ISSN: 0975-3583, 0976-2833 VOL14, ISSUE4, 2023

Original Research Article

ROLE OF ZN MICROSCOPY AND CBNAAT FOR DIFFICULT DIAGNOSIS OF CLINICALLY SUSPECTED CASES OF TUBERCULOSIS IN TERTIARY CARE HOSPITAL

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Abstract

Background: Tuberculosis (TB) remains one of the world's noxious communicable diseases that is caused by the bacterium *Mycobacterium tuberculosis* (MTB). Generally, lungs (pulmonary TB) are affected by this bacteria and spread by air droplet transmission from one person to another. Improvement in diagnosis of MDR -TB highly required because MDR-TB gives difficult challenges due to its complex diagnosis methods and treatment.

Objective: The aim of this study was considering the Gene Xpert/CBNAAT techniques for detection of MTB in patients, and the rapid detection of RIF resistance from samples of Pulmonary TB and Extra Pulmonary TB cases. Results were compared with microscopic observation results of same samples..

Methodology: Total 700 patients of all age groups having either pulmonary or extra pulmonary tuberculosis were included in the study. All samples collected were processed by GeneXpert MTB/RIF machine. Which also simultaneously detect Rifampacin resistance. Samples were also examined microscopically by ZN Staining technique.

Result: According to the study, 61% (n=427) samples were reportedpositive for *Mycobacterium tuberculosis*. Out of total sample tested 36.28% (n=254) found to be refampin sensitive which means bacteria is susceptible to rifampacin. 2.71% (n=19) samples were found to be resistant to the rifampacin.

Conclusion: We can conclude that the Xpert MTB/RIF assay is the rapid technique for quick diagnosis of MTB from specimens to be tested for MTB which may missed by microscopy due to scanty numbers. It simultaneously helps to diagnose susceptibility of Mycobacterium tuberculosis with rifampacin.

Keywords: MTB/RIF, Gene Xpert techniques, Rifampacin resistance, Mycobacterium tuberculosis.

1. INTRODUCTION

Tuberculosis (TB) remains one of the world's noxious communicable diseases that is caused by the Bacteria Mycobacterium tuberculosis (MTB). Generally, lungs (pulmonary TB) are affected by this bacterium and spread by air droplet transmission from patient to healthy person.[1]

The progress to reduce the number of deaths by Tuberculosis is increasing year by year since 2005 but the COVID-19 pandemic has reversed this total number. Now, the total number of

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE4, 2023

deaths in 2020 are almost equal to the number deaths in 2017. The same trend was evident in the global Tuberculosis mortality rate. (Deaths per 100 000 population per year).The global number of deaths officially classified as caused by Tuberculosis (1.3 million) in 2020 was almost double the number caused by HIV/AIDS (0.68 million), and Tuberculosis mortality has been more severely impacted by the COVID-19 pandemic in 2020 than HIV/AIDS. In contrast to Tuberculosis, deaths from HIV/AIDS continued to decline between 2019 and 2020.[2]

Improved diagnosis of tuberculosis is a global priority for tuberculosis control which requires early case detection particularly in cases of smear-negative disease which are often associated with the HIV co-infection and young age. HIV-associated TB is often misdiagnosed due to the limitations of conventional diagnostic techniques. Improvement in diagnosis of MDR -TB highly required because MDR-TB gives difficult challenges due to its complex diagnosis methods and treatment. A quick technique for diagnosis is urgently needed due to the alarming increase in MDR-TB cases, the global emergence of extensively drug-resistant TB (XDR-TB), institutional spread that has been observed, and the increased mortality of patients who have MDR-TB or XDR-TB and HIV co-infection. [3]
Therefore, new techniques of molecular diagnostic methods in the field of TB have been introduced into practice, which are playing an important role in early diagnosis thus facilitating prompt treatment of TB. One such method is GeneXpert MTB/RIF assay which is now commonly available and being used widely. This molecular method is more subtle in detecting the presence of TB bacilli in sputum samples and other extra pulmonary samples like CSF, Synovial fluid, pericardial fluid, pleural fluid etc. where bacterial count is low; in comparison to microscopic examination. Both presence of *Mycobacterium tuberculosis* and genetic mutations associated with Rifampacin resistance can be detected simultaneously in a short duration of just two hours.[4]
GeneXpert MTB/ RIF assay is asemi-automated closed cartridge system, easy to operate and user friendly with consistently better sensitivity than sputum microscopy. It is based on a hemi nested real time PCR assay utilizing five molecular beacon technology each labelled with a differentially coloured fluorophore spanning the rpoB gene 81-bp rifampacin resistance determining region (RRDR) of M. Tuberculosis. The test concurrently determines MTB and rifampacin susceptibility, which can be used as a surrogate marker for multidrug resistance (MDR–TB) [5]. Thus, it can detect TB along with rifampacin resistance in less than two hours, directly from untreated samples.[6, 7]
The aim of this study is to assess the Gene Xpert techniques for detection of MTB among clinically suspect casesand the rapid detection of RIF resistance in smear-positive and smear-negative pulmonary and extra-pulmonary clinical specimens.

