ORIGINAL RESEARCH PAPER

Pulmonary Medicine

A STUDY OF THE DIAGNOSTIC UTILITY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST OF VARIOUS BODY FLUIDS OTHER THAN SPUTUM IN DIFFERENT FORMS OF TUBERCULOSIS

KEY WORDS: CBNAAT, Sensitivity, Specificity, BAL, Pleural Fluid, Pus, Lymph Node, Gastric Aspirate

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ABSTRACT

Introduction: Diagnosis of TB is difficult in specimens having low number of bacilli. In recent times, CBNAAT is used due to its rapidity, sensitivity and specificity The heterogeneous clinical presentations, pauci-bacillary nature and difficulty in obtaining specimens (often requiring invasive procedures) make the diagnosis of TB, a challenging task and hence the requirement for a rapid, simplified and cost effective diagnostic tool arises. **Objectives:** To determine the sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT for diagnosis of Tuberculosis from specimens other than sputum. **Methodology:** Study Design-Longitudinal prospective study, Duration of Study-1 year study period, Study Setting -RNTCP Cell in the Department of Respiratory Medicine, MMCHRI, Sample Size-60, Study Population-Sputum Negative Presumptive Pulmonary and Extra pulmonary Tuberculosis Patients, Sample Collection- BAL, Pleural Fluid, Pus Aspirate, CSF, Lymph Node aspirate, Ascitic Fluid, Gastric Aspirate, Synovial Fluid. **Results:** In our study Majority of study population were in the age group of Less than 30(30%). About 25% were in the age group of more than 61 years. Nearly 12% were in the age group of 41-50 years. About 15% were in the age group of 31-40 years. Nearly 18% were in the age group of 51-60 years. In our study 63% were males and 37% were females. In our study 40% are smokers and 60% are nonsmokers. In our study 27% are diabetics and 73% are non-diabetics. In our study 3.4% had HIV and 96.6% are HIV negative. 10% of study population had previously taken ATT and 90% had not taken ATT. About 25% tested positive for CBNAAT test and 75% tested negative for the test. **Conclusion:** The diagnosis of Tuberculosis remains challenging since the number of M.tb bacilli present in tissue at the site of disease is often low and clinical specimen from deep seated organs may be difficult to obtain. CBNAAT provides an edge over other methods by reducing the time required for diagnosis and precision in detection of M.tb bacilli not only in sputum samples but also in extra-pulmonary specimens.

INTRODUCTION

Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Millions of people continue to fall sick with TB each year. In 2017, TB caused an estimated 1.3 million deaths (range, 1.2–1.4 million) among HIV-negative people and there were an additional 300 000 deaths from TB (range, 266 000–335 000) among HIV-positive people.

Globally, the best estimate is that 10.0 million people (range, 9.0–11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women and 1.0 million children. There were cases in all countries and age groups, but overall 90% were adults (aged ≥15 years), 9% were people living with HIV (72% in Africa) and two thirds were in eight countries, among which India accounts for 27%.

Diagnosis of TB is difficult in specimens having low number of bacilli. In recent times, CBNAAT is used due to its rapidity, sensitivity and specificity. Gene Xpert-MTB/RIF Assay is a cartridge based, semi-automated, rapid molecular assay, which permits rapid TB diagnosis through detection of the DNA of Mycobacterium tuberculosis and simultaneous identification of mutation that confers Rifampicin resistance (which is highly predictive of MDR TB) It is very important to diagnose and treat TB to cut down its transmission.

METHODOLOGY

- Study Design-Longitudinal prospective study
- Duration of Study-1 year
- Study Setting -RNTCP Cell in the Department of Respiratory Medicine, MMCHRI
- Sample Size-60
- Study Population-Sputum Negative Presumptive Pulmonary and Extra pulmonary Tuberculosis Patients
- Sample Collection- BAL, Pleural Fluid, Pus Aspirate, CSF, Lymph Node aspirate, Ascitic Fluid, Gastric Aspirate, Synovial Fluid

INCLUSION CRITERIA-

- All Sputum Negative Patients of Presumptive Pulmonary and Extra Pulmonary Tuberculosis of pediatric and adult age groups

EXCLUSION CRITERIA-

- Sputum for AFB positive on smear microscopy

All Sputum Negative Patients of Presumptive Pulmonary and Extra Pulmonary Tuberculosis of pediatric and adult age groups were subjected to:

a) Data collection based on demographics, Smoking and Alcohol habits, comorbidities, past H/O TB, HIV status
b) After data collection, sample collection was done depending on the type of presentation of the case
c) The sample collected was stored in Falcon's Tube for CBNAAT and one more tube for Culture
d) The CBNAAT report was then compared with that of Liquid Culture and the analysis was done
e) Sensitivity and Specificity of CBNAAT was done for the sample specimens.

