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ORIGINAL ARTICLE

Isolation and characterization of *Mycobacterium tuberculosis* from extra pulmonary specimens in a tertiary referral hospital of north India

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ABSTRACT

Background: Tuberculosis is one of the major public health challenges globally. Extra pulmonary tuberculosis (EPTB) is defined as an isolated occurrence of TB at the body sites other than lungs. The aim of this study was to see the frequency of isolation of mycobacterium from different EPTB samples.

Materials and Methods: A total of 510 different extra pulmonary samples were collected during the period of August 2014 to July 2015. All the samples were subjected to Ziehl Neelsen staining. Samples were decontaminated by NALC-NaOH method and inoculated on a pair of Lowenstein-Jensen (LJ) media and one p-Nitrobenzoic acid (PNB). Cultures were incubated at 37°C and observed for 8weeks for any growth.

Results: Mycobacteria were isolated from 60 samples of which the highest number of isolates was obtained from pus samples. Upon biochemical characterization, 6 isolates were characterized as non tuberculous mycobacteria. The frequency of isolation was 11.76%.

Conclusion: Our result suggests that due attention and emphasis should be given on diagnosis and management of EPTB.

INTRODUCTION

Tuberculosis (TB), a specific human disease caused by *Mycobacterium tuberculosis*, has emerged as the greatest killer worldwide due to a single infectious agent alongside HIV/AIDS[1]. There were 9.0 million new TB cases in 2013 and 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people) as reported by WHO in the year 2014[1]. The most common manifestation of the disease is pulmonary TB but extra pulmonary tuberculosis (EPTB) can't be overlooked as it accounts for 15-20 per cent of all the cases of tuberculosis. This percentage is much higher in HIV-positive patients, where EPTB accounts for more than 50 per cent of all cases of TB [2].

The term EPTB refers to isolated occurrence of tuberculosis at body sites other than lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints bones and meninges[3]. The non-specific clinical presentation of the disease makes the diagnosis complicated. In resource limited settings the diagnosis of EPTB is based on AFB and Lowenstein-Jensen (LJ) culture. Furthermore the paucibacillary nature of specimens often leads to the low

sensitivity of AFB smear. The LJ culture positivity is found to be 12% to 80% in different extra pulmonary samples however it is considered as gold standard for diagnosis of tuberculosis[4]. Diagnostic delay may affect the treatment of the patients and due to lack of appropriate therapy drug resistance develops even in extra pulmonary tuberculosis[5].

The data for prevalence of a disease is an essential measure for studying the epidemiology. Very few studies have been done on EPTB which is not sufficient to figure out the exact data of the disease from a high TB burden country like India. In this regard we designed our study to see the prevalence of EPTB among the patients of north India attending a tertiary care center of Varanasi.

MATERIALS AND METHODS

Ethics statement

This study has been ethically approved by the institute ethical committee of Institute of Medical Sciences (ECR/526/Inst/UP/2014), Banaras Hindu University, Varanasi. The data used in this study were collected as part of routine medical care in the hospital clinical chart. The given ethics committee approved the study and waived the need for written consent since all data used were previously collected during the course of routine medical care and did not pose any additional risks to the patients.

Study design

This study was undertaken in the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Extra pulmonary samples were collected from patients attending indoor and outdoor facility of Sir Sunderlal hospital during the period of August 2014 to July 2015. Patients were included in the study based upon their clinical symptoms like fever, night sweats, fatigue, loss of appetite, weight loss along with complaints specific to the body site. A total of 510 different extra pulmonary samples were analyzed during the period. Single sample was collected from each patient. The sample distribution was pus (304), gastric aspirate (91), pleural fluid (62), cerebrospinal fluid (18), urine (14), knee aspirate (4), fine needle aspirates (4), bone marrow (6), cold abscess (3), ascitic fluid (4).

Specimen collection and processing

All the samples were subjected to microscopy prior to decontamination. Smears were stained by the Ziehl Neelsen (ZN) method and examined for acid fast bacilli (AFB). Pus, gastric aspirate and other mucopurulent specimens were decontaminated by N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) method. In this procedure, the initial concentration of NaOH was 4%. This 4% NaOH solution was mixed with an equal quantity of sodium citrate solution (2.9%) to make a working solution (NaOH concentration in this solution is 2%).

