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EVALUATION OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) IN THE DIAGNOSIS OF SMEAR NEGATIVE EXTRA PULMONARY TUBERCULOSIS IN A TERTIARY CARE HOSPITALKOTA, SOUTH-EAST RAJASTHAN, INDIA

Medical Microbiology

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ABSTRACT

Background: Tuberculosis in India is a major health problem, causing about 220,000 deaths every year. In 2020, the Indian government made statements to eliminate tuberculosis from the country by 2025 through its National TB Elimination Program. The Gene-Xpert-MTB/RIF is cartridge-based nucleic acid amplification assay, that amplifies and detects mycobacterial DNA using RT-PCR. The results are available within 100 minutes. **Objectives:** This study was conducted to isolate theSmear negative cases in the diagnosis of Extra Pulmonary Tuberculosis and also to detect Rifampicin resistance in Smear negative and CBNAAT positive cases in the diagnosis of Extra Pulmonary Tuberculosis. Methodology: The present study was conducted in the Govt. Medical College, Kota from period of June 2023 to May 2024.The EPTB samples collected was uses for Fluorescent staining and Cartridge Based Nucleic Acid Amplification Test (CBNAAT) Clinical specimens will be processed by standard microbiological methods. **Results:** Out of total 300 samples 266(88.6%) were detected negative and 34 (11.4%) were detected positive by CBNAAT, Among EPTB positive cases no rifampicin resistance was detected by CBNAAT in cases was observed. Mostly samples were Pleural fluid (60%) followed by Cerebrospinal fluid (17%), Pus (15%) and Ascitic fluid (8%) was the leastPus (58.8%) was the most common positive sample followed by Ascitic flud (12.5%) , Pleural fluid (5%) and CSF (3.8%) was the least positive. **Conclusion:** The easy availability of results also makes it ideal for the Indian health care settings. Although negative Xpert Ultra results are not sufficient to rule out TB, positive Xpert Ultra results may be useful in rapidly identifying EPTB cases, thus suggesting that Xpert Ultra is a useful rule-in rapid diagnostic test that can improve the definitive diagnosis of EPTB.

KEYWORDS

CBNAAT, Extra Pulmonary Tuberculosis (EPTB), Gene-Xpert-MTB/RIF, RT-PCR

INTRODUCTION

Tuberculosis in India is a major health problem, causing about 220,000 deaths every year. In 2020, the Indian government made statements to eliminate tuberculosis from the country by 2025 through its National TB Elimination Program. Interventions in this program include major investment in health care, providing supplemental nutrition credit through the Nikshay Poshan Yojana, organizing a national epidemiological survey for tuberculosis, and organizing a national campaign to tie together the Indian government and private health infrastructure for the goal of eliminating the disease. India bears a disproportionately large burden of the world's tuberculosis rates, with World Health Organization (WHO) statistics for 2011 giving an estimated incidence figure of 2.2 million cases for India out of a global incidence of 9.6 million cases.[1]Pulmonary tuberculosis is the most common presentation because it affects mostly the lungs however; it is a multi-systemic disease with a variable presentation.[2]

EPTB refers to a case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, and meninges. Diagnosis of EPTB is challenging due to high rates of smear negativity[3]. Diagnosis of Extra Pulmonary Tuberculosis (EPTB) remains especially challenging since the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deepseated organs may be difficult to obtain.[4-7]

Tuberculosis is a major public health problem and socioeconomic issue in India. Early diagnosis and treatment are the most important armour against this deadly infectious disease. A rapid diagnostic test with high sensitivity and specificity is a long-awaited dream and is quite essential to win the battle against this 'Captain of Men of AllDeaths, So, the present study will be undertaken to assess the importance of CBNAAT in SmearNegativeTuberculosis in the field of diagnosis by comparing with culture and to find out RifampicinResistance if present by CBNAAT.

The Gene-Xpert-MTB/RIF or cartridge-based nucleic acid amplification test (CBNAAT) is a semi automated, cartridge-based nucleic acid amplification assay, that amplifies and detects mycobacterial DNA (deoxyribonucleic acid) using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The results are available within 100 minutes [8].

