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Original Research Article

Prevalence of *M. Tuberculosis* Complex Isolates amongst Cases of Extrapulmonary Tuberculosis in a Tertiary Care Centre in Central Kerala

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ABSTRACT

Introduction: Extrapulmonary tuberculosis (EPTB) is an important health problem. About 25% of all the tuberculosis cases are manifested as extrapulmonary. This study was aimed to determine the prevalence and drug resistance profile of *Mycobacterium tuberculosis* complex in patients with extrapulmonary tuberculosis attending a tertiary care centre in Central Kerala.

Materials and methods: A prospective study was conducted in Pushpagiri Medical College, Tiruvalla, Kerala from November 2015 to October 2017. During this period, 216 site-specific samples were collected from patients with clinical suspicion of EPTB. Specimens were subjected to Ziehl-Neelsen (Z-N) staining and culture using liquid (BD BACTEC^{TM} Micro MGIT^{TM} {Mycobacteria Growth Indicator tube}) and solid (Lowenstein Jensen) media. Drug susceptibility testing (DST) for streptomycin (S), isoniazid (I), rifampicin (R), ethambutol (E) and pyrazinamide (PZA) were performed on all culture positive isolates using the Micro MGIT.

Results: Out of 216 extrapulmonary samples, *Mycobacterium tuberculosis* complex was isolated in 21 (9.7%) cases by MGIT, 14 (6.4%) by LJ and MGIT while only 4 (1.8%) of the culture-confirmed samples were found to be positive by Z-N staining method. Drug susceptibility testing was done on all the culture positive isolates, of which 7 (33%) were sensitive to all drugs while 3 (14.2%) were multi-drug resistant.

Conclusion: Early diagnosis and timely treatment is of paramount importance for the efficient management of the disease

Keywords: Extrapulmonary tuberculosis (EPTB), MGIT (Mycobacterial growth indicator tube), MDR-TB (Multi-drug resistant tuberculosis).

INTRODUCTION

Tuberculosis (TB) remains a global health problem and is the seventh leading cause of death worldwide. [1] In the year 2017, globally there were 10.4 million incident cases of tuberculosis reported and in India, there were 2.8 million cases accounting for a quarter of the world's tuberculosis cases. [2] Tuberculosis can affect almost any part of the body and

although pulmonary is the most common presentation, extrapulmonary disease is not rare. [3] Worldwide, the burden of Extrapulmonary tuberculosis (EPTB) is high, ranging from 15-20 per cent of all TB cases in HIV-negative patients, while in HIV-positive people, it accounts for 40-50 per cent of new TB cases. [3] In India, EPTB accounts for approximately 15-20% of all types of tuberculosis. [3] Most common forms are lymph node TB (35%) followed by pleural (20%), bone (10%) and genitourinary (9%). Cerebrospinal, abdominal, skin accounts for remaining 26%. [4]

Diagnosis of EPTB remains challenging because clinical samples obtained from relatively inaccessible sites may be paucibacillary, thereby decreasing the sensitivity of diagnostic tests and also due to the atypical presentation of the illness. [5] The timely detection and accurate diagnosis of any form of EPTB are necessary for the proper treatment of EPTB. The diagnostic delay may affect the treatment of the patients and lack of appropriate therapy leading to the development of drug resistance even in extrapulmonary tuberculosis.

To the best of our knowledge, the data on the prevalence of extrapulmonary tuberculosis in the central part of Kerala is limited. This study was thus designed to determine the prevalence and drug resistance profile of *Mycobacterium tuberculosis* complex in patients with extrapulmonary tuberculosis attending a tertiary care centre in Central Kerala.

MATERIALS AND METHODS

Study design

A cross-sectional study was undertaken in the Department of Microbiology, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla from November 2015 to October 2017. A total of 216 samples were collected from patients with clinical suspicion of EPTB in this study. There were 81 pus or aspirates, 43 tissue biopsies, 26 pleural fluids, 19 urine specimens, 17 cerebrospinal fluid, 11 lymph node aspirates and 19 other fluids such as peritoneal fluid, ascitic fluid.

