Original Research Article

Utility of cartridge based nucleic acid amplification test in the diagnosis of extra pulmonary tuberculosis

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ABSTRACT

Background: Tuberculosis is one of the top 10 cause of death globally. Extra-pulmonary tuberculosis is an important clinical problem. Extra-pulmonary tuberculosis range from 30%-53% in India. Diagnosis of extra-pulmonary tuberculosis is still challenging despite many investigations. World Health Organization recommends Gene-Xpert Mycobacterium Tuberculosis/Rifampicin (Cartridge Based Nucleic Acid Amplification Test-CBNAAT) over conventional tests for diagnosis of extra-pulmonary tuberculosis which permits rapid tuberculosis diagnosis through detection of the genetic sequence of DNA of mycobacterium tuberculosis and simultaneous identification of a majority of the mutations that confirm Rifampicin resistance which is highly predictive of multi-drug resistant tuberculosis.

Methods: Study was carried out over a period of one year. Patients with suggestive of extra-pulmonary tuberculosis were included in study. Diagnosis of extra-pulmonary tuberculosis carried out by clinical, radiological, biochemical analysis, cytological, bacteriological confirmation. Based on mycobacterium tuberculosis result, the study population were divided into ‘Mycobacterium Tuberculosis detected’ and ‘Mycobacterium Tuberculosis not detected’ groups. Mycobacterium Tuberculosis detected group was further divided into ‘Rifampicin resistant’ and ‘Rifampicin sensitive’.

Results: Total 220 patients were included. Among extra-pulmonary tuberculosis, there were 83.64% were pleural fluid. 65.91% patients where be <45 years of age. Mostly patients were from rural areas and illiterate. Diabetes Mellitus found as the most common co-morbidities. CB-NAAT was able to detect mycobacterium tuberculosis in 35% (77) extra-pulmonary samples, out of which 6 were rifampicin resistant. Out of 184 samples of pleural fluid, 53 were rifampicin sensitive and 4 were found rifampicin resistant.

Conclusions: CB-NAAT has to be endorsed in every health care centres as the test gives rapid result and also detection of rifampicin resistance which is the major concern for every clinician.

Keywords: Cartridge based nucleic acid amplification test, Extra-pulmonary tuberculosis, Multi-drug resistant tuberculosis, Mycobacterium tuberculosis, Pulmonary tuberculosis

INTRODUCTION

Tuberculosis has existed for millennia and remains a major global health problem. It causes ill-health in millions of people each year and is one of the top 10 causes of death worldwide; ranking above HIV/AIDS.1 Pulmonary tuberculosis is the most common presentation and practically the only form of the disease that is infectious. Extra-pulmonary tuberculosis is also an important clinical problem.2 Extra-pulmonary...
Tuberculosis represented 15% of the 6.1 million incident cases that were notified, ranging from 8% in the Western Pacific Region to 23% in the Eastern Mediterranean Region. The percentage of patients with extra-pulmonary tuberculosis in tertiary care centres in India was between 30% to 53%, while the percentage estimated by the national control program in India for HIV-negative adults is between 15%-20%. Among extra-pulmonary tuberculosis, lymph node tuberculosis is the most common type constitutes about 35% cases followed by pleural effusion (20%), bone and joint (10%), genitourinary tuberculosis (9%), tubercular meningitis (5%) abdominal tuberculosis (3%), other (10%).

Tuberculosis affecting other sites-known as extra-pulmonary tuberculosis is rarely smear-positive; it is generally accepted that the contagious potential of this form is negligible and it has, therefore, never been a priority in the campaigns undertaken by national tuberculosis control programs. Also, the literature on the various forms of extra-pulmonary tuberculosis is scant and this lack of evidence is of particular concern in the case of treatment guidelines.

The diagnosis of extra-pulmonary tuberculosis is 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 extra-pulmonary tuberculosis. Clinical presentation of extra-pulmonary tuberculosis is atypical. Therefore, the clinicians more often rely upon the clinical impression, radiological and endoscopic appearances and non-conventional diagnostic methods as evidence to diagnose extra-pulmonary tuberculosis.