AIM AND OBJECTIVES

This study was conducted to isolate the Early Detection of Smear Negative cases in the Diagnosis of Extra Pulmonary Tuberculosis that helps in guiding Clinicians for appropriate Treatment thus reducing morbidity and mortality and alsoto detect Rifampicin Resistance in Smear Negative and CBNAAT Positive cases in the Diagnosis of Extra Pulmonary Tuberculosis. The ultimate goal of this study was to identify EPTB infection in early phase of disease and prevent future complications

MATERIALAND METHOD

Study Design: The present study was conducted in the Govt. Medical College-Kota.

Study Type– Cross-sectional (Observational) study

Study Period– Sample collection and analysis was done after approval from institutional ethical committee from June 2023 to May 2024 (1YEAR)

Study Population– All Suspected TB Patients

Type of Sample– Pleural fluid, Ascitic fluid, Cerebro Spinal Fluid (CSF) and Pus

Sample Size – 300

Sample size was calculated by using formula as follows: $N=\frac{z^2pq}{d^2}$

Inclusion Criteria

1. Suspected extra pulmonary tb patient.
2. Patients who give Consent.

Exclusion Criteria

1. All pulmonary TB patient.
2. All patients who are on anti Tuberculosis Treatment.
3. Patients who do not give consent

Sample Collection

All samples from NMC Hospital of Government Medical College, Kota was received,collected and transported immediately to the department of Microbiology in an appropriate and sterile container where they was further processed.The samples collected was uses for Fluorescent staining and Cartridge Based Nucleic Acid Amplification Test (CBNAAT) Clinical specimens will be processed by NTEP guidelines Government of India.

Auramine- Rhodamine Staining

The fluorochrome dye, Auramine-Rhodamine, used combine with the

mycolic acid on the cell wall of the bacteria, which is then fixed by steamed heat. A decolorizing agent, acid alcohol is used to rinse off the none stained dyes. The counterstain Potassium Permanganate functions to stain the non-fluorescent tissues and the cell debris thus reducing possibilities of artifacts. When the cells are observed under an Ultra-violet Light, they appear bright yellow-green or red-orange[9]

Cartridge-based Nucleic Acid Amplification Test (CBNAAT)

Cartridge-based Nucleic Acid Amplification Test (CBNAAT) is used to detect Mycobacteria tuberculosis and rifampicin-resistance using GeneXpert IV Dx system and the Xpert MTB/ RIF cartridge.The CBNAAT system integrates and automates sample processing, nucleic acid amplification, and detection of Mycobacteria tuberculosis and rifampicin resistance. The system utilises the use of single-use disposable CBNAAT cartridges that hold the Polymerase Chain Reaction (PCR) reagents and hosts the PCR process.[10-11]

The process involves the following steps: **1. Sample Processing:** Specimens are processed by adding the sample reagent at 2:1 (v/v) ratio to the sputum sample, mixing and incubation for 15 minutes at room temperature. The sputum sample get liquified after the processing step. **2. Loading Sample into Cartridge:** Liquefied sample is added to the cartridge using a transfer pipette. **3. Setting up the Machine to Run the Test:** After switching on the system, "Create Test" (Figure A) is clicked in the GeneXpert Dx system window, test details are added, the barcode label of the cartridge is scanned (Figure B) and "Start Test" (Figure C) is clicked to initiate testing. **4. Loading the Cartridge:** The instrument module door which displays the blinking green light is opened to load the cartridge. The door of module is closed firmly (an audible click sound should be heard). The green light stops blinking when the test starts.**5. Obtaining the Results:** When the test is finished, the green light turns off. It takes around 1 hour 55 minutes to complete test run. On completion of test run, the result is generated automatically on the monitor. **6. Ending the Test Run:** When the system releases the door lock at the end of run, the module door is opened to remove the cartridge. The used cartridge is discarded.

Compilation of Data: All demographic and clinical data was collected and documented in pre-structured Performa.

Statistical Analysis of Data: Collected Data was entered into Microsoft Excel spreadsheet. Categorical variables were expressed as percentage basis and analyzed as a chi-square test, continuous data expressed as mean and Standard deviation and p value less than 0.05 was taken as statistically significant for all statistical analysis done.

RESULTS

Out of total 300 samples 266(88.6%) were detected negative and 34 (11.4%) were detected positive by CBNAAT, Among EPTB positive cases no rifampicin resistance was detected by CBNAAT in cases was observed. Mostly samples were Pleural fluid (60%) followed by Cerebrospinal fluid (17%), Pus (15%) and Ascitic fluid (8%) was the least.Pus (58.8%) was the most common positive sample followed by Asciticflud (12.5%) , Pleural fluid (5%) and CSF (3.8%) was the least positive.

