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UTILITY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) IN THE DIAGNOSIS OF TUBERCULAR PLEURAL EFFUSION

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ABSTRACT

Introduction: TB is one of the top 10 causes of death globally. PTB is most common presentation. Lymph node TB is the most common type of EPTB constitutes about 35% cases followed by pleural effusion(20%), bone and joint(10%), genitourinary TB(9%), TB Meningitis(5%), abdominal tuberculosis(3%), other(10%). WHO also recommends Gene Xpert MTB/RIF over conventional tests which permits rapid TB diagnosis through detection of the genetic sequence of DNA of mycobacterium TB and simultaneous identification of a majority of the mutations that confirm Rifampicin resistance which is highly predictive of MDR-TB.

Methodology: Study was carried out over a period of one year in a tertiary care centre. Patients with suggestive of pleural effusion were included in study. Pleural fluid was drawn using standard protocol and sent for CBNAAT test and bacteriological examination. Based on MTB result, the study population were divided into 'MTB detected' and 'MTB not detected' groups. MTB detected group was further divided into 'Rif' Resistant and 'Rif' sensitive group. 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.

Results: 203 patients were included with a male to female ratio of 2.98:1. 65.91% patients were below 45 years of age. Mostly patients were from rural areas and illiterate. 38.64% had different type of substance abuse habit. DM (29.03%) found as the most common co-morbidities. CBNAAT test was able to detect MTB in 76 (37.44%) extra-pulmonary samples, 70 were Rifampicin sensitive and 6 were 'Rifampicin' resistant.

KEYWORDS : MTB, RIF, CBNAAT, MDR

INTRODUCTION

Tuberculosis has existed for millennia and remains a major global health problem. In 2017, TB causes an estimated 1.3 million death among HIV negative people and about 3 lakh deaths from TB among HIV positive people. Globally, the best estimate is that 10 million people developed TB disease in 2017. 30 high TB burden countries accounted for 87% of the world cases according to WHO. The six countries that stood out as having the largest number of incident cases (60%) in 2015 were India, Indonesia, China, Nigeria, Pakistan and South Africa.[1]

The incidence rate in India was 211 per lakh population according to WHO global report 2016. The best estimate is that there were 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among HIV positive people.

Pulmonary tuberculosis is the most common presentation and it is the only form of the disease that is infectious but EPTB is also an important clinical problem.[2] EPTB represented 15% of the 6.1 million incident cases that were notified. The percentage of patients with EPTB in tertiary care centres in India was between 30%-53%, while the percentage estimated by the national control program in India for HIV negative adults is between 15%-20%**.** Lymph node tuberculosis is the most common type of EPTB constitutes about 35% cases followed by pleural effusion(20%), bone and joint(10%), genitourinary TB(9%), TB Meningitis(5%) abdominal tuberculosis(3%) and other(10%).[3]

TB affecting other site is rarely smear-positive; because of its negligible contagious potential, therefore, it is never been a priority in the campaigns undertaken by national TB control programs.[4] Also, the literature on the various forms of EPTB is scant and this lack of evidence is of particular concern in the case of treatment guidelines.

The diagnosis of EPTB is still challenging because of paucibacillary in nature, lack of specific sign and symptoms and often negative acid fast bacilli smear of biological specimens. Indirect methods like tuberculin skin test and interferon gamma release assay are adjunctive diagnostic tools but it may be negative in presence of disease. In developing countries like India where tuberculosis is highly endemic, tuberculin skin test and gamma interferon result alone is not sufficient evidence to diagnose EPTB.[5] Clinical presentation of EPTB is atypical. Histo-cytological examination also has its limitation. Therefore, the clinicians more often rely upon the clinical impression, radiological and endoscopic appearances and nonconventional diagnostic methods as evidence to diagnose EPTB. Tuberculin test and PCR assays are another test for diagnosis of EPTB but sensitivity and specificity of these test are variable.