RESULTS

In our study Majority of study population were in the age group of Less than 30 (30%). About 25% were in the age group of more than 61 years. Nearly 12% were in the age group of 41-50 years. About 15% were in the age group of 31-40 years. Nearly 18% were in the age group of 51-60 years. In our study 63% were males and 37% were females. In our study 40% are

smokers and 60% are nonsmokers. In our study 27% are diabetics and 73% are non-diabetics. In our study 3.4% had HIV and 96.6% are HIV negative. 10% of study population had previously taken ATT and 90% had not taken ATT. About 25% tested positive for CBNAAT test and 75% tested negative for the test.

Table 1: Age wise distribution of study participants

Age in Years	Frequency	Percentage	Mean ± S.D
≤ 30	18	30	42.27±22.35
31-40	9	15	
41-50	7	11.67	
51-60	11	18.33	
≥61	15	25	
Total	**60**	**100**	

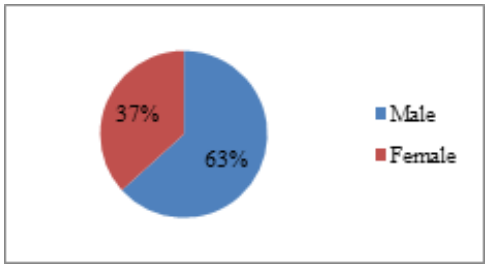

Figure 1: Sex wise Distribution of study participants

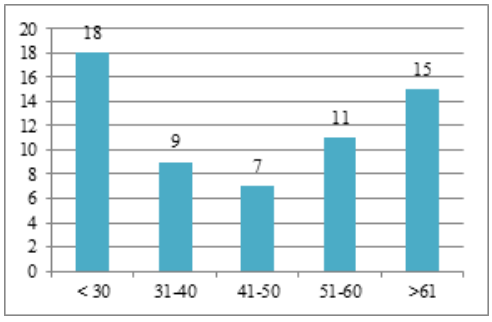

Figure 2: Age wise distribution of study participants

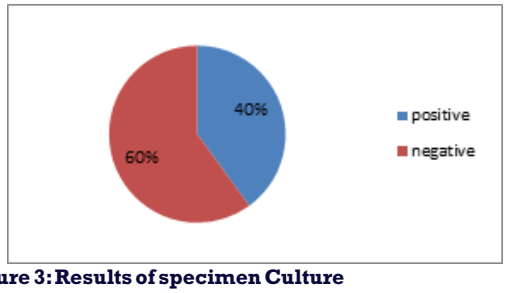

Figure 3: Results of specimen Culture

Table 2: Samples collected among study participants

Sample	Frequency	Percentage
Pus aspirate	4	6.67
Ascitic fluid	8	13.33
Bronchoalveolar lavage	20	33.33
Cerebrospinal fluid	1	1.67
Gastric aspirate	8	13.33
Lymph node	6	10
Pleural fluid	10	16.67
Synovial fluid	3	5
Total	**60**	**100**

Table 3: Rifampicin Sensitivity among CBNAAT Positive patients

Drug Sensitivity	Frequency	Percent
Rifampicin Sensitive	14	93.33
Rifampicin Resistance	1	6.67
Total	**15**	**100**

Table 5: Validity of CBNAAT amongst specimen

CBNAAT	Specimen		Total
	Positive	Negative	
Positive	15	0	15
Negative	9	36	45
Total	24	36	60

- Sensitivity =62.50%
- Specificity =100%
- Positive predictive value=100%
- Negative predictive value=80%

DISCUSSION

The present study is a longitudinal study involving 60 patients attending respiratory department of Meenakshi Medical college Hospital and Research Institute. All Sputum Negative Patients of Presumptive Pulmonary and Extra Pulmonary Tuberculosis of pediatric and adult age groups having symptoms of fever, cough with expectoration, loss of weight are included in this study.

