Original Article

Patterns of Extrapulmonary Tuberculosis and the Role of Cartridge-Based Nucleic Acid Amplification Test in the Diagnosis of Extrapulmonary Tuberculosis

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Abstract

Background: Extrapulmonary tuberculosis (EPTB) was underdiagnosed due to its varying and nonspecific symptoms and limited diagnostic tools. Recent data suggest that there is an increase in the incidence of EPTB due to the availability of the newer diagnostic tools. **Objectives:** This study aims to evaluate the patterns and role of newer diagnostic tools, such as cartridge-based nucleic acid amplification test (CBNAAT), in EPTB. **Methods:** Six hundred and thirty-six patients with a presumptive diagnosis of EPTB were subjected to diagnostic tools, such as cartridge-CBNAATs, Ziehl–Neelsen (Z–N) stain, and cytology or histopathology. **Results:** Out of 636 presumptive cases of EPTB, 116 cases were positive for tuberculosis. The cartridge-CBNAAT showed positivity in all 116 cases**.** Out of these 116 EPTB cases, only 10 cases were positive on Z–N stain, and 24 cases were positive on cytology and histopathology. The most common sites affected were the lymph nodes and the pleural cavities. **Conclusion:** Cartridge-CBNAAT gives a rapid result and plays a significant role in the diagnosis of EPTB.

Keywords: *Cartridge-based nucleic acid amplification test, extrapulmonary tuberculosis, Ziehl–Neelsen stain*

Introduction

Tuberculosis (TB) is a global health problem, and it is one of the most common causes of death worldwide. In 2023, 8.2 million new cases were reported globally, surpassing the previous record of 7.5 million in 2022 and 7.1 million in 2019. The increase in TB cases in 2022 and 2023 suggests improved access to diagnostic facilities.[1]

Any clinically diagnosed cases or bacteriologically confirmed cases of TB that involve organs other than the lung are categorized as extrapulmonary tuberculosis (EPTB).[2] EPTB can present as a primary form of the disease without the involvement of the lung, or it can occur by lymphatic and hematogenous spread from primary TB in the lungs to organs other than the lungs.[3] Worldwide, EPTB accounts for 15% to 20% of all TB cases.[4] National-level EPTB data indicate that EPTB in India comprises 15 to 24% of total TB cases.[5] EPTB occurs in various parts of the body, such as the pleura, lymph nodes, bones and joints, the central nervous system, the genitourinary system, the gastrointestinal system, and the skin.[2,4]

EPTB is more common in immunocompromised individuals, as immune suppression allows organisms to disseminate from the lungs to other organs.[2,6] Diagnosis of EPTB is complex and challenging due to its varying and nonspecific symptoms. Common symptoms of TB, such as fever and cough, are often absent, and these patients usually present with subtle symptoms, such as headache, abdominal pain, backache, fatigue, or systemic symptoms depending on the organs affected, which may lead to misdiagnosis or underdiagnosis of EPTB.[4]

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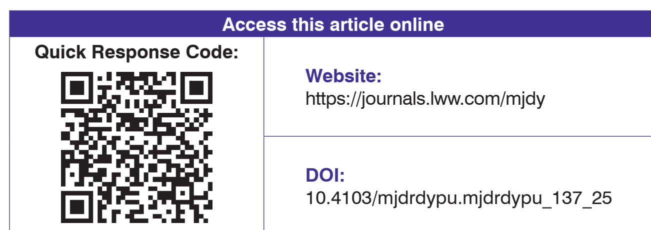

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EPTB samples received for diagnosis are usually paucibacillary, which may lead to misdiagnosis or underdiagnosis.[4] In EPTB, detection of acid-fast bacilli by microscopy also has limited sensitivity, ranging from 0 to 40%.[7] In patients having presumptive diagnosis of EPTB, a Cartridge-based nucleic acid amplification test (CBNAAT) can be used for samples, such as lymph node aspirate, lymph node biopsy, and cytology samples, such as pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid, or urine specimens, in GeneXpert MTB/RIF equipment as the initial diagnostic test. There is a paucity of data on precise demographics, clinical manifestations, organs affected, and diagnostic and therapeutic evaluation of EPTB.[4] Hence, this study was undertaken to evaluate the commonly affected organs and therapeutic outcomes in EPTB patients, and to assess the role of CBNAAT in diagnosing EPTB.

