Original Research Article

Prevalence of positivity of CBNAAT & rifampicin resistance in extra-pulmonary tuberculosis in tertiary care Centre of Gujarat, India

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Abstract

Background: Tuberculosis (TB) is a significant health problem in developing countries like India and remains a key challenge to public health due to inadequate diagnostic assays. While considerable advances have been made in diagnosing pulmonary tuberculosis, EPTB diagnosis and management remain challenging. Moreover, due to the paucibacillary nature of extrapulmonary specimens and sample collection often requiring invasive procedures, diagnosis of EPTB is often delayed.

Aims and Objectives: Determine the prevalence of positivity of CBNNAT and detection of rifampicin resistance in EPTB samples

Materials and Methods: This prospective observational study was conducted in a tertiary care hospital in Bhavnagar, Gujarat, from January 2021 to January 2022. The investigation comprised 470 extrapulmonary clinical samples from patients suspected of having EPTB, including lymph nodes, pus, pleural fluid, CSF, ascitic fluid, and tissue aspirate analyzed. The samples were divided into two parts—Ziehl stained one part–Neelsen stain and auramine-rhodamine stain and examined under the conventional microscope. The second part used CBNAAT to detect MTB and rifampicin resistance.

Results and Observations: Out of 470 samples, MTB was detected in 54(11%) samples. Of these 54 samples, 47 were sensitive to rifampicin. 3(5.5%) were resistant to rifampicin, and 4(7.40%) were resistant indeterminate. While 16(3.4%) samples showed AFB by Ziehl–Neelsen stain, 26 samples (5.53%) were detected positive by a fluorescent stain. Out of these 470 samples, 19(12.4%) samples of pleural fluid, 12(21.4%) samples of lymph node aspirate, 2(10%) samples of empyema, 4(9.09%) samples of ascitic fluid, 11(14.8%) samples of pus, 6(5.9%) samples of CSF showed MTB detected

Conclusion: CBNAAT has the potential to significantly improve and escalate the diagnosis of smear-negative body fluid specimens in regions not only with high TB burden but also with overlapping HIV. Also, the detection of Rifampicin resistance aids in the prompt initiation of appropriate therapy, thus improving the overall quality of TB care.

Keywords: Cartridge base nucleic amplification test (CBNAAT), extrapulmonary tuberculosis (EPTB), Acid fast bacilli (AFB)

Introduction:Tuberculosis (TB) is a contagious disease, predominantly involving the lungs, caused by Mycobacterium tuberculosis (MTB).[1] Traditionally, it was believed that pulmonary TB constitutes around 85% of total TB cases, whereas the remaining 15% are extrapulmonary tuberculosis (EPTB) cases.[2] But, current data from around the world show a massive variation in the proportion of EPTB among all TB cases, ranging from 15% to 53%.[3] Tuberculosis (TB), Before the COVID-19 pandemic, was the world's leading cause of death from a single infectious agent. As per The Global TB Report 2022[4], 10.6 million people fell ill with TB worldwide in 2021, whereas there were 1.4 million TB deaths among HIV-negative people and an additional 1,87,000 among HIV-positive people.[5]

While significant advances have been made in diagnosing pulmonary tuberculosis, EPTB diagnosis and management remain a considerable challenge. EPTB, which can involve almost any system of the body, along with the ambiguity regarding clinical management, has made it a formidable enemy in the war against TB—clinical manifestations of EPTB range from nonspecific to mimicking any other disease. Moreover, due to the paucibacillary nature of extrapulmonary specimens and sample collection often requiring invasive procedures, diagnosis of EPTB is often delayed due to varying responses to treatment, and its duration further compounds the management of EPTB. Therefore, diagnosing and managing EPTB remains a challenge and a significant bottleneck in achieving TB elimination. [5]

The cartridge-based nucleic acid amplification test (CBNAAT) assay is a real-time polymerase chain reaction (PCR) cartridge-based assay for simultaneously detecting Mycobacterium tuberculosis complex and rifampicin resistance from biological specimen samples within two hours. This technology was endorsed by the World Health Organization (WHO) in December 2010 and is recognized as a significant advancement in global TB control. The WHO issued policy recommendations to perform the CBNAAT assay on respiratory samples in 2011.[6] In the recently published meta-analysis by the WHO, the sensitivity of CBNAAT in diagnosing various EPTB, like lymph node TB, CNS TB, and pleural TB, are 84.9%, 79.5%, and 43.7%, respectively, as compared to culture.[7] However, clinicians in India have concerns regarding the yield of CBNAAT in EPTB, as it has often been seen that the yield does not match with the data in the WHO meta-analysis. Hence, we conducted this study to assess the diagnostic accuracy of CBNAAT in EPTB in our setting. [7]

Materials and Methods: This prospective observational study was conducted in a tertiary care hospital in Bhavnagar, Gujarat, from January 2021 to January 2022. During this period, all nonrespiratory clinical samples from clinically suspected patients with symptoms of suspected EPTB attending the outpatient department or admitted in the chest ward or other departments—Medicine and Pediatrics Departments of Bhavnagar Civil Hospital were collected and sent to our laboratory for further processing.