Sample processing

All specimens were subjected for direct smear microscopy using Ziehl-Neelsen (ZN) staining method. Non-sterile samples like pus, aspirates and other mucopurulent specimens were processed using N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) digestion, decontamination technique. One drop of the specimen after decontamination was used for smear preparation. Sterile body fluids like cerebrospinal fluid were inoculated directly without the decontamination process. The BBL Mycobacteria Growth Indicator Tube (MGIT BD BACTECTM) tube containing 7 ml modified Middle Brook 7H9 broth was used as a culture medium, to which an enrichment supplement, as well as a mixture of antibiotics consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin, were added. Five hundred µl of processed specimens were inoculated into BBL MGIT tube as per the manufacturer's protocol. The BBL MGIT tubes were incubated at 37°C and examined daily in a 365nm wavelength UV light source fluorescence detector (Bactec TM MicroMGIT device) up to 8 weeks of incubation. A positive and a negative control tube were included with every batch of test tubes examined to validate the decontamination process. Tubes were checked daily using microMGIT for the presence of fluorescence which indicates the presence of growth. All positive tubes were confirmed for the presence of acid-fast bacilli by Ziehl Neelsen (ZN) staining and were subcultured on to blood agar plate to rule out contamination. The smears were observed for the presence of acid-fast bacilli and for the presence of cording which is a characteristic feature of *M. tuberculosis* complex. *M. tuberculosis* complex isolates were are further confirmed using BD MGIT MTBc identification test (TBc ID). This is a rapid chromatographic immunoassay for the

qualitative detection of *M. tuberculosis* complex antigen from AFB smear-positive BD MGIT tubes. The assay was performed according to the manufacturer's instructions.

Simultaneously LJ slants were inoculated with the 0.1 ml of the processed sample and were incubated at 37°C for a maximum of eight weeks. They were checked twice weekly for first two weeks and then once every week for a maximum period of eight weeks.

The confirmed *M. tuberculosis complex* isolates were further subjected to drug susceptibility testing using the drugs streptomycin (S), isoniazid (I), rifampicin (R), ethambutol (E) and also pyrazinamide (PZA) BD BACTEC MGIT 960 SIRE kits were used for drug susceptibility testing. For pyrazinamide, separate PZA medium tube containing 7 ml of broth was used. Drugs were diluted with sterile distilled water to obtain the critical concentration of 1.0 µg/ml for streptomycin, 0.1 µg/ml for isoniazid, 1.0 µg/ml of rifampicin, 5.0 µg/ml of ethambutol and 100 µg/ml of pyrazinamide. Susceptibility testing was performed according to the protocol provided by the manufacturer. For each sample, five drug-containing tubes and one control tube without drug were inoculated. The tubes were incubated at 37°C and were examined daily for fluorescence with a 365-nm wavelength light. The results were interpreted only after the growth control tube fluoresced.

RESULTS

Among 216 extrapulmonary specimens, *M tuberculosis* complex was found to be positive in 21 (9.7%) cases by any one of the three methods i.e. MGIT, LJ and ZN stain (Table 1). All of these were culture positive by microMGIT showing the highest recovery rate (9.7%). Among the 21 culture positive specimens, smear positivity was seen only in four specimens i.e., in 1.8% of the samples. The present study showed that *M. tuberculosis* complex was most commonly isolated from lymph nodes, (36.3%) followed by pus or aspirates (12.3%), urine (10.5%), pleural fluid (7.6%) and tissue biopsies (4.6%) (Table 1). The mean time to detection of growth by Micro MGIT was 23.3 days and 35 days by the L.J medium.

Table: 1 Comparison of detection by different methods among extrapulmonary tuberculosis

Sample	Positive by		
	Z-N stain	MGIT	LJ medium
Pus/Aspirate n=81	3 (3.7%)	10 (12.3%)	8 (9.8%)
Tissue/biopsy n=43	0 (0%)	2 (4.6%)	1 (2.3%)
Pleural fluid n=26	0 (0%)	2 (7.6%)	1 (3.8%)
Urine n=19	0 (0%)	2 (10.5%)	1 (5.2%)
CSF n=17	0 (0%)	0 (0%)	0 (0%)
Lymph node n=11	1 (9.0%)	4 (36.3 %)	2 (18.1%)
Other fluids n=19	0 (0%)	1 (5.2)	1 (5.2%)
Total n=216	4 (1.8%)	21 (9.7%)	14 (6.4%)

Antimycobacterial susceptibility testing of 21 *M tuberculosis* complex isolates was done (Table 2).