Demographic results:

In our study Majority of study population were in the age group of Less than 30(30%). About 25% were in the age group of more than 61 years and nearly 12% in the age group of 41-50 years. About 15% were in the age group of 31-40 years. Nearly 18% were in the age group of 51-60 years. Mean age is 42.27 and the standard deviation is 22.35.

In our study 63% were males and 37% were females. In our study 40% are smokers and 60% are nonsmokers. In our study 27% are diabetics and 73% are non-diabetics. In our study 3.4% had HIV and 96.6% are HIV negative.

10% of study population had previously taken ATT and 90% had not taken ATT.

In our study 35% of population weighed between 51-60kg, 30% weighed between 61-70 kg. About 28% weighed below 50 kg. About 7% weighed more than 71 kg. Mean weight is 53.02 and standard deviation is 17.29

In our study, about 25% of study population had tested positive for CBNAAT. Amongst that, Rifampicin sensitivity was 93.33% and Rifampicin Resistance was 6.67%. About 33% of sample is collected from bronchoalveolar lavage. 13.33% of sample is collected from ascetic fluid and 13.33% of sample from gastric aspirate. About 40% had positive results in specimen and 60% had negative results to specimen About 25% tested positive for CBNAAT test and 75% tested negative for the test.

Rifampicin resistance detection by CBNAAT has greater advantage in treatment of the patients with shorter turnaround time (2 hours) which is not possible with FNA and LED even though FNA is cost effective in the diagnosis of EPTB, combining with CBNAAT has an advantage of detection of FNA missed cases and it can be integrated into a routine diagnostic protocol.

As the number of Mycobacterium Tuberculosis Bacilli (MTB) in extra pulmonary sites is often low, diagnosis of EPTB still remains challenging. Cytology and conventional smear microscopy have been used as the initial diagnostic tools in the extra pulmonary tuberculosis in resource poor settings.

Comparison with other studies:

	Sensitivity	Specificity
Sharma etal[1]	95.7	99.3
Kumar anshuetal[2]	92.7	98.9
Sanjayetal[3]	95.7	99.3
Kapleshetal[4]	87.5	94.4
Present study	62.5	100

	Sensitivity	Specificity	PPV	NPV
Kumar anshuetal[2]	92.7	98.9	97.1	97.2
Present study	62.5	100	100	80

	Gayathri et al[5]	Malakar et al[6]	Singh et al[8]	Present study	Narang et al[7]
Positive	58	90	77	25	85
Negative	42	10	23	75	15

CONCLUSION

The heterogeneous clinical presentations, paucibacillary nature and difficulty in obtaining specimens (often requiring invasive procedures) make the diagnosis of TB, a challenging task and hence the requirement for a rapid, simplified and cost effective diagnostic tool arises.

This is where CBNAAT plays an important role leading to early initiation of appropriate therapy, improved treatment outcomes, minimizing morbidity and mortality.

CBNAAT is a semi-quantitative nested nucleic acid amplification test based on molecular detection of mutated gene. It is simple, rapid, cost effective and doesn't require technical expertise. It can be carried out in automated manner including bacterial lysis, nucleic acid extraction, and amplification and detection. It can diagnose TB within 2 hours and gives accurate results due to use of disposable closed cartridges preventing cross contamination. [1]

In settings where resources are limited for facilities like Culture, DST, CBNAAT is extremely useful, simple and reliable test. It also has a significant role to play in the diagnosis of tuberculosis. Its potential in TB detection has been underutilized due to lack of awareness regarding the same. Hence, the study was conducted the study to determine effectiveness of this rapid and logistically simplified test in the diagnosis of TB.

The main advantages of the test are, for diagnosis, reliability when compared to sputum microscopy and the speed of getting the result when compared with the culture test. For diagnosis of TB, although sputum microscopy is both quick and cheap, it is often unreliable. It is particularly unreliable when people are HIV positive. Although culture gives a definitive diagnosis, to get the result usually takes weeks rather than the hours of the GeneXpert test. The main advantage in respect of identifying rifampicin resistance, is again the matter of speed. Normally to get any drug resistance result takes weeks rather than hours.

In a low-resource high-burden setting, CBNAAT may have greatest impact where the clinician's pretest confidence in TB is low and empirical treatment has not been started. It is expected from the Study that CBNAAT will show a high sensitivity and specificity.

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