Although the culture remains the gold standard for the diagnosis of the tuberculosis but it can take up to 8-10 weeks using a solid media. The Gene Xpert MTB/RIF (CBNAAT) is an automated real time PCR assay designed for the rapid and simultaneous detection of Mycobacterium tuberculosis and Rifampicin resistance within 2 hours.[6] Based on systematic review, WHO recommends Xpert over conventional tests for diagnosis of EPTB which permits rapid TB diagnosis through detection of the genetic sequence of DNA of mycobacterium TB and simultaneous identification of a majority of the mutations that confirm Rifampicin resistance which is highly predictive of MDR TB.[7]

AIMS AND OBJECTIVES

To determine utility of gene Xpert MTB/RIF test in detection of tubercular pleural effusion.

MATERIAL AND METHODS

It is a prospective type of study which was conducted for a period of one year (January 2016 to December 2016) at Department of Respiratory Diseases and Tuberculosis, R.N.T. Medical College, Udaipur from an informed and written consent was taken from all patients prior to study after proper counselling with symptoms, signs and radiological examination suggestive of tubercular pleural effusion were included. Their detailed clinical history, demographic profile, socioeconomic status and anthropometric data and contact number was taken and recorded. Previous history of tuberculosis, history of contacts with PTB, past history of medical illness and history of co-morbid illnesses were also taken. General physical examination as well as complete systemic examination was done carefully with more emphasis on involved system. Mantoux test with 10 TU was done and reading was recorded after 72 hours. A fresh digital chest radiograph was advised to study population with suspected pleural effusion, hydro-pneumothorax or pyo-pneumothorax. Sputum samples from study population, who had cough with expectoration for any duration, were sent for AFB examination by light microscopy under RNTCP. After clinico-radiographic suspicion, pleural effusion was confirmed

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by ultra-sonography. 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. After pleural aspiration, a check X-ray was advised to see the parenchymal involvement which was curtained by pleural effusion.

Pleural fluid sample was sent for biochemical, cytological, gram stain and pyogenic culture and sensitivity in addition to routine investigations. 2 ml of pleural fluid or pus sample also sent for Gene X-pert test. After confirmation of diagnosis, ATT started under RNTCP.

We were contacted study population 2 months after starting ATT under RNTCP to know the well being. If patients had no clinical improvement or had diagnosed other than tuberculosis then we excluded those patients from our study. 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. 'Rif' Resistant and 'Rif' sensitive. All the collected information was filled in excel sheet. Chi squire test or suitable formula was applied to know the significance of our study. Patients with tubercular pericardial effusion, transudative pleural effusion and ascites, malignant pleural effusions and hemothorax, contraindication to thoracocentasis and who had denied for study were excluded from this study.

RESULT

Table 1: Distribution of Study Population according to Age and Sex

Age Sex	Male	Female	Total
< 45 yrs	108 (76.59%)	33 (23.41%)	141 (69.46%)
>45 yrs	44 (70.97%)	18 (29.03)	62 (30.54%)
Total	152 (74.88%)	51 (25.12%)	203 (100%)

There were 74.88% male and 25.12% were female. Male to female ratio was 2.98:1. Majority of patients (65.91%) were < 45 years of age. 130 (64.04%) patients were illiterate and 73 (35.96%) patients were literate.

Table 2: Distribution of study population according to their substance abuse

Substance abuse	Number	Percentage
Smoker	32	41.02 %
Gutkha chewer	23	29.49%
Smoker and Alcoholic	18	23.08 %
Alcoholic	3	3.85 %
Smoker, alcoholic and Gutkha chewer	02	2.56 %
Total	**78 (38.42%)**	**100 %**

Out of 203 subjects, 78 (38.42%) had different substance abuse habit and among these 41.02% patients had smoking habit followed by 29.49% gutkha chewer, 23.08% both smoker and alcoholic, 3.85% alcoholic and 2.35% smoker, alcoholic and gutkha chewer.