Table 2: Drug susceptibility pattern of *M. tuberculosis* complex in Extrapulmonary tuberculosis cases

Pattern of drug resistance	Number (%) N=21
Sensitive to all five drugs	8 (38%)
Resistance to	
To all five drugs	1 (4.8 %)
Streptomycin only	1 (4.8%)
Isoniazid only	0
Rifampicin only	0
Ethambutol only	0
Pyrazinamide only	6 (28.5%)
Streptomycin & Isoniazid	1 (4.8%)
Isoniazid & Pyrazinamide	1 (4.8%)
MDR	3 (14.3%)

Eight isolates (38%) were sensitive to all the five anti-tuberculous (ATT) first-line drugs. Seven isolates (33.3%) showed mono-resistance and two isolates (9.5%) showed dual resistance to Isoniazid + Streptomycin and Isoniazid+Pyrazinamide. PZA resistance was seen in the maximum number of isolates 6(28.5%) followed by S resistance in 1 (4.8%). None of the strains showed monoresistance to rifampicin, isoniazid and ethambutol. Three (14.3%) of the isolates showed multi-drug resistance (resistance to I and R).

DISCUSSION

Tuberculosis is one of the oldest encountered diseases of mankind. Although the incidence of pulmonary tuberculosis is showing a downtrend, [6] the incidence of extrapulmonary tuberculosis has remained

steady. [6] This is mainly due to the fact that extrapulmonary tuberculosis manifests with a variable clinical picture and obscure inaccessible sites making diagnosis difficult. [6]

The percentage of EPTB cases estimated in India is between 15-20% according to Revised National Tuberculosis Control Programme (RNTCP). [3] In our study, of the 216 patients evaluated, 21 (9.6%) were confirmed to have EPTB by culture. These findings are similar to the studies conducted in various parts of India like Varanasi and Tamil Nadu where the positivity rate was 11.6 % and 10.6% respectively. [7,8] In contrast to this, a study conducted by Gaur PS *et al.*, in Lucknow, North India showed a higher prevalence rate of 45.6 %. [9] The reasons for these differences could be related to different epidemiological patterns and the difficulties in EPTB diagnosis and the lack of access to adequate diagnostic infrastructure. [10]

The diagnostic techniques used in this study for the detection of EPTB included Ziehl Neelsen staining, liquid culture using BACTEC^{TM} Micro MGIT^{TM} and solid culture with Lowenstein Jensens medium. All the specimens were screened initially for the presence of acid-fast bacilli by ZN staining. Even though smear microscopy is a simple, cheap and quick method of detecting acid-fast bacilli in tissues and smears, it has limited diagnostic value in extrapulmonary specimens due to the paucibacillary nature of the disease and also requires the presence of more than 10^6 bacteria/g tissue. [11] Also, smear microscopic methods are unable to detect drug resistance. [12] Overall smear positivity of direct smears in this study was 1.8%. In a study conducted by S. Chakravorty et al., 2005 the smear positivity detected was 3.9%. [13]

The present study demonstrated that Micro MGIT system provided better isolation rate of mycobacteria i.e., 21/216 (9.7%) from the clinical samples compared to solid media which yielded only 14 (6.4%). In our study, considering culture as the gold standard, we obtained higher isolation rates with liquid culture, 100%, amongst the total positive specimens (21/21) by MGIT and 66.6% were positive with LJ (14/21). This is similar to the observations made by Chien *et al.*,2000 where the recovery rates were 94% (117/124) with BACTEC MGIT 960 and 75.8% (94/124) with LJ. [14] The reason for the high recovery rate in MGIT system is due to the fact that it is a liquid based system hence bacteria can grow and spread more easily in liquid media than solid media. [15] Thus, MGIT was found to be most rapid and efficient system to isolate *M. tuberculosis* complex. [16]