2. METHODOLOGY

Subject: Total 700 patients of all age groups having either pulmonary or extra pulmonary tuberculosis were included in the study. The study was conducted from January 2019 to December 2022 at Pacific Institute of Medical Sciences (PIMS) Udaipur (Raj.), all samples fulfill inclusive criteria were included in the study.
Inclusive area: Smear positive and Smear negative retreatment case, MDR contact, Pulmonary TB, TB-HIV Co-infected caseswere included in the study.
Exclusive area: Sputum sample which were either blood stained or Contain food particles were excluded from the study.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE4, 2023

Data collection and Examination: Good quality spot sample or early morning sampleswere used for Gene Xpert. In pediatric cases gastric lavage was collected in place of sputum. In extra pulmonary cases the samples collected were CSF, lymph node fluid, pleural fluid, synovial fluid, pericardial fluid, pleural and ascitic fluid. The Xpert assay was performed according to the manufacturer's instructions [CEPHEID, Sunnyvale, CA, USA]. The results are interpreted by the GeneXpert® Dx System from measured fluorescence signals and embedded calculation algorithms and are displayed in the "View Results" window.

3. RESULTS

Total 700 patients are included in the study. 71.86% are male participants followed by 28.14% of female participants.

Table 1: CBNAAT result of samples tested

CBNAAT	No. of cases	Percentage
Samples found Negative	427	61%
Samples found Positive	273	39%
Total samples tested	700	100%

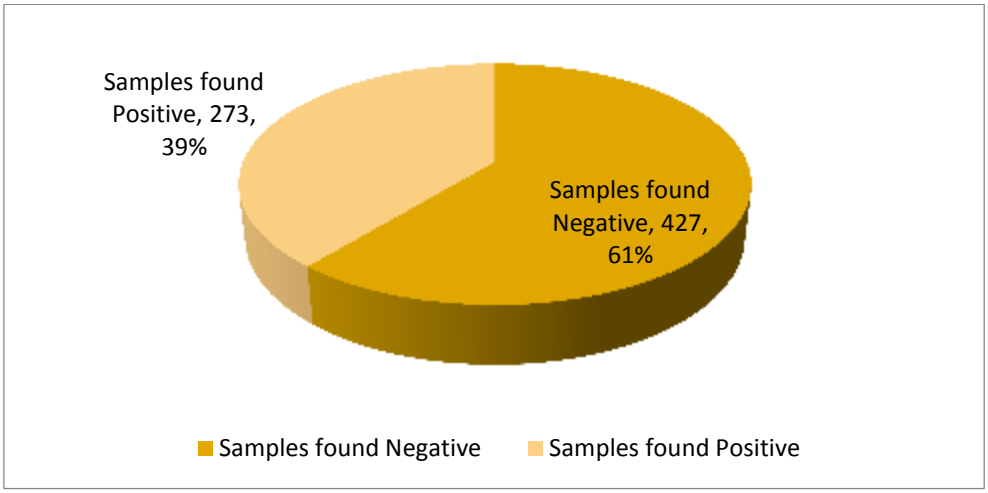

Fig No. 1; CBNAAT Test Result of Samples Tested

Table 1 and fig.1 indicates the results of samples tested by CBNAAT. According to the test 61% (n=427) samples were found to be negative and remaining 39% (n=273) were showing the presence of *Mycobacterium tuberculosis* i.e., positive result.

