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ROLE OF XPERT MTB/RIF IN DIAGNOSIS OF SMEAR-NEGATIVE PULMONARY TUBERCULOSIS

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ABSTRACT BACKGROUND: Tuberculosis remains one of the major health problems in India. The incidence of pulmonary tuberculosis (PTB) can be reduced by preventing transmission with rapid and precise case detection and early treatment. The Gene-Xpert MTB/RIF assay is a useful tool for detecting Mycobacterium tuberculosis (MTB) with rifampicin resistance within approximately two hours by using a nucleic acid amplification technique. This study was designed to reduce the underdiagnosis of smear-negative pulmonary TB. Aim of the study-To find out the effectiveness of Gene-Xpert MTB/RIF assay in diagnosis of smear negative Pulmonary Tuberculosis. Objective- To find out the Rifampicin resistance in suspected Pulmonary Tuberculosis patients.

METHODS:We retrospectively reviewed the sputum samples of suspected pulmonary tuberculosis of 150 patients attending OPD at Institute of Respiratory Diseases, Jaipur from April 2020 to March 2021 for ZN stain, and GeneXpert (Xpert® MTB/Rif assay). Patient related information was collected from the Test Requisition Forms (TRF), received with the sample. Extra pulmonary Tuberculosis patients were excluded from the study. The sensitivity and specificity of GeneXpert and ZN microscopy were calculated and Prevalence of Rifampicin resistance is calculated.

RESULTS: Out of 150 patients, 39(26%) and 61(40.67%) with sputum samples were positive by smear microscopy and GeneXpert respectively. The prevalence of Rifampicin resistance was 12.24% and 8.33% in smear positive and negative pulmonary tuberculosis patients respectively. Overall Prevalence of Rifampicin resistance in Pulmonary Tuberculosis was 11.47%. The sensitivity of GeneXpert was 100% for sputum positive cases and sensitivity was 13.48% for smear negative cases. **CONCLUSION:** Sputum ZN smear microscopy is a highly specific but moderately sensitive test for the diagnosis of pulmonary tuberculosis. This study recommends the sputum GeneXpert MTB/RIF test to avoid a missed diagnosis of smear-negative pulmonary TB. The other major advantage of Gene Xpert is that it simultaneously detects Rifampicin resistance.

KEYWORDS : Rifampicin resistance, CBNAAT, AFB staining, anti-tubercular treatment.

INTRODUCTION

Tuberculosis (TB) is a communicable disease that is a major cause of ill health. It is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). Globally in 2019, an estimated 10.0 million (range, 8.9–11.0 million) people fell ill with TB. There were 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people and an additional 2,08,000 deaths (range, 1,77,000-2,42,000) among HIVpositive people. [1]. The diagnosis of tuberculosis (TB) still offers big diagnostic challenges related to the detection limit of smear microscopy, long time to culture-confirmation and variable sensitivity of molecular tests. In particular, diagnosing active smear-negative pulmonary TB (PTB), which represent the majority of TB cases is a major concern. Smearnegative pulmonary TB (SNPT) represents 30-60% of all pulmonary TB cases, according to region [2]. Diagnostic delays and poor microbiological accuracy within these cases lead to a late response to clinicians and consequently to a delayed optimal treatment and poorer treatment response . Furthermore, although smear-positive patients are considered to be more infectious, 10-20% of TB transmission at the population level are attributable to SNPT cases [3].

time hemi-nested PCR system which simultaneously detects Mycobacterium tuberculosis complex (MTB) genome as well as mutations that confer rifampicin resistance. It has been recently endorsed by the Scientific and Technical Advisory Board of the World Health Organization as the most sensitive rapid test for TB diagnosis in paucibacillary respiratory samples [4]. A study performed in Canada suggested a limited potential impact of Xpert due to low sensitivity of the assay in the context of less extensive disease in high-resource, low-incidence settings (overall sensitivity 46%; sensitivity in smear-negative 29%) [5]. Otherwise, Opota and colleagues found out an high sensitivity of Xpert (91.5%) in pulmonary TB in a similar setting [6]. In this study we aimed to assess the performance of the Xpert MTB/RIF system on a large number of respiratory samples with particular attention to the confirmation of TB in smear-negative cases.

MATERIALS AND METHODS

This retrospective cross sectional study was carried out by reviewed the sputum samples of suspected pulmonary tuberculosis of 150 patients attending OPD at Institute of Respiratory Diseases, Jaipur from April 2020 to March 2021 for ZN stain, and GeneXpert (Xpert® MTB/Rif assay). Patient related information was collected from the Test Requisition Forms (TRF), received with the sample. Extra pulmonary

Xpert MTB/RIF (Xpert; Cepheid, USA) is a fully automated real

Tuberculosis patients were excluded from the study. The sensitivity and specificity of GeneXpert and ZN microscopy were calculated and Prevalence of Rifampicin resistance is calculated.