Table 3: Distribution of study population according to Co-morbidities

Co-morbidities	Number	Percentage
DM	9	29.03 %
HIV	7	22.58 %
COPD	7	22.58 %
HTN	4	12.90 %
Hypothyroidism	2	6.45 %
DM, HT and Bronchial asthma	2	6.45 %
Total	**31 (15.27%)**	**100 %**

Only 31 (15.27%) patients had co-morbidities. 29.03% patients had DM, 22.58% had obstructive airway disease and 22.58% had HIV Positive, 12.9% had HTN, 6.45% had hypothyroidism and 6.45 % had DM, HT and bronchial asthma.

Table 4: Distribution of tubercular pleural effusion with pulmonary involvement according to their chest radiological presentation

Radiographic presentation	Number	Percentage
Infiltration	23	43.39 %
Infiltration + Consolidation	17	32.07 %
Infiltration + Cavity	8	15.09 %
Infiltration + Pyo-pneumothorax	4	7.54 %
Infiltration + Pyo-pneumothorax + Consolidation	1	
Total	**53**	**100%**

Out of 53 patients with pulmonary involvement in their chest radiograph, most common finding was infiltrations (43.39%) in lung followed by consolidation (32.07%), caviation (15.09%). Only 5 patients had pyo-pneomothorax.

Table 5: Distribution of tubercular pleural effusion with Pleural fluid protein level in pleural fluid analysis

Pleural fluid Protein level (mg/dl)	Number	Percentage
< 2.5	2	0.98 %
2.51 – 3.5	35	17.24 %
3.51 – 4.5	64	31.53 %
4.51 -5.5	69	33.99 %
>5.5	33	16.25 %
Total	**203**	**100 %**

More than 50 % cases had pleural fluid protein level (65.52%) in range 3.51–5.5 mg/dl. Only 2 cases had pleural fluid protein level < 2.5 mg/dl.

Table 6: Distribution of tubercular pleural effusion with Pleural fluid cell count

Pleural fluid cell count (Cells/µL)	Number	Percentage
< 1500	103	50.74 %
1501-3000	65	32.02 %
3001-4500	23	11.33 %
>4500	12	5.91 %
Total	**203**	**100 %**

In majority of patients, cell counts were lymphocytic pleocytosis. 50.74 % patients had < 1500 cell/µL. Only 5.91 % patients had > 4500 cell/µL.

Table 7: Distribution of tubercular pleural effusion with ADA level in Pleural fluid

ADA level in Pleural fluid (U/Litre)	Number	Percentage
40-100	160	78.82 %
101-150	30	14.78 %
151-200	10	4.93 %
>200	3	1.48 %
Total	**203**	**100 %**

It is observed that majority of the patients (78.82%) had pleural fluid ADA level in range of 40-100 U/L followed by 101-150 U/L (14.78%).

Table 8: Distribution of tubercular pleural effusion with various symptoms

Symptoms	Number	Percentage
Chest pain	146	71.92 %
Cough	145	71.43 %
Fever	120	59.11 %
Anorexia	118	58.13 %
Weight loss	111	54.68 %
Dyspnoea	95	46.80 %
Expectoration	59	29.06 %
Night sweats	51	25.12 %
Haemoptysis	9	4.43 %

In study, most common symptom is chest pain followed by cough, fever, anorexia, weight loss and dyspnoea. Only 9 cases had haemoptysis.

Table 9: Distribution of patients with tubercular pleural effusion according to their sputum microscopy and pleural fluid CBNAAT result

Sputum microscopy	Pleural fluid CBNAAT result		Total
	MTB detected	MTB not detected	
Positive	24 (72.73%)	9 (27.27%)	33 (16.26%)
Negative	52 (50.59%)	118 (69.41%)	170 (83.74%)
Total	**76 (37.44%)**	**127 (62.56%)**	**203 (100%)**

Out of 203 tubercular pleural effusions, sputum was positive for AFB in 33 patients and negative in 170 patients. Out of 33, MTB was detected in 24 patients. In sputum negative patients, MTB was detected in 52 patients. There is highly significant correlation between sputum positivity in tubercular pleural effusion and CBNAAT result **(p<0.001)**.