Materials and Methods

Study design is a prospective observational study. All clinically suspected cases of EPTB referred to the nodal center under the Revised National Tuberculosis Control Programme (RNTCP) laboratory at the district hospital in Vijayapura, Karnataka, were included in the study. This study collected data from October 2022 to October 2024 from the laboratory register, treatment cards, and referral registers of RNTCP.

Ethical clearance: Institutional ethical clearance (BLDE (DU)/IEC/1066/2023-2024) and ethical clearance from the district hospital (DHV/DNB/DHV/IEC/04/2024-2025) were obtained for the study.

Sample size: With anticipated sensitivity and specificity of diagnosis of EPTB by CBNAAT and Line Probe Assay of 100% and 73.33%, respectively, and an EPTB prevalence of 15–52%, at a precision of 0.06% and 95% confidence, the required sample size is 601.[7,8] Hence, 636 samples were included in the study.

Sample collection: Clinically suspected cases of EPTB samples, such as pleural fluid, peritoneal fluid, fine needle aspiration cytology (FNAC) samples of lymph node, lymph node biopsies, aspirates of abscess, cerebrospinal fluid, and biopsies of bones and joints received in the RNTCP laboratory of the district hospital, were included in the study. The Ziehl–Neelsen (Z–N) stain was conducted on all samples for acid-fast bacilli. Then, the samples were processed using the GeneXpert MTB/RIF automated real-time polymerase chain reaction (PCR) assay for rapid detection of *Mycobacterium tuberculosis* (*MTB*) and Rifampicin resistance, using single-use plastic cartridges with multiple chambers preloaded with liquid buffers and lyophilized reagent beads. To inactivate clinical samples, sodium hydroxide and isopropanol-containing reagents were used at a 3:1 ratio for sample pellets and a 2:1 ratio for unprocessed samples, depending on the sample type. Then, incubation was conducted at room temperature for 15 minutes. After 15 minutes, samples were transferred to a cartridge and placed into the GeneXpert equipment. Then, the sample was automatically filtered and washed, this process concentrates bacilli and removes inhibitors. In this process, DNA is released through ultrasonic lysis of filter-captured organisms. Dry PCR reagents were combined with the DNA of organisms of *MTB*. Five molecular probes (probes A–E) that overlap and are complementary to the whole 81 bprpo core region were used to detect *MTB*. Out of the five molecular probes, minimum two probes must exhibit positive signals with a cycle threshold (CT) of d”38 cycles to confirm the presence of *MTB*. The difference between the first/early CT and the last/late CT served as the primary method for detecting RIF resistance.

Statistical analysis: The data obtained were tabulated using Microsoft Excel. Mean ± SD and percentages were calculated. Sensitivity and specificity were estimated for the diagnostic tests.

Results

A total of 636 presumptive EPTB cases referred from the public and private sectors to the district hospital were analyzed in this study. The youngest patient in the present study was a newborn, and the oldest study participant was 87 years old. Out of 636 presumptive cases of EPTB, 116 cases, amounting to 18.24%, were positive for TB [Table 1].

The maximum number of samples received in presumptive cases of EPTB in the present study was pleural fluid, amounting to 171 (26.89%) cases, followed by samples of gastric lavage (12.26%), abscess cavity (10.85%), lymph node samples (9.27%), and fine needle aspiration samples from various sites, which were 8.33%. Bronchoalveolar lavage samples were

Table 1: Sociodemographic characteristics of cases of presumptive diagnosis of EPTB and EPTB-positive cases

Characteristics		Cases having presumptive diagnosis of EPTB (*n*=636)	Cases diagnosed as EPTB positive (*n*=116)
Age range (years)	0–20	186 (29.24%)	25 (21.56%)
	21–40	236 (37.11%)	45 (38.79%)
	41–60	141 (22.17%)	33 (28.44%)
	61–80	70 (11.01%)	13 (11.21%)
	81–100	03 (00.47%)	00 (00.00%)
Gender	Male	365 (57.38%)	64 (55.17%)
	Female	271 (42.62%)	52 (44.83%0

EPTB=Extrapulmonary tuberculosis

7.71%, cerebrospinal fluid was 6.76%, and ascitic fluid was 6.29%. Forty (6.29%) samples were received from bones and joints. Nasal swab, synovial fluid, urine, and stool samples were included in the other category, which accounted for 4.87%. All the samples labelled in the other category were negative for TB [Table 2].