150 suspected Pulmonary Tuberculosis (PTB) patients aged >16 years of either sex were enrolled in the study. Suspicion is based on symptoms and signs as well as chest x ray features (strong clinico-radiological suspicion}.

GeneXpert MTB/RIF or CBNAAT

After thorough rinsing of the oral cavity with clean water, all specimens were collected in pre-sterilized Falcon tubes with three layer packing system. Sputum specimens were processed according to the GeneXpert Dx system operator manual given by Central TB Division, Government of India, Guidance Document for Use of CBNAAT under RNTCP(9,10). The assay is designed for extraction, amplification and identification of *rpoB*gene of *M. tuberculosis*, which accounts for more than 95% of mutations associated with RIF resistance. CBNAAT exhibits high degree of specificity by using three specific primers and 5 unique molecular probes [07].

RESULTS

Out of 150 patients, 95 (63.33%) were males. Majority of the patients (95) were between 40- 60 years contributing to 56.67% patients with a mean age of 48 ± 7.20 years in our study.

Table 1 shows that out of 150 patients, 39(26%) and 61(40.67%) with sputum samples were positive by smear microscopy and CBNAAT respectively.

Table 2 shows that out of 150 patients that were subjected to CBNAAT, 61 found CBNAAT positive and 89 found CBNAAT negative. Out of 150 patients that were subjected to AFB staining, 49 found AFB positive and 89 found AFB negative. The Sensitivity, Specificity, PPV and NPV were 100%, 80.18%, 80.32% and 100% respectively.

Table 3 shows that out of 61 CBNAAT positive patients, 49 patients found AFB positive and 12 patients found AFB negative. Overall Rifampicin resistance was found in 7 CBNAAT positive patients contributing to 11.47% of Rifampicin resistance cases.

Table 1: Afb Positive Vs Cbnaat Positive Cases

	No of cases	No of cases	%
	examined	diagnosed	
AFB STAIN	150	39	26%
CBNAAT	150	61	40.67%

Table 2: A	Afb Positive .	And Negative	Vs Cbnaat	Positive A	۱nd
Negative	Cases				

	AFB POSITIVE	AFB NEGATIVE	Total
	(n=49)	(n=89)	
CBNAAT POSITIVE	49	12	61
(n=61)			
CBNAAT NEGATIVE	0	89	89
(n=89)			
Total	49	111	150

Table 3: Afb Positive And Negative Cases Vs Cbnaat Positive R Resistance Detection

CBNAAT POSITIVE (n=61)	R Resistance not	R Resistance
	detected	detected
AFB POSITIVE (n=49)	43 (87.75%)	6 (12.24%)
AFB NEGATIVE (n=12)	11 (91.66%)	1(8.33%)

DISCUSSION

Our study showed mean age of PTB patients was 48 ± 7.20 years (mean \pm SD) with male preponderance (63.33%). Dewan

R et al(8) in their study found that mean age of patients was 35 ± 9 years; 69% of were in 20-40 years age group and 76% were males. Diabetes mellitus (n=35) was a common comorbid condition in this study population.

Sensitivity of sputum smear microscopy by ZN staining was low (26%). Geleta DA et al(12) have found a very low sensitivity (9.3%) of sputum smear for AFB.

In our study, overall CBNAAT was positive in 40.67% PTB cases. Sensitivity of CBNAAT varied significantly between 100% in sputum smear-positive PTB and 13.48% in sputum smear-negative PTB. In studies conducted by Mukherjee S et al[09] and Geleta DA et al[08] showed similar results of very high sensitivity of CBNAAT in smear positive cases have been reported. Mohanty T et al[10] and Dewan R et al[11] reported sensitivity of 32% and 32.58% of CBNAAT in smear negative PTB in their studies which is quite high in comparison to our study.

Overall Sensitivity and Specificity of CBNAAT was 100% and 80.18% respectively. This finding is supported by the study of Sharma SK et al[12] where sensitivity and specificity of CBNAAT was found to be 94.5%-99% and 97.7%- 99.3% respectively.

In our study the overall prevalence of Rifampicin resistance among pulmonary tuberculosis patients was 11.47%. The prevalence of Rifampicin resistance among smear positive PTB and smear negative PTB was 12.24% and 8.33% which is comparable to patel et al[13] study which shows the overall prevalence of Rifampicin resistance was around 4.5% and prevalence of Rifampicin resistance in smear positive PTB and negative PTB were 50% and 6.12%.

CONCLUSION:

Sputum ZN smear microscopy is a highly specific but moderately sensitive test for the diagnosis of pulmonary tuberculosis. This study recommends the sputum GeneXpert MTB/RIF test to avoid a missed diagnosis of smear-negative pulmonary TB. The other major advantage of Gene Xpert is that it simultaneously detects Rifampicin resistance.

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