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. As the diagnosis of extra-pulmonary tuberculosis is often compromise by the paucibacillary nature of the disease, newer diagnostic tools and policies have been eagerly awaited. The Gene Xpert Mycobacterium Tuberculosis/Rifampicin (Cartridge Based Nucleic Acid Amplification Test) is an automated real time polymerase chain reaction assay designed for the rapid and simultaneous detection of Mycobacterium Tuberculosis (MTB) and Rifampicin resistance (RIF) within 2 hours. Based on systematic review, World Health Organization recommends Gene-Xpert over conventional tests for diagnosis of Extra-pulmonary tuberculosis which permits rapid tuberculosis diagnosis through detection of the genetic sequence of DNA of mycobacterium tuberculosis and simultaneous identification of a majority of the mutations that confirm Rifampicin resistance which is highly predictive of multidrug resistant tuberculosis.

Authors objectives was to determine utility of cartridge based nucleic acid amplification test in detection of extra-pulmonary tuberculosis.

**METHODS**

Study design was prospective, and hospital-based study. Study was conducted in outpatient department of Respiratory Medicine Department of R.N.T. Medical College, Udaipur, Rajasthan. From January 2016 to December 2016. EPTB patients (n=220) with their consent were study population.

**Data collection**

Clinical history, demographic profile, socioeconomic status and anthropometric data of EPTB patients were taken with their significant past illnesses. Mantoux test was done and digital chest X-ray was advised to suspected pleural effusion, hydro-pneumothorax or pyo-pneumothorax. Sputum samples were sent for Acid Fast Bacilli (AFB) examination. Pleural effusion was confirmed by ultra-sonography. After that pleural fluid was aspirated by USG guidance.

From involved site of suspected extra-pulmonary tuberculosis patients, samples were taken and sent for laboratory analysis, 2 ml of extra-pulmonary tuberculosis samples were sent for Gene X-pert. Diagnosis of extra-pulmonary tuberculosis carried out by clinical, radiological, biochemical analysis, cytological, bacteriological confirmation and response with anti-tubercular treatment.

Based on mycobacterium tuberculosis result, the study population were divided into ‘**Mycobacterium Tuberculosis detected**’ and ‘**Mycobacterium Tuberculosis not detected**’ groups. Mycobacterium Tuberculosis detected group was further divided into two subgroups i.e. ‘**Rifampicin Resistant**’ and ‘**Rifampicin Sensitive**’.

**Inclusion criteria**

- Patients of both sex and of all age groups.
- Exudative pleural effusion with or without parenchymal infiltration.
- Exudative ascites with or without other abdominal organ involvement.
- CNS tuberculosis (meningitis, tuberculoma, etc).
- Empyema with or without parenchymal lesions.
- Cold abscess.
- Palpable lymphadenopathy in which cytology was suggestive of tuberculosis.
- Spinal tuberculosis with paraspinal abscess.
- Joint tuberculosis with significant synovial fluid.
Exclusion criteria

- Tubercular pericardial effusion, pleural effusion and ascites.
- Malignant pleural effusions and hemothorax.
- Contra indication to thoracentesis.
- Patients who will not want to be a part of this study.

Statistical analysis

The data obtained was filled in excel sheet and statistical analysis was done using SPSS 21. Chi square test was applied to know the significance.

Ethical issue

Ethical clearance from ethical committee has been obtained before starting of the study.

Table 1: Distribution of Extra-pulmonary tuberculosis with sex.

| Type of EPTB                  | Male               | Female  | Total       |
|-------------------------------|--------------------|---------|-------------|
| Tubercular pleural effusion   | 134 (72.83%)       | 50 (27.17%) | 184 (83.64%) |
| Tubercular Empyema            | 18 (94.74%)        | 1 (5.26%)  | 19 (8.64%)  |
| CNS TB                        | 8 (80%)            | 2 (20%)  | 10 (4.54%)  |
| Lymph node TB                 | 1 (25%)            | 3 (75%)   | 4 (1.82%)   |
| Cold abscess                  | 1 (100%)           | 0 (0%)    | 1 (0.45%)   |
| TB Paraspinal abscess         | 1 (100%)           | 0 (0%)    | 1 (0.45%)   |
| Joint TB                      | 1 (100%)           | 0 (0%)    | 1 (0.45%)   |
| Total                         | 164 (74.54%)       | 56 (25.46%) | 220 (100%) |