Demographic and clinical details of EPTB-positive cases

The maximum number of EPTB cases diagnosed as positive for TB was in the age group of 21 to 40 (38.79%), followed by the age group of 41 to 60 (28.44%), indicating that EPTB predominantly affects the economically productive age group. The male-to-female ratio was 1.2: 1, with a slight male preponderance [Table 1]. Out of 116 EPTB-positive cases, in 22 (18.96%) cases, HIV positivity was noted, and in 93 (80.17%) cases, no association of HIV was observed. In four cases (3.45%) of EPTB-positive patients, a history of Type-2 diabetes mellitus was present.

Among the EPTB-positive cases, the most commonly affected sites were the lymph nodes and pleural fluids, with 24 cases (20.68%) each. Sixteen cases (13.79%) of EPTB-positive cases were ascitic fluid. In 14 cases (12.06%), tissue samples were received as samples from the spine. The tissue samples received as abscesses were 13 (11.20%). Other samples were bronchoalveolar lavage, cerebrospinal fluid, and gastric lavage, which were five, four, and three cases, amounting to 4.31%, 3.45%, and 2.59%, respectively. FNAC samples from various EPTB cases showed positivity in 13 cases (11.20%) [Table 2].

Diagnosis of TB was conducted in all 116 cases based on CBNAAT. Of 116 EPTB cases diagnosed by CBNAAT, 10 (8.62%) showed acid-fast bacilli on Z–N staining. In 24 (20,68%) cases, granulomatous lesions were noted on cytology/histopathology. When a comparative statistical analysis was conducted between CBNAAT, Z–N stain, cytology, and histopathology, CBNAAT showed an accuracy of 94.13% as compared to Z–N stain and cytology/histopathology.

Patient type and treatment outcomes

Out of 116 confirmed EPTB cases, 98 (84.48%) were new cases, and in 18 cases (15.51%), relapse was noted. Treatment history data revealed that 64 (55.17%) participants completed treatment, and 35 (30.17%) were still undergoing treatment at the time of the study. During study period, it was observed that a small proportion of cases, amounting to 12 cases (10.34%), responded well to treatment. Two (1.72%) were lost to follow-up, and 2 (1.72%) died during the study period. In one patient, a treatment change was mentioned due to adverse effects. Rifampicin resistance was detected in five cases (4.31%), and in the remaining 111 cases (95.69%), rifampicin was sensitive [Table 2].

Discussion

TB is one of the major causes of morbidity and mortality. If early diagnosis and appropriate treatment is conducted, mortality from TB can be reduced.[1-3] Clinical manifestation of TB occurs in two forms. The most typical form is pulmonary TB, and the other form is EPTB.[9] In the last decade in India, under a RNTCP, the rapid expansion of directly observed short-term treatment centers has been established to facilitate TB treatment and reduce its incidence. However, in these centers, treatment for EPTB cases cannot be effectively provided due to the diagnostic challenges posed by nonspecific symptoms.[10] The estimated incidence of TB in India was 2.1 million cases, out of which 16 percent were new cases of EPTB.[11] In the present study, out of 636 presumptive cases of EPTB, 116 cases (18.24%) were positive for TB on CBNAAT.

EPTB cases were more commonly observed in age groups of 15 to 44, amounting to 50.95% and 45 to 64, amounting to 30.68% as compared to the pediatric and older age groups.[10] One of the studies mentioned that

Table 2: Site-wise distribution of cases of presumptive diagnosis of EPTB and EPTB-positive cases

Organ/site	Number (*n*=636)	Percentage (%)	Positive (*n*=116)	Percentage (%)	Negative (*n*=520)	Percentage (%)
Pleural fluid	171	26.89	24	14.04	147	85.96
Gastric lavage	78	12.26	3	3.85	75	96.15
Abscess	69	10.85	13	18.84	56	81.16
Bronchoalveolar lavage	49	7.71	5	10.20	44	89.80
Cerebrospinal fluid	43	6.76	4	9.30	39	90.70
FNAC samples	53	8.33	13	24.53	40	75.47
Lymph node	62	9.75	24	38.71	38	61.29
Spine, bones, and joints	40	6.29	14	35.00	26	65
Ascitic fluid	40	6.29	16	40.00	24	60
Other	31	4.87	0	00.00	31	100

EPTB=Extrapulmonary tuberculosis; FNAC=Fine needle aspiration cytology

the younger age group of 16 to 25 years has a higher propensity to transmit infection, and this was the most commonly affected age group in EPTB. They also mentioned that the age group of 26–40 years was the next group affected by EPTB.[6] In the present study, the maximum number of EPTB cases was noted in the age group of 21 to 40 (38.79%), followed by the age group of 41 to 60 (28.44%), indicating that EPTB predominantly affects the economically productive age group, with chances of high propensity for infection in this group. EPTB cases are slightly higher in males as compared to females.[10,12] Similar observations were noted in the present study, with a slight male preponderance.

