Original Article

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Detection of Extrapulmonary Tuberculosis from Various Samples in Sputum Smear Negative Patients

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Abstract

Background: Extra pulmonary tuberculosis (EPTB) can occur in isolation or along with PTB in immune-competent patients. EPTB accounts for 15-20% of all cases of TB. EPTB remains a challenging diagnosis for both clinicians and microbiologists. This study is conducted to detect EPTB from various samples in sputum smear negative patients.

Materials and Methods: 100 samples from clinically suspected patients of EPTB received at culture and drug susceptibility testing lab under RNTCP, Andhra Medical College, Visakhapatnam were included in the study. All samples were processed for Ziehl-Neelsen (ZN) stain and culture for *Mycobacterium* TB was done on Lowenstein-Jensen (LJ) medium as per the guidelines of National TB Research Institute, Chennai. The positive cultures were processed for line probe assay-polymerase chain reaction (LPA-PCR) with MTBDR kit as per the guidelines.

Results: Out of the 100 extra-pulmonary samples, processed 9% were positive by ZN staining and 12% were positive by LJ culture. Those samples positive by ZN smear and LJ culture were all positive by LPA (100%). Out of 12 LPA performed samples, 9 (75%) were both rifampcin and isoniazid (INH) sensitive, 1 (8.3%) was rifampcin and INH resistant, 1 (8.3%) was rifampcin resistant and INH sensitive, and another sample was rifampcin sensitive and INH resistant. Out of 12 LPA tests performed two samples (16.7%) were rifampcin resistant indicating multidrug-resistant TB.

Conclusion: EPTB is not given priority earlier in TB control programs in developing countries as the proportion is low and less infectious than PTB. As the incidence of MDRTB is increasing in PTB, an attempt has been made in this study to detect resistance pattern of isolated cultures by LPA and found that it was 16.7% in EPTB.

Key words: Cultures, Extra pulmonary tuberculosis, Program, Sensitive

INTRODUCTION

Tuberculosis (TB) can involve any organ system in the body. While pulmonary TB (PTB) is the most common presentation, extra PTB (EPTB) is also an important clinical problem.[1,2] The term EPTB describes isolated occurrence of TB at body sites other than the lung. However, when an extra pulmonary focus is evident in a patient with PTB, such patients have been categorized under PTB as per the guidelines of WHO.[3]

EPTB constitutes about 15-20% of all cases of TB.[4,5] In HIV-positive patients, EPTB accounts for more than 50% of all cases of TB.[6,7]

In India and other developing countries, lymph node (LN) TB constitutes to be the most common form of EPTB followed by pleural effusion, bone and joint TB, genitourinary TB, TB meningitis, and others.[8-10]

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An accurate diagnosis of TB is desirable before the start of anti-TB treatment. The sensitivity and specificity are low for Ziehl-Neelsen (ZN) stain in EPTB. The turnaround time (4-8 weeks) is long for the gold standard culture in Lowenstein-Jensen (LJ) medium though sensitive and should be followed by identification tests.[11] Though there are limitations an attempt has been made to detect the EPTB in this study by these two methods and culture positive samples were processed for line probe assay to detect *Mycobacterium tuberculosis* (MTB) and the sensitivity pattern of rifampicin and isoniazid (INH).

MATERIALS AND METHODS

The present study was conducted in the culture and drug susceptibility testing (C and DST) Lab under RNTCP, Andhra Medical College, Visakhapatnam during the period of 2012-2013. 100 samples from clinically suspected EPTB patients received at C and DST lab were included in the study.

All cases already on anti-TB treatment or had been confirmed as having PTB were excluded from the study. The sputum smear negative by ZN stain cases which were clinically diagnosed as EPTB were included in the study.

The processing of samples for ZN staining was done as per the guidelines of RNTCP,[12] and the processing for culture was done as per the guidelines of National TB Research Institute (NIRT), Chennai.[13] The processing for line probe assay (LPA) from positive growths was done with MTBDR kit (Hain life sciences).

All the samples were collected under strict aseptic conditions. Sample transportation, processing, and inoculation were done as per the Guidelines of NIRT Chennai. The samples were processed for LPA as per the kit literature.

Inoculated LJ medium slopes were incubated at 37°C.

Culture reading was done:

1. All the cultures were read every week for up to 8 weeks using the same methodology used for pulmonary samples as per the NIRT guidelines.
2. Typical MTB growths on LJ medium were subjected for biochemical tests and LPA.

Biochemical Tests

The mycobacterial isolates obtained in culture were subjected to biochemical testing for species characterization by carrying out nitrate reduction tests and absence of growth on LJ medium with paranitrobenzoic acid.

Quality control was carried out using the MTB H37RV strain as a positive control and a reagent control without organism as a negative control.

LPA-polymerase chain reaction (PCR) was performed as per the kit literature (MTBDR kit Hain life sciences).