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

| Substance abuse | Number | Percentage |
|-----------------|--------|------------|
| Smoker          | 35     | 41.18%     |
| Gutkha chewer    | 25     | 29.41%     |
| Smoker and Alcoholic | 20 | 23.53%     |
| Alcoholic       | 03     | 3.53%      |
| Smoker, alcoholic and Gutkha chewer | 02 | 2.35%      |
| Total           | 85     | 100%       |

RESULTS

In this study 74.54% were male and 25.46% were female. Male to female ratio was 2.93:1. Among extra-pulmonary tuberculosis cases, there were 83.64% were pleural fluid, 8.64% were pus, 4.54% were CSF, 1.82% were lymph node aspirate, 0.45% was synovial fluid and 0.45% was paraspinal abscess and 0.45% was cold abscess.

Majority of patients (65.91%) were <45 years of age. Mostly patients (84.09%) were from rural areas whereas 15.91% patients were from urban areas, 62.27% patients were illiterate, and 37.72% patients were literate (Table 1). Out of 220 subjects, 85(38.64%) had different substance abuse habit and among these 41.18% patients had smoking habit followed by 29.41% gutkha chewer, 23.53% both smoker and alcoholic, 3.53% alcoholic and 2.35% smoker, alcoholic and gutkha chewer (Table 2).

Table 3: Distribution of study population according to co-morbidities.

| Co-morbidities          | Number | Percentage |
|-------------------------|--------|------------|
| DM                      | 10     | 29.41%     |
| HIV                     | 8      | 23.52%     |
| COPD                    | 8      | 23.52%     |
| Hypertension            | 4      | 11.76%     |
| Hypothyroidism          | 2      | 5.88%      |
| DM, HTN and Bronchial asthma | 2     | 5.88%      |
| Total                   | 34     | 100%       |

Only 34(15.45%) patients had co-morbidities, 29.41% patients had Diabetic Mellitus (DM), 23.52% patients had obstructive airway disease and 23.52% patients have HIV. Positive, 11.76% had hypertension, 5.88% patient had hypothyroidism and 5.88% patient had diabetes mellitus, Hypertension and bronchial asthma, 81.82% patients had Body Mass Index (BMI) <18.5 kg/m² and 14.09% had BMI between 18.5-24.9 kg/m² and 4.09% had >25 kg/m² (Table 3). Out of 99 patients with pulmonary involvement in their chest radiograph, 70.71% had infiltrations, 17.17% had pyo-pneumothorax, 6.06% had pulmonary cavity and 6.06% had consolidation (Table 4).

Out of 220 samples Cartridge Based Nucleic Acid Amplification Test was able to detect mycobacterium tuberculosis in 77(35%) extra-pulmonary samples, out of which 6 were rifampicin resistant. Out of 184 samples of pleural fluid, 53 were rifampicin sensitive and 4 were found rifampicin resistant. Out of 10 cerebro-spinal fluid...
samples, 2 samples found mycobacterium tuberculosis detected (Table 5).

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

| Radiographic presentation | Number | Percentage |
|---------------------------|--------|------------|
| Infiltrations             | 70     | 70.71 %    |
| Pyo-pneumothorax          | 17     | 17.17 %    |
| Cavity                    | 6      | 6.06 %     |
| Consolidation             | 6      | 6.06 %     |
| Total                     | 99     | 100%       |

Out of 184 pleural fluid samples, 98(53.3%) had pulmonary involvement. This test was more successful in detection of mycobacterium tuberculosis in patients with tubercular pleural effusion who had pulmonary involvement in their chest radiograph. There is statistically significant correlation between result of cartridge based nucleic acid amplification test and pleural effusion with pulmonary lesions (p <0.001) (Table 6).

Out of 184 tubercular pleural effusions, sputum was positive for acid fast bacilli in 29 patients and negative in 155 patients. Out of 29, mycobacterium tuberculosis was detected in 20 patients. In sputum negative patients (155), mycobacterium tuberculosis was detected in 37 patients.

