

EVALUATION OF CBNAAT IN THE DIAGNOSIS OF SMEAR NEGATIVE TUBERCULOSIS IN A TERTIARY CARE HOSPITAL

Rachamadugu Hymavathi¹, Gundela Swarnalatha², Beldona Nagajyothi³, Basireddy Srikanth Reddy⁴

¹Assistant Professor, Department of Microbiology, Kurnool Medical College, Kurnool.

²Professor and HOD, Department of Microbiology, Kurnool Medical College, Kurnool.

³Associate Professor, Department of Microbiology, Kurnool Medical College, Kurnool.

⁴Assistant Professor, Department of Microbiology, Kurnool Medical College, Kurnool.

ABSTRACT

BACKGROUND

Tuberculosis is world's one of the deadliest communicable diseases, which requires early diagnosis and treatment. As smear confirmation requires at least 10000 bacilli/mL and conventional culture methods take 4 - 8 weeks, nucleic acid amplification methods are better option for early diagnosis of the disease.

MATERIALS AND METHODS

In the present study, we evaluated the diagnostic yield of CBNAAT (Cartridge Based Nucleic Acid Amplification Technique) in the detection of Tuberculosis in smear negative patients attending Government General Hospital, Kurnool, Andhra Pradesh.

RESULTS

Out of the total 3157 suspected Tuberculosis cases 2850 were smear negative samples, out of which 353 samples were diagnosed as CBNAAT positive with 19 Rifampicin resistance cases.

CONCLUSION

From this study, NAA technique is found to be a better diagnostic method when compared to the microscopy.

KEYWORDS

CBNAAT, Fluorescent Microscopy, Sensitivity, Specificity.

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BACKGROUND

Tuberculosis is an ancient and potentially fatal communicable disease caused mainly by *M. tuberculosis* complex (MTC), that can affect any part of the body with lungs being the most common organ involved. It is estimated that 1/3 of the world's population (2 Billion) is infected with this organism⁽¹⁾; 8 to 9 million new cases appear every year and 3 million persons die from this disease. India is one of the worst affected countries and having > 40% population infected, out of which 3 million are highly infectious open cases. Half million people die from this infection every year in India- one every minute, which reflects the magnitude of this highly fatal disease.⁽²⁾

If TB is to be eliminated as a global problem, earlier diagnosis and treatment will be essential. Tuberculosis can be diagnosed by Smear microscopy either by Ziehl-Neelsen's or Fluorescent staining; conventional culture either liquid or solid culture methods or molecular methods like GeneXpert, PCR and CBNAAT. Even though smear microscopy is easy and economical, it has some problems in detecting tuberculosis in patients with low bacterial load and differentiation is also

difficult with other mycobacteria. Conventional culture will take 2 to 8 weeks to obtain the growth, which hampers the early diagnosis and treatment.^(3,4) Drug resistant tuberculosis has become a problem in high TB countries including India.

Keeping in view of all the above problems, WHO has recently recommended a real time PCR test called CBNAAT as a primary aid for earlier and better diagnosis of the infection including Drug resistance.

Aims and Objectives

1. To evaluate the efficacy of CBNAAT in smear negative cases in the diagnosis of tuberculosis.
2. To detect Rifampicin Resistance.

MATERIALS AND METHODS

The present study was a prospective observational study conducted in the Department of Microbiology, Kurnool Medical College and Government General Hospital, Kurnool from January 2016 to July 2017. All the suspected tuberculosis patients attending GGH were selected, came out to be a total of 3157. These cases were included irrespective of age and sex. The study group includes pulmonary and extrapulmonary samples including sputum, pleural fluid, CSF, pus, lymph nodes, paraspinal and gastric aspirates, ascitic and pericardial fluids and biopsy tissues.

Sample Processing

After the informed consent taken from the patients, samples were collected with suspected TB in a sterile container and transported to the laboratory within one hour. Samples were

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Corresponding Author:

Dr. Gundela Swarnalatha,

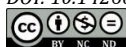
Professor and HOD,

Department of Microbiology,

KMC, Kurnool-518002.

E-mail: swarnalathagundela@gmail.com

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screened by microscopic examination (Ziehl-Neelsen’s stain and fluorescent stain). All the smear negative cases were subjected to CBNAAT testing.