RESULTS

In our study, the majority of the patients (48%) were between the age group of 21 and 40 years, followed by 11-20 years (17%) and 41-50 years (15%) (Table 1), with a male:female ratio of 1.8:1 (64:36) (Table 2). Most of the samples received were of LNs (30) followed by pleural fluid (28), cerebrospinal fluid (18), bone and synovial fluid (10), ascetic fluid (6), pericardial fluid (4), and endometrial tissue (1) (Table 3). Out of the 100 samples, 9 (9%) were positive for acid-fast bacilli (AFB) by ZN staining and 12 out of 100 samples (12%) were culture positive on LJ medium. All the 12 culture-positive samples were positive for MTB by LPA. Out of 12 LPA results, 9 (75%) were both rifampicin and INH sensitive, 1 (8.3%) sample was both rifampicin and INH resistant, 1 (8.3%) was

Table 1: Age wise distribution (*n*=100)

Age (years)	Number
1-10	2
11-20	17
21-30	26
31-40	22
41-50	15
51-60	6
>61	12
Total	100

Table 2: Gender wise distribution (*n*=100)

Gender wise distribution
Males=64
Females=36
Total=100

Table 3: Sample wise distribution

Nature of clinical samples	Number of samples	Number of TB detected positive and percentage
LN	30	7 (23.3)
Pleural fluid and pus	28	3 (10.7)
CSF	18	0 (0)
Bone and synovial fluid	10	1 (1)
Ascitic fluid	6	1 (16.6)
Urine	4	0 (0)
Pericardial fluid	3	0 (0)
Endometrial tissue	1	0 (0)
Total	100	12

LN: Lymph node, CSF: Cerebrospinal fluid, TB: Tuberculosis

Table 4: Distribution of LPA results

Biochemical Tests	*n*=12	Percentage
Rifampicin and INH sensitive	9	75
Rifampicin resistant and INH sensitive	1	8.3
Rifampicin sensitive and INH resistant	1	8.3
Rifampicin and INH resistant	1	8.3

INH: Isoniazid, LPA: Line probe assay

rifampicin resistant and INH sensitive, and 1 (8.3%) was rifampicin sensitive and INH resistant (Table 4). Out of the 12 samples, 2 samples (16.7%) were rifampicin resistant indicating MDR-TB. Out of 30 LN samples, 7 (23.3%) were MTB positive; out of 28 pleural fluid samples, 3 (10.7%) were positive; out of 10 bone and synovial fluid samples, 1 (10%) was positive; and out of 6 ascitic fluid samples, 1 (16.6%) was positive. Samples from other sites were negative for MTB (Table 4).

DISCUSSION

TB remains a major global public health problem. It is estimated that about one-third of the world's population is infected with MTB.[14] Extra pulmonary forms have been increasingly reported, accounting for 20-50% of all cases of TB in recent studies.[15-17] ETB remains a challenging diagnosis for both clinicians and microbiologists.[18] Signs and symptoms are most often non-specific, and obtaining material for culture often requires an invasive procedure that cannot be easily repeated.

The early diagnosis of EPTB is challenging because of the paucibacillary nature of these infections, resulting in a very rarely positive smear microscopy finding and a long incubation time required for growth. Hence, procedures such as nucleic acid amplification tests (NAATS) with enhanced sensitivity is required and to be available for diagnosis of EPTB.

In the present study, most of the clinically suspected patients were between the age groups of 21 and 40 years, with male:female ratio of 1.8:1 which correlates with the study of Siddiqui *et al.*,[11] who reported 2.03:1 and Chakravorty *et al.*[19]

In the present study, 9% of the samples were positive for AFB by ZN staining, whereas Siddiqui *et al.*,[11] reported 5% positivity.

MTB growth on LJ medium was positive in 12% of samples which correlates with Siddiqui *et al.*,[11] who reported 15% positivity. Of 9 cases which were positive by microscopy, all 9 (100%) showed growth on LJ medium. In addition, the LJ medium could detect 3 out of 91 (3.3%) cases which were negative by microscopic examination in our study, which correlates with Siddiqui *et al.*[11]

Out of the 9 samples that showed presence of AFB on microscopy all 9 (100%) were positive by LPA (PCR) and out of 12 cases which showed growth on LJ media all 12 (100%) were positive by LPA (PCR) in our study which correlates with Siddiqui *et al.*[11]

In the present study, LNs were the most common site of EPTB, 7 (23.3%) out of 30 cases were positive followed by pleural fluid 3 (10.7%) out of 28 cases and 1 (10%) case out of 10 cases from bone and synovial fluid and 1 (16.6%) case out of 6 cases from ascetic fluid which correlates with Sreeramareddy *et al.*,[20] who reported 16.4% positivity for LN samples, Sharma and Mohan[21] reported 35% for LNs, pleural fluid 20%, bone and joint 10%, and Siddiqui *et al.*[11] reported 17.4% for ascitic fluid.

Out of 12 LPA positive samples 9 (75%) were both rifampicin and INH sensitive 1 (8.3%) sample was both rifampicin and INH resistant, 1 (8.3%) was rifampicin resistant, and INH sensitive, 1 (8.3%) was rifampicin sensitive and INH resistant.

CONCLUSION

EPTB is not given priority earlier in TB control programs in developing countries as the proportion is low and less infectious than PTB.

As the incidence of MDRTB is increasing in PTB, an attempt has been made in this study to detect resistance pattern of isolated cultures by LPA and found that it was 16.7% in extra PTB.

As early diagnosis of EPTB is challenging because of the paucibacillary nature of the infections, procedures such as NAATS with enhanced sensitivity is required and to be available for diagnosis of EPTB.

As the incidence of EPTB is increasing because of HIV and in view of multidrug-resistance, the EPTB in RNTCP programme is enhanced for early diagnosis and treatment to decrease morbidity and mortality.

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