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Prevalence of positivity of CBNAAT in Extra Pulmonary Tuberculosis and non sputum producing pulmonary TB

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Abstract

Background: TB is the 9th leading cause of death worldwide and the leading cause from a single infectious agent. TB menifests clinically as pulmonary and EPTB. It is estimated that 15 to 20% of all TB cases are extra pulmonary.

Methods: This was a prospective observational study conducted in SPMC RDH Bikaner.Patients of EPTB diagnosed on the basis of clinico radiological basis attending the OPD. Patients who attended department of pulmonary medicine S.P. Medical College with signs and symptoms and radiological examination suggestive of extra-pulmonary tuberculosis and non sputum producing pulmonary tuberculosis were included in this study.

Results: In this study 6(100%) patients of pleural fluid AFB positive were CBNAAT positive and 22(13.58%) patients of pleural fluid AFB negative were CBNAAT positive and this difference was found to be statistically significant (p=0.0001)

Conclusion- Present study revealed that more positivity rate by CBNAAT in comparision to smear staining in pleural fluid which indicate that it is a more sensitive technique as compared to conventional methods. It also detect resistant to Rifampicin which we could have missed with smear staining or by other conventional methods.

Keywords: CBNAAT, Pleural fluid, Extrapulmonary tuberculosis.

Introduction

TB Is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, there were an estimated 1.3 million TB deaths among HIV negative people (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive people.[1] Globally, the TB mortality rate is falling at about 3% per year. TB incidence is falling at about 2% per year; this needs to improve to 4-5% per year by 2020 to reach the first milestones of the End TB Strategy. [2]

The CBNAAT uses a cartridge containing all elements necessary for the reaction, including lyophilized reagents, liquid buffers and wash solutions. With observing aseptic technique, sample (2 ml

mucuprulent) was collected in a falcon tube. The sample was loaded into cartridge and analyzed for presence of mycobacteria and rifampicin resistance in GX4 System (with 4 modules)[3]

In a significant development, WHO Global TB Programme has included an Indian Molecular assay True Nat as initial test for TB and MDR-TB in view of its high diagnostic accuracy, in the rapid communication document on Molecular assays released by WHO's Global TB Programme. The True Nat TB test is a new molecular test that diagnosis TB as well as testing for resistance to the drug rifampicin in about 90 minutes.

In an effort that was supported by DHR (department of health research) Ministry of Health and Family welfare, Govt. of India and DBT, various indigenous technologies developed by Indian scientists/companies for detection of MDR/XDR TB were reviewed. Most promising kits were selected by the 'Expert Group' and were subjected to a double blind validation in comparison to standard tests at 4 national reference laboratories of the country.

After a stringent review and a series of validation and subsequent feasibility studies and continuous follow-up, the 'TrueNat M.TB & Rif' assay was found to be at par with the internationally recognized molecular assay Gene Xpert in terms of sensitivity and specificity and detection of rifampicin resistance. This was taken up by National TB Elimination Programme after recommendations from ICMR (Indian council of medical research).

The TrueNat assay kit is highly cost effective as compared to GeneXpert and can be used in peripheral centers without an AC lab and runs on battery which can be solar powered.

"Endorsement of the TrueNat by WHO would enable other low and middle income countries to procure TrueNat for TB and Rifampicin resistance thus supporting TB elimination in developing countries".

Material and Methods

The study was conducted in Sardar Patel Medical College, Associate Group of Hospital, Bikaner in department of Respiratory Medicine. A tertiary care center for respiratory diseases in western part of Rajasthan, India.

Study Design: This was a prospective observational study.

Consent: Patients of extrapulmonary Tuberculosis diagnosed on the basis of clinico radiological basis attending the outpatient clinic of the department of pulmonary medicine of our hospital and The protocol was explained to the patient/care provider before enrolment and informed consent were taken from each patient.