CBNAAT

Using the sterile pipette provided with the kit, sample was drawn from the container 1 cm past the minimum mark and transferred into the large opening at the bottom right of the cartridge slowly without shaking. After closing the lid, the program was started. Cartridge was put in the module after the initialisation and the module door was closed. After the program completed, the cartridge was removed and disposed off in the Biohazard Waste disposal bin and the results were recorded electronically.

Statistical Analysis

Data was entered in Excel version 2007. Data analysed in percentages and accuracy tests like sensitivity, specificity, false positives, false negatives, positive predictive value and negative predictive value were done. Results were recorded and interpreted in Bar charts and Pie diagrams.

RESULTS

A total of 3157 suspected tuberculosis patients were screened by microscopy. Out of them, 2850 were smear negative (Table 1 and Fig. 1). Out of these 2850 samples tested by CBNAAT 353 samples were detected positive by CBNAAT positive, 2389 cases as negative and 108 samples had errors (Table 2 and Fig. 2).

Microscopy	Number of Samples	Percentage (%)
Smear Positive	307	9.72
Smear Negative	2850	90.27
Total Samples	3157	100

Table 1. Percentage of Smear Positive Cases

CBNAAT Positive	No. of Samples	(%)
CBNAAT Positive	353	12.38
CBNAAT Negative	2389	83.8
Errors (Invalid and No results)	108	3.78
Total Smear Negative Samples	2850	100

Table 2. Percentage of CBNAAT Positive Cases

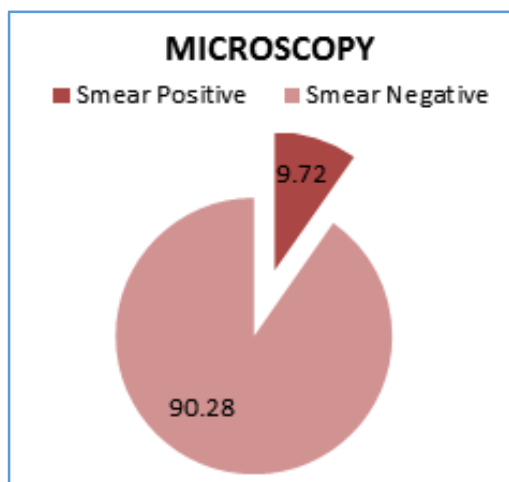


Figure 1. Microscopy Results

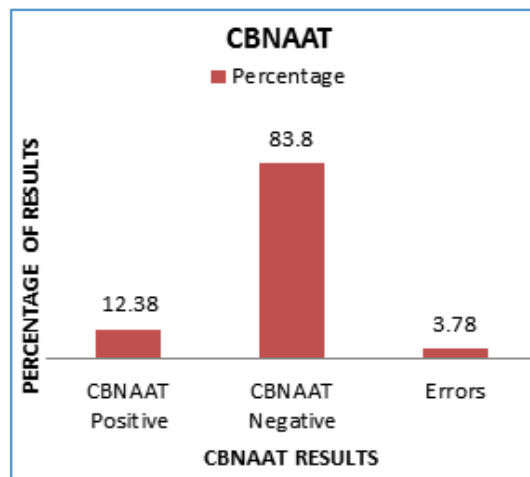


Figure 2. CBNAAT Results

Out of the 2850 smear negative cases, 2585 were pulmonary samples and 265 were extrapulmonary samples (Table 3 and Fig. 3).

Sample	Number of Samples	(%)
Pulmonary Samples	2585	90.7
Extrapulmonary Samples	265	9.3
Total Smear Negatives	2850	100

Table 3. Distribution of Pulmonary and Extrapulmonary Samples

Out of 2585 pulmonary specimens screened, 335 (%) were positive by CBNAAT, whereas 18 out of 265 extrapulmonary samples were identified as positive by CBNAAT (Table 4 and Fig. 4).

