [96] In our study, the sensitivity of Fluorescent microscopy when compared with Gene Xpert MTB/RIF reported to be 53.57% while specificity was 98.27%. Although Gene Xpert MTB/RIF has been recommended by WHO for testing EPTB samples recently, validation for Gene Xpert MTB/RIF is available mostly for PTB.

A study done by Tortoli et al, concluded that the sensitivity of fluorescent microscopy was 47% in both PTB and EPTB when compared with Gene Xpert. In our study the sensitivity of fluorescent smear microscopy in combined PTB and EPTB is 47.61%, which is similar to previous published literature.[11]

Another study done by Khan et al, on EPTB samples, the sensitivity of fluorescent microscopy was found to be 40%, as compared to Gene Xpert. In our study, the sensitivity of fluorescent smear microscopy in EPTB was 30%, which has low sensitivity compared to the referred study.[12]

Another study conducted by Kudi et al, the sensitivity of fluorescent microscopy was 35% in both PTB and EPTB, compared to Gene Xpert. In our study, the sensitivity of fluorescent smear microscopy in both PTB and EPTB was 46.7 %, which has higher sensitivity.[13]

A study done by Taddese et al, documented the diagnostic accuracy in presumptive TB cases in both PTB and EPTB samples for fluorescent microscopy -21.55% and for Gene Xpert – 41.64%. In our study this diagnostic accuracy for fluorescent microscopy-23.5% and Gene Xpert – 42%. Our study had higher diagnostic accuracy.[14]

Another study done by Kumar et al reported the diagnostic accuracy in all sputum samples as 38% by fluorescent microscopy and 53% by Gene Xpert. In our study, the diagnostic accuracy in sputum samples was 41% by fluorescent and 64% by Gene Xpert i.e. our study had higher diagnostic accuracy.[15]

Another study done by Iram et al, found out diagnostic accuracy of Gene Xpert in PTB and EPTB samples was 41.3% and diagnosed MDR TB in 6.5% cases. In our study, diagnostic accuracy of Gene Xpert in PTB and EPTB samples was 42%, which is higher in our study.[16]

In the present study, we report 7.1% of rifampicin resistance (6/84) indicating multidrug resistant (MDR) tuberculosis. In a study done by Iram et al reported MDR prevalence in both PTB and EPTB as 6.5%. [16]

Similar study was also done by Gupta et al, in which rifampicin resistance in PTB was detected in 5.8% cases, while in our study, 6.25% MDR TB were detected among pulmonary TB.[17]

Xpert MTB/RIF test is a major advance in TB diagnostic testing, but has limitations, including the limited shelf-life of the diagnostic cartridges, some operating temperature and humidity restrictions, requirement for electricity supply, unknown long-term robustness, and the need for annual servicing and calibration of each machine.

CONCLUSION

The Xpert MTB/RIF assay is a new test that is revolutionizing tuberculosis (TB) control by contributing to the rapid diagnosis of TB disease and drug resistance. Major advantages of the Xpert MTB/RIF assay are that results are available quickly (2 hrs), and minimal technical training is required to run the test. Additionally, the Xpert MTB/RIF assay can quickly identify possible multidrug-resistant TB. From our study we conclude that Gene Xpert MTB/RIF is simple and reliable technique for diagnosing pulmonary and extra pulmonary tuberculosis with high sensitivity and specificity not only in smear positive cases but also in smear negative cases. It is a game changer not only in pulmonary tuberculosis control but probably also in extra pulmonary tuberculosis.

Table No. 1: Gender distribution among all samples.

Gender	No of Patients (N=70)	(%)
Female	76	38%
Male	124	62%
Total	200	100%

Table 2: Number of pulmonary and extra pulmonary samples that are detected by smear microscopy, Gene Xpert MTB/RIF and MDR cases

Type of sample	Number of samples	%	Fluorescent microscopy Positive	Gene xpert mtb/rif positive	Rifampicin resistant
Sputum	100	50	41	64	4

Pleural fluid	25	12.5	1	4	0
Pus	25	12.5	4	11	2
Ascitic fluid	25	12.5	0	1	0
CSF	25	12.5	1	4	0
Total	200	100	47	84	6

Table 3: Age Wise Distribution Of Positive Samples

Age groups (years)	Number of Patients (N=84)	Percentage (%)
11-30	27	32.14
31-50	39	49.42
51-70	12	14.28
>70	6	7.14
Total	84	100

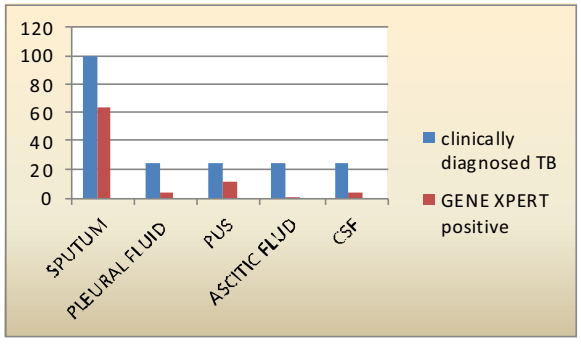
Table 4: Comparative evaluation of fluorescent microscopy and Xpert MTB/RIF in pulmonary Tuberculosis.

FLOUROSCENT SMEAR	GENE XPERT		TOTAL
	POSITIVE	NEGATIVE	
Positive	39	2	41
Negative	25	34	59
Total	64	36	100

Table 5: Comparative evaluation of fluorescent microscopy and Xpert MTB/RIF in Extrapulmonary tuberculosis.

FLOUROSCENT SMEAR	GENE XPERT		TOTAL
	POSITIVE	NEGATIVE	
Positive	6	1	7
Negative	14	79	93
Total	20	80	100

Figure 1: -GENE XPERT positive cases among all clinically presumptive pulmonary and extrapulmonary TB cases.



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