All patients of both genders up to 80 years attending the hospital in this period with features of EPTB were included in the study. All sputum, urine, and blood samples are excluded from the study.

The investigation comprised 470 extrapulmonary clinical samples from patients suspected of having EPTB, including lymph nodes, pus, pleural fluid, CSF, ascitic fluid, and tissue aspirate.

The samples were divided into two parts—one part was stained by Ziehl–Neelsen stain and auramine–rhodamine stain and examined under the conventional microscope under oil immersion (100 ×) magnification for 300 fields. Smears were similarly stained by auramine dye which enters the bacteria's cell wall, making it glow golden-yellow when examined by fluorescence microscopy under ultraviolet (UV) light for AFB.

The second component was a sample that was tested using CBNAAT. It was placed in universal falcon tubes (30 mL capacity) with sampling reagent (NaOH and isopropanol at a 2:1 ratio) and maintained at room temperature for 15 minutes with intermittent shaking. The result was read two hours later. Cartridge-based nucleic acid amplification test (CBNAAT) identifies the TB bacilli and the presence/absence of RIF resistance. It has a highly specific primer and five unique molecular probes that target the rpoB gene—identifying the TB bacilli and RIF resistance. Data were entered into Microsoft Office Excel and analyzed using CDC Epi info software.

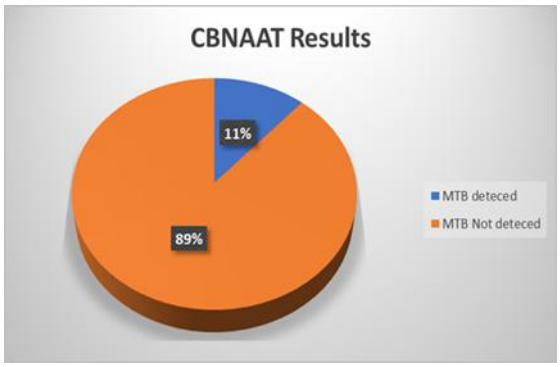
Results and Observations: A total of 470 samples of suspected EPTB were received in the study period from different extrapulmonary sites.Most of the cases were of age group 20 to 50 years of age.55.6% were males and 44.4% were females. Mean age of the study population was 40.816.3 years with age ranging from 5 to 78years. In which 20.3% of the study population had diabetes mellitus, 14.4% had smoked previously, and 16.3% had a history of alcoholism. Diabetes mellitus was the most prevalent comorbidity. ESR was 41.22mm/hour on average.

Out of 470 samples, samples from pleural fluid being the highest (153) and lymph node (56) Of the lymph nodes, the cervical lymph node was the most affected, site (53%), followed by the axillary(25%) and inguinal (16%).

Out of 470 samplesMTB was detected in 54(11%) sample [Figure 1]. Of these 54 samples, 47 were sensitive to rifampicin and 3(5.5%) were resistant to rifampicin and 4(7.40%)were resistant

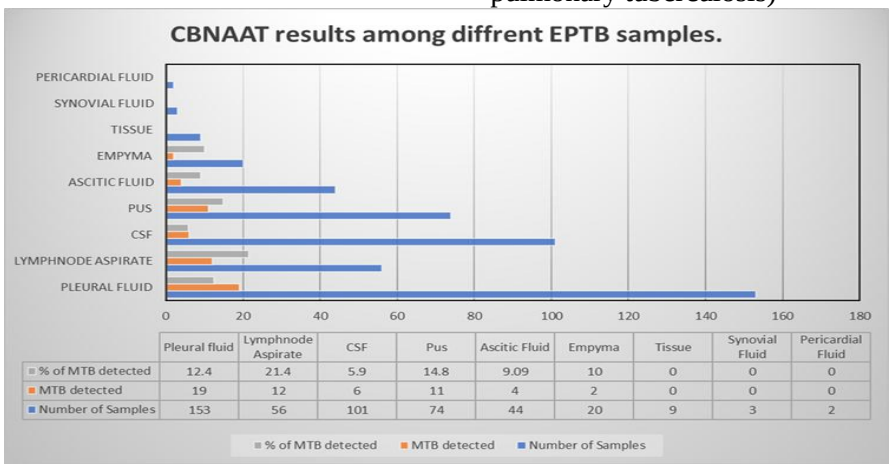
indeterminate.While 16(3.4%) samples showed AFB by the Ziehl–Neelsen stain, 26 samples (5.53%) were detected positive by fluorescent stain.

Figure 1: Positivity of CBNAAT results among samples
(CBNAAT-Cartridge base nucleic amplification test; MTB –mycobacterium tuberculosis)



Out of these 470 samples, 19(12.4%) patients of pleural fluid, 12(21.4%) patients of lymph node aspirate, 2(10%) patients of empyema, 4(9.09%) patients of ascitic fluid, 11(14.8%) patients of pus, 6(5.9%) patients of CSF showed MTB detected and from no samples of synovial fluid, pericardial fluid and tissue samples mycobacterium tuberculosis (M TB) detected [Figure 2].