Positive Sample Types	Number of Samples	(%)
Pulmonary Positive	335	94.9
Extrapulmonary	18	5.1
Total Positives	353	100

Table 4. CBNAAT Positive Samples in Pulmonary and Extrapulmonary Samples

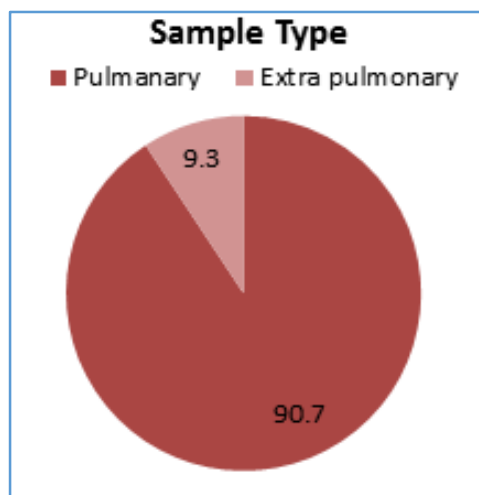


Figure 3. Distribution of Samples

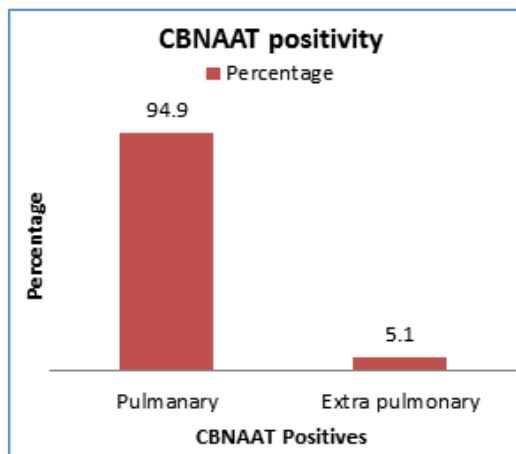


Figure 4. CBNAAT Positive Percentage

Out of 353 positives identified by CBNAAT 330 were sensitive to rifampicin, while MDR TB was detected in 19 samples (5.4%); 4 samples have shown indeterminate results (Table 5 and Fig. 5, 6).

Drug Resistance Pattern	No. of Samples	(%)
Rifampicin Sensitive (RS)	330	93.4
Rifampicin Resistant (RR)	19	5.4
Rifampicin Indeterminate (RI)	4	1.2
Total Positives	353	100

Table 5. Percentage of Rifampicin Resistance Detected by CBNAAT

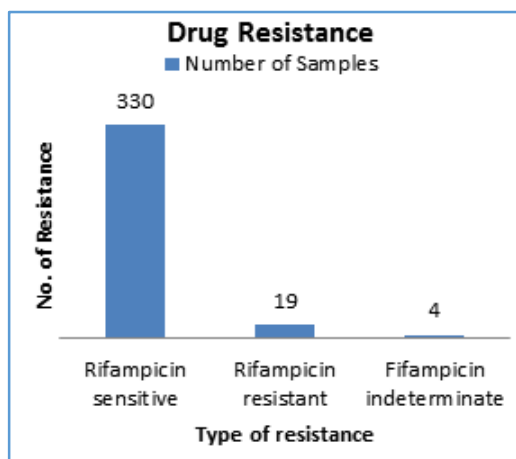


Figure 5. Drug Resistance

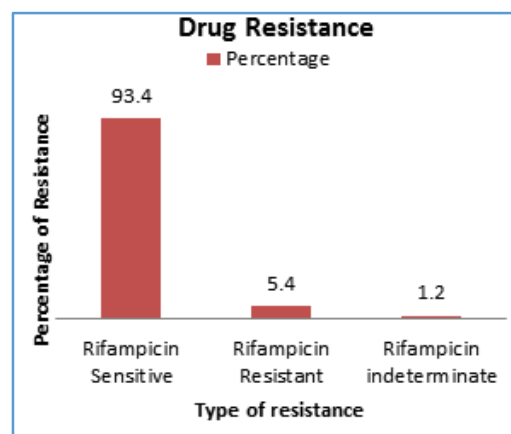


Figure 6. Percentage of Drug Resistance

HIV patients constituted 1252 out of the 2850 cases. Out of these 1252 HIV patients, 129 were identified positive by CBNAAT (Tables 6 and 7).