Besides higher rates of isolation, the mean time to detection of growth by Micro MGIT was shorter (23.3 days) when compared to L.J medium (35 days). This is similar to the previously reported studies where the time to detection for the MGIT system was 3-40 days (mean 14.2) compared with 8-42 days for LJ (mean 23.2). [16] This faster detection time would enable clinicians to establish a definitive diagnosis, and institute appropriate therapy within the shortest possible time. [11]

Higher contamination rate is the major drawback of the liquid culture system but in this study, the contamination rate was found to be lower with MGIT system (2.3%) as compared to LJ medium (4.1%). This is in concordance with the results by Saini *et al.*, 2017, [17] where the contamination rate of BACTEC MGIT 960 system (3%) was less as compared to the solid medium (8%). The reasons for the lower rates of contamination may be mainly due to the experienced hands of laboratory personnel, better disinfection protocols and the use of antibiotic mixture in the liquid medium and also due to the use of the N-acetyl L-cysteine sodium hydroxide (NALC-NaOH) method. [17]

Various studies have suggested that the sites of EPTB may vary according to geographic location, population groups and a wide variety of host factors. [18] In our study the most common site of EPTB was

lymph nodes (36.3%), followed by pus aspirates (12.3%), urine (10.5%), pleural fluid (7.6%) and tissue biopsies (4.6%). These results are comparable with studies from India which states that India and other developing countries, where lymph node tuberculosis continues to be the most common form of EPTB. [3] Reports have shown that Asians and Hispanic patients and African American also seem to have a high predilection for developing mycobacterial lymphadenitis. [3]

Drug susceptibility testing was performed on the 21 *Mycobacterium tuberculosis* complex isolates. It was found that eight (38%) isolates were sensitive to all five drugs. Similar observations from North India (Sunil Sethi *et al.*, 2012) reported 47.5% pan-sensitive isolates. [19] In the present study, the overall resistance of 61.9% to one or more drugs was observed in EPTB isolates. This is similar to the observations made by the NIRT, 2015 (National Institute of Research in Tuberculosis), Chennai, Tamil Nadu, where 59% resistance was observed. [20] From Puducherry and surrounding Tamil Nadu, South India overall resistance to one or more drug reported was 28.1%. [8] Though increasing resistance to EPTB has been reported in India, Korea (Lee *et al.*, 2015) has reported no significant difference in resistance among the pulmonary and extrapulmonary tuberculosis cases. [5]

In our study, we had a prevalence of 14.3% multi-drug resistant (MDR) isolates in EPTB. Though few data are available on the drug resistance patterns of EPTB in India, previous studies have reported 10% MDR- EPTB in Delhi and 27.5% in Varanasi. [7] Studies conducted in NIRT, Chennai in 2015 showed an MDR-EPTB prevalence of 19%. [20] Studies from Nepal have reported 12.5% MDR-EPTB. [7] In patients with extrapulmonary manifestations, the diagnosis is usually based upon clinical, radiological or histopathological findings, rather than on bacteriologic evidences. [21] Therefore, there are very few reports available on drug susceptibility patterns of EPTB as these patients are usually not included in drug resistance surveys, which focus mainly on pulmonary TB. [21] Usually, drug sensitivity testing is done only if there is no response to the first line therapy, leading to considerable loss of time in the administration of appropriate treatment. An important finding of the present study was the high rate of MDRTB in EPTB cases and thereby the need for drug-susceptible testing at the time of diagnosis especially in a tertiary care setting likes ours.

CONCLUSION

There is an overall increase in the incidence of EPTB contributing to the overall burden of Tuberculosis in developing countries like India. Extrapulmonary tuberculosis (EPTB) is frequently a diagnostic and therapeutic challenge; therefore, timely diagnosis and initiation of appropriate therapy are crucial. The alarming increase in drug resistance in EPTB cases necessitate drug susceptibility testing of all EPTB cases. A standardized surveillance programme for the better understanding of the problem of drug resistance in EPTB is the need of the hour.

Conflict of interest- Nil

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