Figure 2: CBNAAT results among different EPTB samples
(CBNAAT-Cartridge base nucleic amplification test; MTB –mycobacterium tuberculosis; EPTB –extra pulmonary tuberculosis)



	Pleural fluid	Lymphnode Aspirate	CSF	Pus	Ascitic Fluid	Empyma	Tissue	Synovial Fluid	Pericardial Fluid
% of MTB detected	12.4	21.4	5.9	14.8	9.09	10	0	0	0
MTB detected	19	12	6	11	4	2	0	0	0
Number of Samples	153	56	101	74	44	20	9	3	2

The sensitivity and specificity of CBNAAT in comparison with the Ziehl–Neelsen smear are 100% and 91.62 %, respectively [Table 1]. At the same time, the sensitivity and septicity of CBNAAT and fluorescent stain are 100% and 93.69%, respectively [Table 2]. Overall positivity is shown in Table 3, which is 11% in CBNAAT. In males, MTB was detected in 30 (10.7%), and in females, MTB was detected in 24 (12.6%). Out of 470 patients, only 7 were HIV positive. MTB was detected in 3 (42.9%) patients.[Figure 3]

Figure 3: CBNAAT results among HIV Positive patients
(CBNAAT-Cartridge base nucleic amplification test; HIV- Human Immunodeficiency Virus)

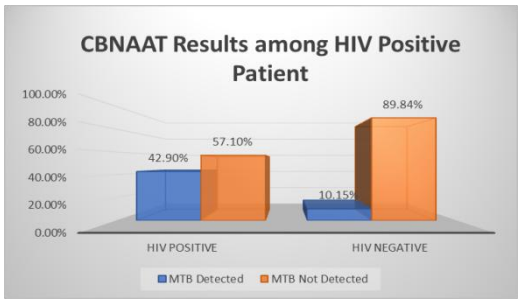


Table 1: Comparison of the result of CBNAAT and ZN stain
(CBNAAT-Cartridge base nucleic amplification test; ZN –Ziehl–Neelsen)

CBNAAT status	ZN stain smear status		Total
	Smear Positive	Smear negative	
CBNAAT positive	16	38	54
CBNAAT negative	0	416	416
Total	16	454	470

Table 2: Comparison of the result of CBNAAT and Florescent stain
(CBNAAT-Cartridge base nucleic amplification test)

CBNAAT status	fluorescent stain smear status		Total
	Smear Positive	Smear negative	
CBNAAT positive	26	28	54
CBNAAT negative	0	416	416
Total	26	444	470

Table 3: Distribution of positivity in samples of CBNAAT and Microscopy
(CBNAAT-Cartridge base nucleic amplification test; ZN –Ziehl–Neelsen)

Total EPTB sample	CBNAAT positive	ZN stain Positive	fluorescent stain positive
470	54	16	26
%	11.4	3.4	5.53

Discussion: Extra pulmonary TB accounts for approximately 25% of TB cases caused by Mycobacterium complex worldwide, [8] thus being responsible to a great extent for the morbidity and mortality due to the bacteria. Because EPTB infection is usually deep-seated, biopsy by surgery is required to collect a sample for testing, making the diagnosis further difficult. Culture by liquid media by mycobacteria growth indicator tube (MGIT) system is costly and needs a lot of expertise to be done by trained laboratory technicians.[9][10]

Cartridge-based nucleic acid amplification test is a simple, rapid, closed system working on the principle of nested semi-quantitative nucleic acid amplification method, which can be done quite quickly, giving early accurate results within 2 hours. [11]

In this 470-participant study, the study population had a mean age of 40.8 years and a slight male predominance of 53.8%. It was comparable to what had been seen in studies by N Nishal et al. [12] According to NTEP reports, men are more likely than women to develop TB in India, with 62% of notified TB patients being men. [13]

In this study, lymph node aspirate showed more positivity (21.4%) in CBNNAT compared to pleural fluid (12.4%), which is similar to the study done by Chattopadhyay et al. [14] Tubercular pleural effusion was the most common form of EPTB in the study conducted by Mukherjee et al., being found in 58.17% of cases, followed by lymphadenopathy (22.71%) which is similar to this study. [15]

A study by Singh et al. 2020 detected RIF resistance by CBNAAT in 05 (6.8%) samples. They also found that out of 46 samples stained by Ziehl–Neelsen, which were negative, CBNAAT found MTB in 27 samples—thus proving the fact that CBNAAT is a very sensitive test that could identify correctly false negative results given by Ziehl–Neelsen stain.[16] while in this study, similar results were found with 11% and rifampicin resistance in 5.5% samples**.**

Conclusion: The detection of Rifampicin resistance facilitates prompt initiation of appropriate therapy, Improving the overall quality of TB care, and the CBNAAT has the potential to significantly improve and escalate the diagnosis of smear-negative body fluid specimens in regions not only with a high TB burden but also with overlapping HIV**.**

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Conflict of interest:None

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