Finding Out of Occult TB Cases using Extra Pulmonary Sample Aspirates by CBNAAT (Cartridge Based Nucleic Acid Amplification Test) in A & N Islands

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Abstract: _Background_ _EPTB is tuberculosis in localized areas other than lungs like lymph nodes, genitourinary tract, pleura, bones, joints, meninges, central nervous system, peritoneum, and other abdominal Organs. EPTB diagnosis is often missed due to lack of classical symptoms and they are potential source of infection. Thus, EPTB also need attention for proper management and prevention of further propagation of TB cases. We investigated the samples aspirated from various sites like body fluids, FNACs etc. and processed them in CBNAAT._ _Materials &Methods_ _Samples from OPD and IPD both were included in this study .all samples received from FNAC lymph node aspirate, CSF, Ascitic fluid, Pleural fluids. These samples were processed in CBNAAT technique ._Results_ we collected 770 samples from all the departments from Jan 2019 till June 2019 at GB Pant hospital. Out of 770 samples 75 were extra pulmonary samples, 19 tested positive for Mycobacterium, 17 showed RIF sensitive while 2 samples were RIF resistance._ _Conclusion_ _EPTB samples are significant in finding out TB cases which were otherwise missed out by routine investigations. It is strongly recommended to process all extra pulmonary samples for CBNAAT to use as the initial diagnostic test in individuals suspected of TB. It also helps to find out MDR cases._

Keywords: Extra pulmonary tuberculosis, Multidrug-resistant tuberculosis, CBNAAT.

1. Introduction

Tuberculosis (TB) affects one-third of the global population in developing countries, with annual estimates of 9.0 million new cases and 1.5 million deaths. [1]While pulmonary TB (PTB) is the most common presentation, extra PTB (EPTB) is also an important clinical condition.[2] which is often undiagnosed with routine investigations. However, the bacterium can also affect extra pulmonary sites such as lymph nodes, pleura, abdomen (peritoneum and gastrointestinal tract), bones and joints, genitourinary tract, central nervous system, and other multiple organs of the body. [3]Extra burden of occult TB cases can be detected by CBNAAT.

Approximately 10 to 15% of tuberculosis (TB) cases in India are estimated to have extra pulmonary disease, they often remain undiagnosed and untreated. EPTB has also significantly contributed in disease load and TB‑related morbidity .Thus, EPTB needs sensitive method to focus for proper diagnosis and management of TB cases Hence, we used extra pulmonary samples and processed them in a sensitive method of diagnosis i.e. CBNAAT to find out maximum possible cases of TB. Collection of extra pulmonary material often requires invasive procedures, expertise, and it is not easy to obtain additional samples. The early detection and treatment of _Mycobacterium tuberculosis_ and MDR TB is the priority to reduce burden of TB in community by CBNAAT in EP samples .In addition to diagnosing of new cases we are also finding out RIF resistance cases which are labelled as multi-drug resistant (MDR-TB) [4, 5, 6].

CBNAAT technology
This technology was recommended and implemented by WHO in December 2010 in developing nations as a substitution over conventional methods for rapid detection of MTB CBNAAT system is based on molecular beacon technology and performs ultrasensitive hemi nested PCR on clinical samples with an accuracy (99–100%) and detects RIF resistance in single assay. [7]Being a closed system, this assay does not require much expertise and there is minimal risk of contamination and biohazard. Initially, the WHO endorsed the assay specifically for diagnosing pulmonary TB patients; recently attempts have been made to explore other affected areas including EPTB clinical samples. [8, 9]

Implementation and Impact of CBNAAT Technology in Developing Nations:
The TB project managed by WHO global TB program along with other STOP TB partners procured above 1000 CBNAAT machines by Government of India is also planning to introduce more machines for initial diagnosis of TB cases. The Revised National TB Control Program me is expanding CBNAAT testing in a big way. [10]. In Andaman and Nicobar islands 5 CBNAAT machines launched in various locations since last 2 years including mobile van for active TB cases finding. We at GB PANT hospital also processing EP samples in CBNAAT.

2. Materials and Methods

Samples from OPD and IPD both were included in this study .all samples received from FNAC lymph node aspirate, CSF, Ascitic fluid, Pleural fluids, purulent aspirates. These samples were processed by CBNAAT technique in addition to routine.

Clinical Sample Collection and Processing

Samples comprising cerebrospinal fluid (CSF), Ascitic fluid, pleural fluid, and lymph node aspirates were collected from various sections had sent to TB lab. All the samples were received in sterile containers. Fine needle aspirate (FNA) samples were collected by a pathologist, while other body fluid samples were collected by physicians during patient investigations and sent to the TB laboratory in GB PANT Hospital.

Test procedure

As per the manufacturer's protocol. Sample reagent was added in a 2:1 ratio to unprocessed sample in 15ml falcon tube and the tube was manually agitated twice during a 15-minute incubation period at room temperature. From the treated sample 2ml was transferred into disposable plastic cartridges having multiple chambers, preloaded with buffers and reagents, these are employed for sample processing, purification, DNA extraction and amplification.

Treated samples are manually loaded into the cartridge which is then inserted into the CBNAAT machine [8] The results were visualized and printable in the view results window. Results were transferred in the report format and issued to the patients.