Table 2: Rifampacin Resistance wise Distribution of Patients

Rifampacin Resistance	No. of cases	Percentage
CBNAAT Negative	427	61%
CBNAAT reported Resistant to Rifampacin	19	2.71%
CBNAAT reported Sensitive to Rifampacin	254	36.28%
Total	700	100%

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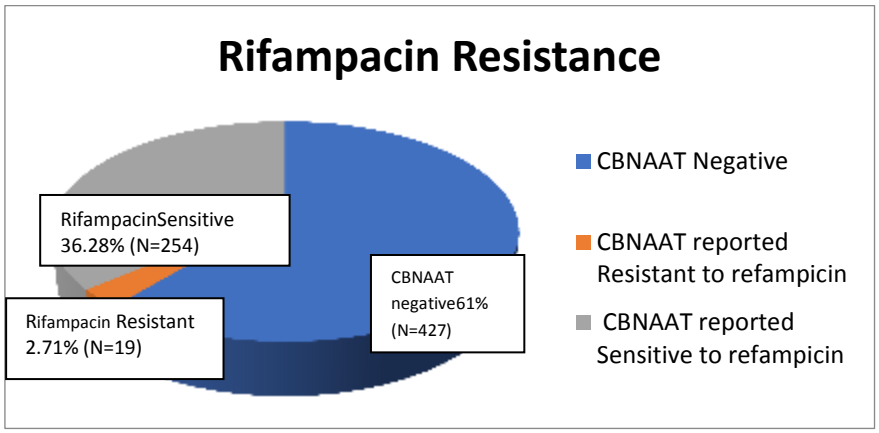

Fig No. 2: CBNAAT drug sensitivity result

Table 2 and figure2 indicates the results of Rifampacin Resistance. According to this, 36% samples were showing sensitivity to rifampacin. 2.71% samples were found to be resistant for refampicin.

4. DISCUSSION

Rifampacin resistance is a reliable predictor of multidrug resistance and a precursor to the development of multidrug resistant tuberculosis (MDR-TB). In the present study, 19 (2.71%) samples were found resistant to rifampacin. This is in accordance with the Global TB Report 2021[8], India TB Report 2021.[9]

Other studies found findings that were comparable. According to the Global TB Report 2021, 132,222 (6.29%) of the 2.1 million individuals with bacteriologically confirmed pulmonary TB in the year 2020 were rifampacin resistant.[8]India TB Report states that 2,858,713 tests on CBNAAT and 125923 tests on TrueNat were performed, out of which 53,826 (7%) and 340 (3.1%) samples were rifampacin-resistant, respectively. Out of 116 MTB positive individuals, 6.9% were rifampacin resistant, according to Okonkwo et al.[10]

Rifampacin resistance rates have been reported to range from 8.75% to 27.9% in a few studies, which are higher than the current study. sputum samples that tested positive for MTB by CBNAAT had a higher incidence of rifampacin resistance (16.8%), according to Kumar and Bhardwaj (2019).[11]According to research by Shilpa et al., 8.75% of 137 sputum samples that tested positive for MTB also had resistance. [12]In research, Mavenyengwa et al. (2017) found that rifampacin resistance was present in 27.9% of the samples.[13]

The rates of rifampacin resistance described in studies other than ours, however, ranged from 1.8% to 3.1%. Rifampacin resistance was only found 3.1% in a study by Ndubuisi et al. (2016), and 1.9% in a study by Sowjanya et al. (2014). Seven (3.7%) of the 18 (6.54%) samples that were found to be resistant to rifampacin in the current research came from newly diagnosed cases of tuberculosis, while 11 (12.7%) came from cases that had already been treated. Similar results were observed in a study by Shrestha et al. (2018), where 3.7% rates of rifampacin resistance were discovered in new cases while 19.04% resistance was discovered in cases that had already been treated.[14-16]

5. CONCLUSION

The present study was carried out to know the efficiency of CBNAAT for the diagnosis of tuberculosis in an area of Rajasthan.In the current study rifampacin resistance was found higher among males and previously treated cases as compared to new cases.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE4, 2023

The successful management of mycobacterial infection depends on an accurate and prompt diagnosis. The Xpert MTB/RIF assay was shown to be a useful tool in the current research for MTBC diagnosis. Given that it takes little time and is inexpensive, it may be very helpful in developing nations for regular rapid diagnosis.

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