Patients who attended department of pulmonary medicine S.P. Medical College with signs and symptoms and radiological examination suggestive of extra-pulmonary tuberculosis and non sputum producing pulmonary tuberculosis were included in this study. Their detailed clinical history, demographic profile, socioeconomic status and contact number was taken and recorded. Previous history of tuberculosis, past history of medical illness and h/o co-morbid illnesses were also recorded. General physical examination as well as complete systemic examination was done carefully with more emphasis on involved system. A fresh digital chest radiograph was advised to study population with suspected pleural effusion, hydro-pneumothorax or pyo-pneumothorax. A new chest X-ray was also advised for patients who did not had their old chest radiograph.

To look any pulmonary lesions in extra-pulmonary TB other than pleural involvement, a new digital chest radiograph also ordered. Sputum samples from study population, who had cough for any duration, were sent for AFB examination by light microscopy under RNTCP, If any patients found pulmonary involvement or sputum positive excluded from the study and any patients only have pulmonary involvement suggestive by symptomatic and chest X-ray but non sputum producing patients also included in the study.

After clinico-radiographic suspicion, pleural effusion was confirmed by ultrasonography (USG). After that pleural fluid was aspirated by USG guidance. In case of fibrous band in pleural effusion or pleural fluid organization were detected in USG, pleural fluid was aspirated from the largest pocket visualized sonographically,

Pleural fluid was sent for protein, cell count, cell type and ADA. 2 ml of pleural fluid or pus was sent for CBNAAT. After diagnosis of tubercular pleural effusion DOTS cat-1 under RNTCP was initiated. Tubercular pleural effusion or tubercular empyma was diagnosed on clinical, radiological, biochemical analysis of pleural fluid and response with ATT.

Cold abscess was drained from depended site and the sample was sent for CBNAAT. In case of palpable lymph node, material was aspirated with the help of wide bore needle. After processing of sample using WHO guideline, sample was sent for CBNAAT.

Ultra-sonography of abdomen was done for patients with suspected ascitic form of abdominal tuberculosis. After radiological confirmation ascitic fluid was tapped and was sent for routine as well as for CBNAAT.

In patients who had symptoms pertaining to CNS involvement like headache, vomiting, neurological deficit and neck rigidity, was looked and after neuroimaging (CECT brain or MR I), lumber puncture was done with aseptic precautions and 2 ml of CSF was drawn and send for CBNAAT. CNS tuberculosis was diagnosed based on neuroimaging findings, CSF findings and response to ATT.

Sample taken for pleural fluid, ascitic fluid, lymph node aspirate and BAL were minimum 3-5 ml. and for cerebrospinal fluid is 2-2.5 ml.

These patient were taken in the study with strong clinical suspicion of Extrapulmonary Tuberculosis:-

- Ascites with lymphocyte predominance
- Chronic lymphadenopathy (cervical,axillary etc)
- CSF lymphocytic pleocytosis with elevated protein and low glucose
- Exudative pleural effusion with lymphocyte predominance.
- All extra-pulmonary tuberculosis cases with HIV infection

Based on MTB result, the study population were divided into 'MTB detected' and 'MTB not detected' groups. MTB detected group was further divided into two sub groups i.e. rifampicin Resistant and rifampicin sensitive.

All the collected information was filled in predesigned proforma in excel sheet for final analysis. Chi squire test or suitable formula was applied to know the significance of our study.

Inclusion - Criteria

1. All cases of extra-pulmonary tuberculosis like pleural effusion, cold abscess,, meningitis, lymphadenopathy, ascites & with the samples being pleural fluid., pus, CSF, lymph tissue, peritoneal fluid and BAL of non sputum producing pulmonary tuberculosis diagnosed on the basis of clinico radiological and/or histopathological findings.
2. Patient who gave informed consent.

Exclusion Criteria

1. Cases of

- pyogenic meningitis
- non-tubercular effusion (for eg. cardiogenic, traumatic, hypoproteinaemia, cirrhosis, renal, collagen vascular disorders and malignancy)
- malignant & inflammatory lymphadenopathy
- non-tubercular ascites (for ex.trauma, hypoproteinaemia, renal,hepatic causes, fungal infection & non-specific peritonitis)