EPTB can occur anywhere in the body.[3] Sites of EPTB may differ across geographic areas, population categories, and other factors.[10] In one of the studies conducted on EPTB, the lymph node was the most common site, accounting for 50% of cases, followed by the pleural cavity, accounting for 28.03%. In 13% of cases, genitourinary TB was noted. In 6% of cases, bones and joints, as well as the gastrointestinal system, were the site of EPTB. In the central nervous system and spine, 3% of EPTB cases were noted.[3] In one study, the pleural cavity was the most common site, accounting for 28.03%, followed by the lymph nodes, which accounted for 28.03%. In their study, EPTB in the CNS, bones and joints, and abdomen were reported to be 12.50%, 12.31%, and 9.66%, respectively.[10] In the present study, the lymph nodes and pleural cavities were the most common sites, each accounting for 20.68%.

EPTB is one of the most common opportunistic infections in HIV patients.[11] Individuals with immunocompromised states, such as HIV infection, diabetes mellitus, and malnutrition, are prone to EPTB.[12] In one of the studies, it was mentioned that 15%−20% of patients with TB had immunocompromised disorders. Further, it was mentioned that in 50% of reported HIV cases, associated TB positivity was noted.[13] In one of the studies, it was mentioned that 28.9% cases of EPTB coinfection with HIV were noted.[2] In the present study, in 18.96% of cases, EPTB coinfection with HIV was observed.

In one of the studies of EPTB, it was mentioned that the CBNAAT assay, when compared to AFB culture and histopathology as a diagnostic modality, had a sensitivity of 32.3%. It was also mentioned that when the diagnostic yield of CBNAAT against the composite reference standard was studied, 100% specificity was noted.[14] In the present study, when the CBNAAT yield for the diagnosis of TB was compared with the combined diagnostic yield of TB diagnosis on Z–N stain, cytology, and histopathology, the sensitivity of CBNAAT was 77.33%, and specificity was 98.87%.

In one of the studies, it was observed that 84.4% of participants completed the treatment course, 11.1% died, and 4.2% lost to follow-up. Their study also reported that 84.4% of participants in EPTB responded to treatment. Cure after the treatment is challenging to decide in EPTB due to nonspecific clinical findings in EPTB.[2] In the present study, 64 participants completed the treatment. Of these 64 cases, 12 (18.75%) patients responded to treatment. In these patients, the follow-up CBNAAT investigation was negative for TB after completion of the course of treatment. In the present study, 1.72% of participants died, and 1.72% lost to follow-up. This analysis was based on the treatment card and referral registers of RNTCP.

Conclusion

EPTB commonly occurs in the productive age group of 21 to 40 years and is most commonly noted in organs, such as lymph nodes and pleura. Detection of EPTB is challenging due to variable clinical presentations; however, newer, rapid diagnostic modalities, such as CBNAAT, which has higher sensitivity and specificity compared to other modalities, such as Z–N stain, cytology, and histopathology, are increasingly used for EPTB diagnosis. CBNAAT may help in early diagnosis and to plan the strategies for diagnosis and treatment of EPTB and to reduce the social burden of TB.

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Authors contribution list

- Concept and design of study were done by Dr Surekha U. Arakeri and Dr Shivanad Gundali
- Data acquisition, data analysis, statistical analysis, and interpretation of data were done by Dr Shivanad Gundali and Dr Surekha U. Arakeri
- Definition of intellectual content, literature search, clinical studies, and experimental studies were done by Dr Surekha U. Arakeri and Dr Shivanad Gundali
- Manuscript preparation, manuscript editing, and manuscript review were done by Dr Surekha U. Arakeri and Dr Shivanad Gundali
- Dr Surekha U. Arakeri and Dr Shivanad Gundali helped in drafting the article or revising it critically for important intellectual content
- Final approval of the version to be published was done by Dr Surekha U. Arakeri and Dr Shivanad Gundali

Data availability statement

Data supporting study were collected from the nodal center of the RNTCP laboratory at district hospital Vijayapura, Karnataka. Data supporting the findings are available from the authors and can be shared upon reasonable request.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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