Sample	Number of Samples	Percentage (%)
HIV Positive	1252	43.9
HIV Negative	1598	56.1
Total Samples	2850	100

Table 6. Distribution of HIV Cases

CBNAAT Results in HIV Positives	Number of Samples	Percentage (%)
CBNAAT Positive	129	10.3
CBNAAT Negative	1123	89.7
Total HIV Positive	1252	100

Table 7. Percentage of CBNAAT Positives in HIV Patients

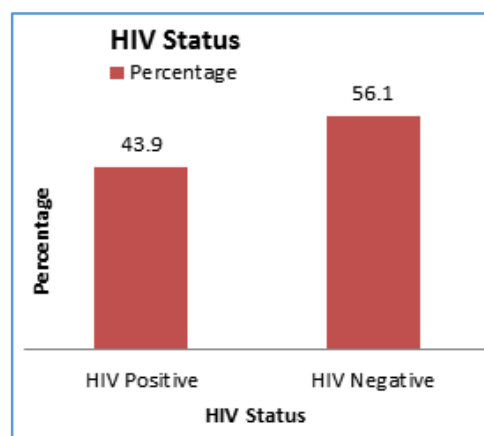


Figure 7. Distribution of HIV Status

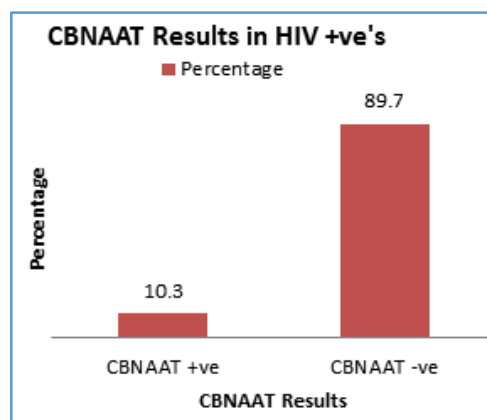


Figure 8. CBNAAT Results in HIV +ve's

DISCUSSION

Tuberculosis is one of the deadliest diseases ever known to the mankind. With the emergence of HIV, the incidence of TB has risen in the past few decades. Though there are many diagnostic tests available, the diagnosis is always a tedious procedure and has its limitations. Staining methods like ZN and fluorescent stains are rapid but lack the sensitivity, whereas culture is highly sensitive but is time consuming. Above this, there is emergence of drug resistance with MDR TB and XDR TB spreading rampantly in the community. Early diagnosis and prompt treatment is the motto for the tuberculosis control programs, which prevents the spread and emergence of drug resistance. In such a scenario, nucleic acid based diagnostic tests have emerged as a boon for the TB

patients. With the introduction of CBNAAT, the diagnosis of TB has been eased. It not only helps in the early diagnosis of the patients, but also helps in identifying the rifampicin drug resistance within 2 hours which is extremely helpful for the categorical treatment of the patients.

In the present study the sensitivity, specificity, PPV and NPV of smear microscopy were 45.9%, 99.7%, 97.7% and 87.6%; whereas the sensitivity, specificity, PPV and NPV of GeneXpert were 54%, 100%, 100% and 89.3%. Our study results correlated well with the study results of many other researchers. In majority of the studies, microscopy sensitivity ranged from 22% to 41% and specificity from 95% to 100% which is similar to our study findings.^(5,6,7)

In Agarwal et al⁽⁸⁾ study for smear negative samples, the sensitivity and specificity of GeneXpert was 79.1% and 93.1%. Many other studies have the sensitivities and specificities in the range of 57% - 75% and 97% - 100% respectively.⁽⁹⁻¹²⁾

In the present study rifampicin resistance was detected in 5.4% of the total cases, which is similar to the study conducted by Habte et al¹³ where the resistance rate was 4.2%.

The present study highlights the importance of nucleic acid based testing methods, especially in smear negative cases. In extrapulmonary cases and HIV cases, it is often difficult to identify the pathogen by routine acid fast staining. In such cases, the GeneXpert plays pivotal role in the detection of the *M. tuberculosis* and also in the detection of the drug resistance to rifampicin.

CONCLUSION

Both conventional microscopy and GeneXpert are important tools in the rapid identification of tuberculosis. But as the sensitivity of the GeneXpert is higher than the microscopy and it also detects drug resistance to rifampicin, the utility of the CBNAAT is much more accountable especially in the smear negative tuberculosis cases. With extended coverage of GeneXpert services in both urban and rural level in the future will help not only in the early diagnosis and prompt treatment but also prevents the spread of infection and emergence of drug resistance.

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