Table 5: Distribution of study populations according to their CBNAAT result.

| Extra pulmonary tuberculosis sample | Mycobacterium tuberculosis detected | Mycobacterium tuberculosis not detected | Total |
|-------------------------------------|------------------------------------|---------------------------------------|-------|
|                                    | Rifampicin Sensitive | Rifampicin Resistant | Total |                         |                    |                  |
| Pleural fluid                      | 53 (28.8%)             | 4 (2.17%)                 | 57 (30.98%) | 127 (69.02%) | 184 (83.64%)          |
| Pleural pus                        | 12 (63.16%)            | 1 (5.26%)                 | 13 (68.42%) | 6 (31.58%)    | 19 (8.64%)            |
| Cerebrospinal fluid               | 2 (20%)                | 0 (0%)                    | 2 (20%)    | 8 (80%)      | 10 (4.54%)            |
| Lymph node aspirate               | 2 (50%)                | 1 (25%)                  | 3 (75%)    | 1 (25%)      | 4 (1.82%)             |
| Cold abscess                      | 1 (100%)               | 0 (0%)                    | 1 (100%)   | 0 (0%)       | 1 (0.45%)             |
| Paraspinal abscess                | 1 (100%)               | 0 (0%)                    | 1 (100%)   | 0 (0%)       | 1 (0.45%)             |
| Synovial fluid                    | 0 (0%)                 | 0 (0%)                    | 0 (0%)     | 1 (100%)     | 1 (0.45%)             |
| Total                              | 71 (32.27%)            | 6 (2.72%)                 | 77 (35%)   | 143 (65%)    | 220 (100%)            |

Table 6: Distribution of tubercular pleural effusion with or without pulmonary involvement according to CBNAAT result.

| Pulmonary Involvement | Mycobacterium tuberculosis detected | Mycobacterium tuberculosis not detected | Total |
|-----------------------|------------------------------------|---------------------------------------|-------|
|                       | Rifampicin Sensitive | Rifampicin Resistant | Total |                         |                    |                  |
| Yes                   | 41 (41.8%)             | 4 (4.08%)                 | 45 (45.92%) | 53 (54.08%) | 98 (53.3%)            |
| No                    | 12 (13.95%)            | 0 (0%)                    | 12 (13.95%) | 74 (86.05%) | 86 (46.7%)            |
| Total                 | 53 (28.8%)             | 4 (2.17%)                 | 57 (30.98%) | 127 (69.02%) | 184 (100%)            |

Table 7: Distribution of patients with tubercular pleural effusion according to their sputum microscopy and pleural fluid CBNAAT result.

| Sputum microscopy result | Pleural fluid CBNAAT result | Mycobacterium tuberculosis not detected | Total |
|-------------------------|-----------------------------|---------------------------------------|-------|
|                         | Mycobacterium tuberculosis detected |                                              |       |
| Positive                | 20 (68.96%)                 | 9 (31.03%)                           | 29 (15.76%) |
| Negative                | 37 (23.87%)                 | 118 (76.13%)                        | 155 (84.24%) |
| Total                   | 57 (30.98%)                 | 127 (69.02%)                        | 184 (100%) |

There is statistically significant correlation between sputum positivity in tubercular pleural effusion and cartridge based nucleic acid amplification test result (p <0.001) (Table 7).

DISCUSSION

In this study 74.54% were males and 25.46% were females. In the study by Mavila R et al, out of 187 extra-pulmonary tuberculosis cases; 112 (59.9%) were male
and 75 (40.1%) were female.\(^8\) Aysel et al, studied that men were more frequently affected by pulmonary tuberculosis (59.6%), while extra-pulmonary tuberculosis was more commonly detected in women (52.2%).\(^9\)

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 tuberculosis patient by members of the community. The higher rates of tuberculosis among male is due to a higher prevalence of infection among men. Above reasons may be the cause of sex difference in the study.

Mostly patients (84.09%) were from rural areas whereas 15.91% patients were from urban areas in our study. Abdallah TEM et al, studied that among 224 extra-pulmonary tuberculosis patients, 74.6% (167) patients were rural residence.\(^10\) Patients attending our institution were mostly from rural areas because rural population is dominating in Udaipur Zone. This might be reasons for higher rural population in our study.

There were 62.27% patients were illiterate and 37.72% patients were literate in our study. 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.