2. Patient who did not give informed consent

TB detection was done by CBNAAT, made by Cepheid-Sunnyvale-USA. Extrapulmonary specimens were processed according to the CBNAAT system operator manual given by Central TB division, Government of India, Guidance document for the use of cartridge-based nucleic acid amplification test (CBNAAT) under RNTCP. Our machine contains 4 cartridges, so 4 samples were processed for each run. According to standard operating procedure, the sampling reagent (containing NAOH and isopropanol) was added at 2:1 ratio to the sample and kept for 15 min at room temperature with intermittent shaking. 3 ml of this treated sample was transferred to the cartridge and the cartridge was inserted in the module of CB-NAAT machine. An automatic process completed the remaining assay steps and the results were displayed on the monitor of CBNAAT after 1 h and 50 min. CBNAAT cartridge is a disposable, single self-enclosed test unit in which all steps of NAAT, i.e.. Sample processing, PCR amplification and detection were automated and integrated. The manual steps involved in the assay were adding reagent and sample loading.

Observations

Table 1: Type of Extra Pulmonary Tuberculosis in study population

Type	Number	Percentage
PLEURAL FLUID	168	52.17
ASCITIC FLUID	19	5.90
BAL	37	11.49
COLD ABSCESS	8	2.48
CSF	48	14.91
EMPYEMA	6	1.86
L.N PUS	36	11.18
Total	322	100.00

In present study 168 (52.17%) were pleural fluid, 48 (14.91%) were CSF, 37 (11.49%) were BAL, 36 (11.18%) were L.N. PUS, 19 (5.90%) were ascitic fluid, 8 (2.48%) were cold Abscess and 6 (1.86%) were Empyema.

Table 2 : Age and Sex distribution of study population

Age group {years}	Female		Male		Total	
	N	%	N	%	N	%
< 15	8	5.67	16	8.84	24	7.45
16-30	50	35.46	59	32.60	109	33.85
31-45	35	24.82	33	18.23	68	21.12
46-60	22	15.60	39	21.55	61	18.94
>60	26	18.44	34	18.78	60	18.63
Total	141	100.00	181	100.00	322	100.00

In this study in both male & female group, maximum patients were in age group 16-30 years. As age advanced, number of patients decreased in both female and male groups. Females were affected relatively more in younger age group as compared to males and this difference was found to be statistically not significant (P<0.358)

Table 3: CBNAAT result relation to FNAC result in Lymph node sample

FNAC result	CBNAAT positive		CBNAAT negative		Total	
	N	%	N	%	N	%
Suggestive of TB	16	64.00	9	36.00	25	100.00
Not suggestive of TB	6	54.55	5	45.45	11	100.00
Total	22	61.11	14	38.89	36	100.00

Chi-square = 0.027 with 1 degree of freedom; p = 0.869 (NS)

In present study 16(64.00%) patients suggestive of TB by FNAC were CBNAAT positive and 9(36.00%) were CBNAAT negative. 5 patients (45.45%) were NOT suggestive of TB by FNAC were CBNAAT negative and 6(54.55%) were CBNAAT positive. This difference however is not found to be statistically significant (p=0.869).

Table 4: ADA in CBNAAT positive and negative patients of pleural effusion cases.

CBNAAT result of pleural fluid	N	Mean	Std, Deviation
CBNAAT positive	28	56.928	13.559
CBNAAT negative	140	35.442	11.206
Total	168	39.023	14.098

t = 8.936 with 166 degrees of freedom; P = 0.0001 {HS}

In this study CBNAAT positive patients had significantly higher mean ADA 56.928 as compared to CBNAAT negative patients who had mean ADA 35.442. This difference however was found to be statistically significant (p=0.0001).

Table 5: CBNAAT result in relation to Fluid AFB in Pleural fluid sample

Pleural Fluid AFB	CBNAAT positive		CBNAAT negative		Total	
	N	%	N	%	N	%
Positive	6	100.00	0	0.00	6	100.00
Negative	22	13.58	140	86.42	162	100.00
Total	28	16.67	140	83.33	168	100.00

Chi-square = 25.200 with 1 degree of freedom; p = 0.0001 {HS)

In this study 6(100%) patients of pleural fluid AFB positive were CBNAAT positive and 22(13.58%) patients of pleural fluid AFB negative were CBNAAT positive and this difference was found to be statistically significant (p=0.0001)