Table 1-Fluorescent Stain vs CBNAAT Distribution of EPTB Samples

	TOTAL	NEGATIVE	POSITIVE
FLURUORESCENT SATIN	300	300	0
CBNAAT	300	266	34

Table 2-Sample Wise Distribution EPTB

SAMPLE	VALUE	TOTAL	PERCENTAGE
PF	178	300	60%
CSF	52	300	17%
PUS	46	300	15%
ASF	24	300	8%

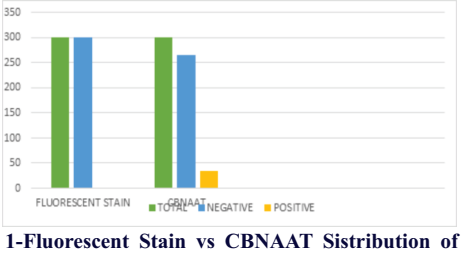

Figure 1-Fluorescent Stain vs CBNAAT Sistribution of EPTB Sample

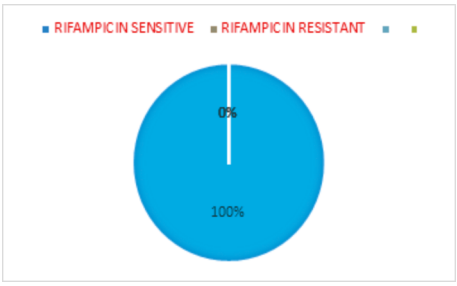

Figure 2-Rifampicin Sensitivity in Positive EPTB Sample

DISCUSSION

The present study was planned to identify Mycobacterium tuberculosis complex & detect rifampicin resistance in smear negative extra pulmonary specimens by Xpert MTB/RIF Ultra.In our study out of the total 300 samples, 34 (11%) were found positive for EPTB which is not similar to a study conducted by Gupta S et al where out of 100 EPTB specimens MTB Complex was found to be positive in 13 % cases.[12].In the present study, 300 samples were clinically suspected patients of EPTB. Out of these samples, the maximum was Pleural fluid (60%) followed by Cerebrospinal fluid (17%), Pus(15%) and Ascitic flud (8%) was the least. Similarly in some studies pleural fluid was found to be the most common sample received followed by ascitic fluid. In contrast lymph node aspirate was the most common sample (32.5%) followed by pleural fluid (29.3%) in some studies,[13] Many other studies have also reported the same.[14-16]In the present study, Out of 34 CBNAAT positive cases, Rifampicin resistance was seen in 0.0% of the patients while remaining 100% of the patients rifampicin resistance was not detected. In a study conducted by Adhikary et al Rifampicin resistance prevalence was 10.75% which may be due to different patient selection criteria, geographic variation, and variation in sample size (smaller the sample size, more likely higher resistance rate). [17-20]

Limitation of Study

Clinical characteristics of the subjects were not taken into consideration in this study. Treatment outcome was not followed up in this study. Co morbidities and vulnerability of the patients were not assessed.

SUMMARY AND CONCLUSION

Gene Xpert MTB/RIF assay is efficient and reliable technique for the rapid diagnosis of extra pulmonary TB, especially in smear negative cases. Its simplicity, sensitivity, speed and automation, make this technique a very attractive tool for diagnosis of Mycobacterium tuberculosis from extra pulmonary samples in MDR cases and smear negative cases of TB suspects. Many studies have shown a potential for the use of Xpert Ultra in extra-pulmonary specimens. The easy availability of results also makes it ideal for the Indian health care settings. Although negative Xpert Ultra results are not sufficient to rule out TB, positive Xpert Ultra results may be useful in rapidly identifying EPTB cases, thus suggesting that Xpert Ultra is a useful rule-in rapid diagnostic test that can improve the definitive diagnosis of EPTB.

DECLARATIONS

Conflict of Interest: The authors have no conflict of interest.

Human and Animal Rights and Informed Consent: This article does not contain any studies with human or animal subjects performed by authors.

Bio-Safety: All standard precautions, bio-safety measures & Biomedical Waste Management in our study according to Biological Waste Management's Rules 2016 and it`s new amendment were observed.

Data Availability: All datasets generated of analyzed during this study are included in the manuscript.

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