Table 10: Distribution of tubercular pleural effusion with or without pulmonary involvement according to CBNAAT result

CBNAAT	No Pulmonary involvement	Pulmonary involvement	Total
Positive	17 (20.99%)	66 (54.1%)	76 (37.44%)
Negative	64 (79.01%)	56 (45.9%)	127 (62.56%)
Total	**81 (39.9%)**	**122 (60.1%)**	**203 (100%)**

In this study out of 203 pleural fluid samples, 122 had pulmonary abnormalities whereas 81 patients had normal chest X-Ray. CBNAAT was more successful in detection of MTB in patients with tubercular pleural effusion who had pulmonary involvement in their chest radiograph. There is statistically significant correlation between result of CBNAAT and pleural effusion with pulmonary lesions. **(p<0.001)**

DISCUSSION

In our study 74.88% were males and 25.12% were females. Xinyu Zhang *et al* (2011) studied 5,684 TB patients, of these 3,332 (58.7%) were males and 2,341 (41.3%) were females.[8] Although there is clear evidence that socioeconomic and cultural factors leading to barriers in accessing health care, may cause under notification in women, particularly in developing countries. Other confounding factors, such as smoking, alcohol and drug use, exposure to outdoor pollution, migration of males to high prevalence areas could be the other reasons of male predominance. In low income countries, women often have a reduced access to economic resources and fewer educational opportunities as compared to male. As a result, many women are unable to locate and reach appropriate health services. The decision regarding a woman's treatment is also made by the husband or senior members of the family. Furthermore, the stigma attached to a positive diagnosis leads many women to forego seeking necessary medical attention. In the low income countries like India, women tend to self medicate or seek out traditional healers instead of accessing public health facility because they are afraid of being recognized as a TB patient by members of the community. The higher rates of TB among male is due to a higher prevalence of infection among men. Above reasons may be the cause of sex difference in our study.

Mostly patients (85.71%) were from rural areas whereas 14.29% patients were from urban areas in our study. In study of TEM Abdallah *et al* found that 74.6% (167) patients were rural residence. Patients attending our institution were mostly from rural areas because rural population is dominating in Udaipur Zone.

64.04% patients were illiterate and 35.96% patients were literate in our study. TEM Abdallah *et al* found that illiterate male were 68.3 % out of 224 patients.[9] People with low health literacy may have access to health information but they often fail to use the health information properly and making medication and treatment errors because of misunderstanding health instructions.

In our study, majority of patients (65.91%) were < 45 years of age. In the study by VK Tiwari *et al*, numbers of patients <40 years were 88 (81.6%) and > 40 years were 20 (18.6%).[10]

We studied that out of 203 subjects, 78 (38.42%) had different substance abuse habit and among these 41.02% patients had smoking habit followed by 29.49% gutkha chewer, 23.08% both smoker and alcoholic, 3.88% alcoholic and 2.35% smoker, alcoholic and gutkha chewer. In the study by Manjusha Sajith *et al*, out of 49 EPTB cases, 6 (12.24%) patients were smoker and 13 (26.53%) were alcoholic. The highest number of smokers (17 patients) and alcoholics (33 patients) had PTB as compared to EPTB.[11] In this study, smoking habit were more, in comparison to other published study. The reason behind that may be due to illiteracy, unaware about health hazards of tobacco use and poor execution of the law for tobacco use may be the other possibility.

In our study, only 31 (15.27%) patients had co-morbidities. 29.03% had DM, 22.58% had COPD and 22.58 % had HIV Positive, 12.9% had HTN, 6.45% had hypothyroidism and 6.45% had DM, HT and bronchial asthma.