3. Results

In this study, out of 770 samples, 75 were extra pulmonary, majority of samples 45(60%) were from female patients and 30(40 %) were from male patients.14 (73.6%) samples were

from lymph node, 4 (21.05%) pleural effusion, 1 (5.26%) ascitic fluid. Out of total 19 tested positive for Mycobacterium tuberculosis,17 showed RIF sensitive while 2 patients had confirmed rifampicin-resistant disease and all were correctly identified by MTB/RIF and no false-positive results were reported[8].None of the CSF samples was reported MTB positive, by CBNAAT.
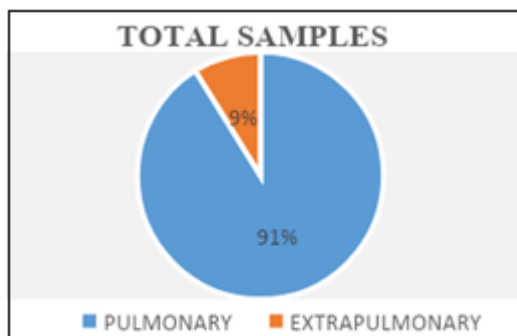

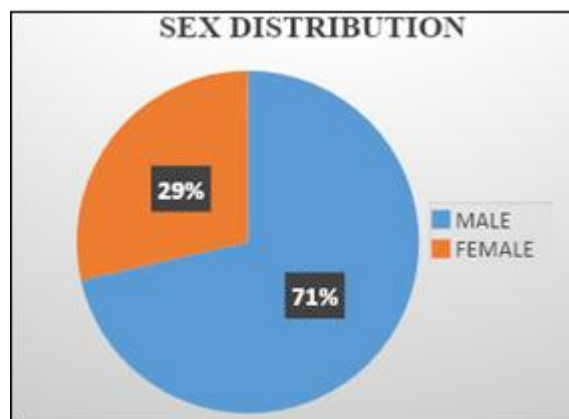**Figure 1:** Proportion of total samples versus extra pulmonary samples

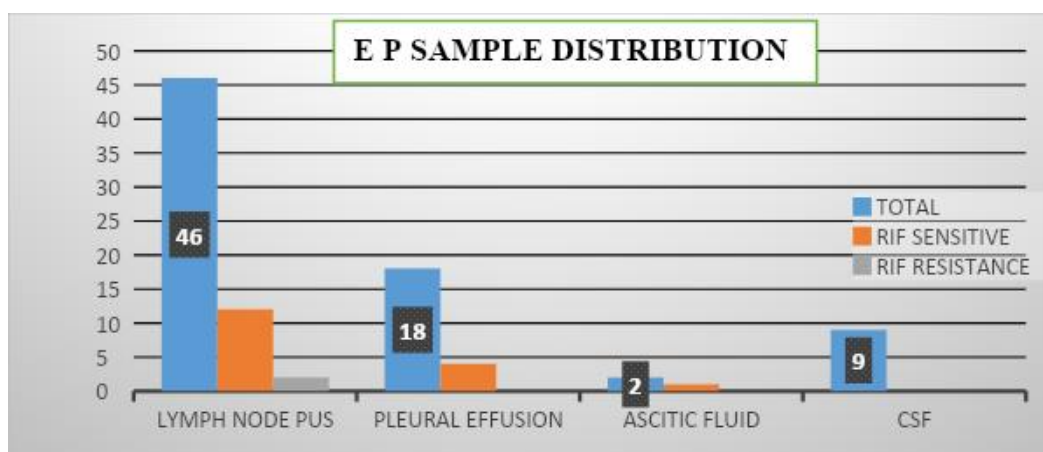**Figure 2:** Sex distribution in extra pulmonary samples

Figure 3: Distribution of Extra Pulmonary samples site wise

4. Discussion

CBNAAT in TB Diagnostic centers enhance the positive cases of TB patients. It has a shorter turnaround time and simultaneously detects MDR TB in less than 3 h with rifampicin sensitive status so that we can start the MDR

regimen thereby preventing spread of TB in the community. CBNAAT is preferred over the conventional techniques for EPTB samples because of its high specificity and sensitivity. Although, conventional laboratory techniques as LED-FM smear microscopy for diagnosis of tuberculosis are cost effective but less sensitive as compared to the CBNAAT

because the large bacillary load (10^5/mL) will be required for a smear to become positive. As per the literature the specificity of the CBNAAT was found to be (99.6%) reported by Boehme et al. (8). Currently culture based methods are essential for monitoring therapy and for performing DST for anti TB agents other than rifampicin including isoniazid and other second line anti TB drugs. Conventional procedures are laborious, tedious and require high infrastructure laboratories with trained personnel, a luxury that is available only in a few reference centers and not in resource-limited settings or decentralized laboratory settings, where they are most required. Emerging data suggests that this assay has the potential for diagnosing EPTB as the sensitivity and specificity were found to be almost similar for both PTB and EPTB cases. Ligthelm*et al.* showed excellent diagnostic accuracy of the CBNAAT in terms of sensitivity (96.7%) and specificity (86.6%) in patients with tuberculosis lymphadenitis. [8] Similarly, Lawn and Zumla demonstrated high sensitivity (81.3% for EPTB) of CBNAAT on large number of extra pulmonary samples. [11]

5. Limitations

A positive test result does not necessarily indicate the presence of viable organisms.

Test results might be affected by antecedent or concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.

Samples like blood, urine, and stool cannot be processed by CBNAAT.

6. Conclusion

1) CBNAAT is the sensitive technique to detect bacilli in low concentration samples.
2) It is recommended to process all extra pulmonary samples for CBNAAT to detect more and resistant TB cases.
3) CBNAAT test not only has good sensitivity and specificity for escalate the diagnosis of TB and detection of RIF resistance in TB cases but also perfectly for EPTB cases in the Indian health care setting.
4) To end the spread of MDR-TB and decreasing mortality and morbidity. Faster methods that allow MDR regimens to be started early are urgently needed. Since this assay is independent of the user's skills and routine staff with minimal training can perform the test.

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