Discussion

Tuberculosis (TB) remains a key challenge in the face of global public health and inadequate diagnostic assays have hampered our chances to tackle this disease effectively. According to WHO there were 10 million new TB cases and 1.5 million TB death in 2018. Extra pulmonary tuberculosis accounts for 20% of total burden of tuberculosis globally it Is estimated that .approximately 70 million people will die from tuberculosis within the next 20 years and it is because of inadequate measures for TB control As the number of bacilli are very less in extrapulmonary samples and because of difficulty in obtaining tissues from deep seated organs; diagnosis is delays In most cases. The CBNAAT marks an important development in the field of rapid molecular TB diagnostics. This assay was rapidly endorsed by WHO in December 2010 as a replacement for sputum smear microscopy, particularly in setting with high rates of HIV-associated TB and multidrug resistant TB developed for testing

sputum samples.[4] This multifunctional diagnostic platform is an automated, closed system that perform real time PCR and can be used by operator with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hours. This test detects DNA specific for Mycobacterium tuberculosis by polymerase chain reaction. Extra-pulmonary TB is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analysis and in the extraction of M. tuberculosis DNA from the samples. As there are limited studies about the use and efficacy of the CBNAAT in extra-pulmonary tuberculosis there is a need for more and more research on this novel approach. This study explores the use of CBNAAT in different non respiratory samples.

In this study, 322 patients were included in which extrapulmonary tuberculosis was suspected by clinico-radiological, cyto-pathological and biochemical analysis. 322 different extra-pulmonary and non sputum producing tuberculosis samples from presumptive or clinically diagnosed cases of TB were examined by CBNAAT test.

In present study 16(64.00%) patients suggestive of TB by FNAC were CBNAAT positive and 9(36.00%) were CBNAAT negative. 5 patients (45.45%) were NOT suggestive of TB by FNAC were CBNAAT negative and 6(54.55%) were CBNAAT positive. This difference however is not found to be statistically significant (p=0.869).

Total number of patients in present study was 36 in which FNA for cytology and CBNAAT was done. CBNAAT was positive from lymph node in 22 out of the 25 FNAC positive patients. In 11 patients in whom FNAC was negative, CBNAAT diagnose TBLN in 6 of them. Means CBNAAT is better than FNAC to diagnose tubercular lymphadenitis because its gives idea about rifampicin resistance also.

In the study by Singh KG et al[5], total number of patients in this study was 57 in which FNA for cytology and CB-NAAT was done. CBNAAT was positive from lymph node in 43 out of the 47 FNAC positive patients, in 10 patients in whom FNAC was negative, CB-NAAT did not diagnose TBLN in 9 of them.

In present study, Out of 168 cases of pleural fluid, 6 cases were smear positive and came out to be positive with CBNAAT, 22 were smear negative but came out to be positive with CBNAAT, 140 cases were negative for both smear & CBNAAT. It is noted that none of the smear positive gave negative results by CBNAAT. On the other hand many of negative samples came to be positive with CBNAAT indicating CBNAAT is highly sensitive and specific technique. We also found out of 28 pleural fluid samples which came positive with CBNAAT, 1 was resistant to Rifampicin which we would have missed with staining or by other conventional methods. The result of the study revealed a maximum positivity rate by CBNAAT which indicated that it is a more sensitive technique as compared to conventional methods.

In the study by Avashia S et al[4] Out of 300 cases, 40 cases were ZN smear positive and came out to be positive with CBNAAT, 71 were ZN smear negative but came out to be positive with CBNAAT, 189 cases were negative for both ZN smear & CBNAAT. It is noted that none of the ZN smear positive gave negative results by CBNAAT On the other hand many of ZN negative samples came to be positive with CBNAAT indicating CBNAAT is highly sensitive and specific technique. We also found out of 111 samples which came positive with CBNAAT, 6 were resistant to

Rifampicin which we would have missed with ZN staining or by other conventional methods.

Conclusion

Present study revealed that more positivity rate by CBNAAT in comparision to smear staining in pleural fluid which indicate that it is a more sensitive technique as compared to conventional methods. It also detect resistant to Rifampicin which we could have missed with smear staining or by other conventional methods.

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