Soham Gupta *et al*, from South India conducted a study and in this study it was observed that 31.8% pf the PTB patients had DM as a co-morbid factor, which was significantly higher than HIV (8.85%).[12] In literatures, HIV and DM is a predominant co-morbidity in extra-pulmonary as well as pulmonary TB. Depressed cellular immunity, dysfunction of alveolar macrophages, low levels of interferon gamma, pulmonary microangiopathy, and micronutrient deficiency have been implicated in the occurrence of tuberculosis in HIV and Diabetic patients.

There were 165 (81.28%) patients had BMI < 18.5 kg/m^2 and 26 (12.81%) had BMI between 18.5-24.9 kg/m2 and 12 (5.91%) had > 25 kg/m^2.

Many studies also show that having a BMI < 18 (kg/m^2) is considered as a risk factor for developing TB, contrarily, having a BMI > 25 (kg/m^2) has a protective effect against TB.[13] It is well known that nutritional status influences the functioning of the cell-mediated immune system. Though the exact pathways are not fully understood, there is no doubt that several nutritional factors also influence the capacity of the cell-mediated immune system to fight TB bacilli.[14]

The co-existence of parenchymal disease in association with pleural effusion has been observed on chest radiograph in up to 60.1% of patients and occurs on the same side in almost all cases. [15] Observed parenchymal changes are in the upper lobes in about three quarters of cases, suggesting reactivation as the cause of TB. In the remaining patients, parenchymal disease is in the lower lobe suggesting primary TB infection.[16]

In this study, we examined 203 pleural fluid samples and out of it CBNAAT was able to detect MTB in 76 (37.44%) samples. Patil Shital *et al* (2014) studied 100 pleural fluid samples subjected for DNA PCR and observed positive in 74 (74%) of the cases.[17] Reechaipichitkul *et al*. (2000) reported a sensitivity of 50% and specificity of 61% and had PCR positive in 100% of culture positive tubercular effusion and only in 30-60% of culture negative pleural fluid samples.[18] Bahador *et al*. (2005) reported a PCR positive in 66 (84%) of 78 patients studied.[19] In this study, out of 76 MTB detected 6 were found Rifampicin resistant. CBNAAT was more successful in detection of MTB in patients with tubercular pleural effusion who had pulmonary involvement in their chest radiograph (most common finding was infiltrations and infiltration with consolidation) and sputum AFB positive patients. In the study by Soma Chakraborty *et al* (2005) out of the 240 extra pulmonary samples 13 (5.41%) were positive for AFB smear and 23 samples (9.2%) were detected as positive for Mycobacterium tuberculosis on Gene Xpert. 2 (8.69%) out of 23 GeneXpert positive extra-pulmonary samples were found to be Rifampicin resistant.[20] Avashia *et al*. examined 300 various extra-pulmonary samples from suspected extra pulmonary tuberculosis and out of it, MTB was detected in 111 samples and 105 samples were 'Rif' sensitive and 6 samples were 'Rif' resistant (5.40%).[21]

LIMITATIONS

The main limitation of our study was the small study population. We were unable to coordinate other medical collages because of distance constrains. Second limitation in our study was that we were unable to perform repeat test because of burden of extra-pulmonary samples using a single machine at our tertiary centre.

CONCLUSION

While the cytology of Pleural Fluid gives a very good estimation of the positivity status of a patient as the higher cell count is suggestive of TB but CBNAAT helps in diagnosing both the positivity status as well as rifampicin resistant state of the patient. CBNAAT had the potential for diagnosis of tubercular pleural fluid specimens for both in HIV positive and HIV negative tubercular patients. Also, detection of Rifampicin resistance aids in prompt initiation of appropriate therapy and thus improving the overall quality of TB patients care. WHO also recommends CBNAAT testing for diagnosis of tubercular pleural effusion and samples should be send using WHO guidelines. Physician and surgeon of other departments should have the knowledge about this diagnostic facility.

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