Majority of patients (65.91%) were <45 years of age. In a study by Xinyu Zhang et al, out of 1449 extra-pulmonary tuberculosis patients, 1011 (69.8%) patients were below 44 years of age and 438 (30.2%) patients were above 45 years of age.\(^11\)

Out of 220 subjects, 85 (38.64%) had different substance abuse habit and among these 41.18% patients had smoking habit followed by 29.41% gutkha chewer, 23.53% both smoker and alcoholic, 3.53% alcoholic and 2.35% smoker, alcoholic and gutkha chewer. In the study by Sajith M et al, out of 49 extra-pulmonary tuberculosis 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 pulmonary tuberculosis as compared to extra-pulmonary tuberculosis.\(^12\)

Smoking habit were more, in comparison to other published study. The reason behind that it may be due to illiteracy, unaware about health hazards of tobacco use and poor execution of the law for tobacco use. Only 34 (15.45%) patients had co-morbidities. 29.41% patients had diabetes mellitus, 23.52 % patients had obstructive pulmonary disease and 23.52 % patients have HIV Positive. 11.76 % had hypertension, 5.88 % patient had hypothyroidism and 5.88 % patient had diabetes mellitus, hypertension and bronchial asthma.

Sanchis I et al, studied that HIV, diabetes mellitus and cancer were seen in 15.8%(20), 6.3%(8) and 4.8%(6) patients respectively as comorbidities.\(^13\) In literatures, HIV and diabetes mellitus a predominant co-morbidity in extra-pulmonary as well as pulmonary tuberculosis. 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.

Majority of patients (81.82%) had Body Mass Index (BMI) <18.5 kg/m\(^2\) and 14.09 % had BMI between 18.5-24.9 kg/m\(^2\) and only 4.09% 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 tuberculosis, contrarily, having a BMI >25 kg/m\(^2\) has a protective effect against tuberculosis.\(^14\)

Out of 184 pleural fluid samples, 98 (53.3%) had pulmonary involvement. This test was more successful in detection of mycobacterium tuberculosis in patients with tubercular pleural effusion who had pulmonary involvement in their chest radiograph.

Out of 220 samples Cartridge Based Nucleic Acid Amplification Test was able to detect mycobacterium tuberculosis in 77 (35%) extra-pulmonary samples, out of which 6 were rifampicin resistant by this test, 184 samples of pleural fluid, 53 were rifampicin sensitive and 4 were found rifampicin resistant and 10 cerebro-spinal fluid samples, mycobacterium tuberculosis detected in 2 samples.

Handojo et al, reported a polymerase chain reaction (PCR) positive in 26 out of 62 patients (41.19%) with 53.35% sensitivity and 93.75% specificity.\(^15\) Nagdev et al, reported PCR positive in 68.18%, with a sensitivity of 68.6% and specificity of 66.16. A study from Pakistan have reported 15.8%, 40.0% and 6.3% respectively, of pleural fluid, cerebro-spinal fluid and ascite fluid samples to be Cartridge Based Nucleic Acid Amplification Test positive for mycobacterium tuberculosis.\(^17\)
In this study, 6 patients were rifampicin resistance (4 pleural fluids, one lymph node aspirate and one pleural pus). The rifampicin resistance was seen among 11% of pulmonary and 38% of extra-pulmonary specimens from the tertiary care centre in Mumbai, India. Avashia S et al, examined 300 various extra-pulmonary samples from suspected extra-pulmonary tuberculosis and out of it mycobacterium tuberculosis was detected in 111 samples and 105 samples were rifampicin sensitive and 6 samples were rifampicin resistant (5.40%).

Though very few data are available on drug resistance in extra-pulmonary tuberculosis, previous studies have reported 12.5% multi drug resistant tuberculosis-extra-pulmonary tuberculosis in Nepal and 10% in Delhi India.

The main limitation of this study was the small study population. We were unable to coordinate other medical collages because of distance constraints. 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 center.

CONCLUSION

The Gene Xpert assay has the potential to rapid detection of mycobacterium tuberculosis and resistance to rifampicin in extra-pulmonary tuberculosis samples and using as a prognostic indicator for treatment and death due to tuberculosis.

Hence Cartridge Based Nucleic Acid Amplification Test has to be endorsed in every health care centers as the test gives rapid result and also detection of rifampicin resistance which is the major concern for every clinician. WHO also recommends Gene Xpert for diagnosis of extra-pulmonary tuberculosis and samples should be send using WHO guidelines.

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