When an equal quantity of NaOH-NALC-citrate and sample are mixed, the final concentration of NaOH in the specimen was 1%.The sediments were resuspended in 1-2 ml PBS and inoculated on a pair of LJ media and one p-Nitrobenzoic acid (PNB). Urine samples were concentrated by centrifugation in 50 ml screw capped centrifuge tubes for 20-25 minutes.

The sediments were resuspended in 5 ml sterile water and then decontaminated with NALC-NaOH method. As the body fluids like CSF, knee aspirate, pleural fluid, fine needle aspirates, bone marrow and ascitic fluid were collected aseptically they were inoculated directly on LJ media[6]. Cultures were incubated and observed for 8 weeks. No growth after 8 weeks of incubation was considered as negative.

Identification of isolates

The isolates were first identified by colony morphology, microscopic examination, sensitivity to p- Nitrobenzoic acid (PNB) and Further biochemical characterization was done by heat stable catalase test and nitrate reductase assay.

RESULTS

Out of 510 samples analyzed 318 were from males and 192 were from females with a mean age of 24 years. The overall positivity observed through Z-N staining and LJ culture was 9.60% (49) and 11.76% (60) respectively. Of 60 isolates 54 were characterized as MTB and 6 as atypical mycobacteria. The number of pus samples was highest among all non-sputum samples processed. Out of 304 pus samples processed 28 turned smear positive and 29 were found to be culture positive. Three cold abscess were processed but none of them turned positive either for smear or LJ culture. The sample distribution and results of culture and smear is enlisted in table 1.

Table 1: Sample distribution and result of culture and microscopy

Sample	No of cases	Z-N smear (%)	Culture (%)
Pus	304	28 (9.21)	29 (9.5)
Gastric aspirate	91	9 (9.89)	16 (17.58)
Pleural fluid	62	5 (8.06)	7 (11.29)
Cerebrospinal fluid	18	1 (5.5)	1 (5.5)
Urine	14	1 (7.14)	1 (7.14)
Fine needle aspirate	4	1 (25)	1 (25)
Ascitic fluid	4	2 (50)	3 (75)
Knee aspirate	4	1 (25)	1 (25)
Cold abscess	3	0 (0)	0 (0)
Bone marrow	6	1 (16.66)	1 (16.66)
Total	510	49 (9.60)	60 (11.76)

DISCUSSION

Non-specific clinical presentation of extra pulmonary tuberculosis often fails to make definitive diagnosis of the disease. Hence laboratory based diagnostic methods are required. Culture is considered as gold standard for the diagnosis of tuberculosis. In our study we reported an isolation rate of 11.6% from 510 extra pulmonary samples which is in affirmation with the previous reports from Nepal and India[7, 8]. A higher positivity of 30.1% was reported by Maurya *et al* which is probably due to use of liquid culture[9].

Out of 510 different extra pulmonary samples collected the highest number was of pus samples (304) and a positivity of 9.21% and 9.5% was obtained for ZN smear and culture respectively, while other reports have suggested the highest positivity from lymph node biopsy or aspirates[10]. Six atypical mycobacterium were obtained in our study. Out of these six 2 each were isolated from pus sample and gastric aspirate. One was isolated from urine and one from pleural fluid. Three samples of cold abscess were collected but none of them resulted in positive on culture. We got one positive culture each from bone marrow, knee aspirate, fine needle aspirate and cerebrospinal fluid.

The limitation of this study is lack of clinical history and histo pathological data of the patients due to which the factors associated with the predisposition of the disease may be missed. Furthermore the use of liquid media could have been improved the isolation rate, as a higher rate of positivity has been observed in case of liquid culture[11].

Other molecular techniques such as Xpert MTB/RIF is widely being used for the diagnosis of EPTB but some reports have suggested a need for reevaluation of this technique due to its false positivity in some cases[12].

In conclusion, we got a significant number of MTB isolated from extra pulmonary cases of our tertiary care center which suggests that this form of TB has also reached an alarming level. The contagious potential of extra pulmonary tuberculosis is negligible, therefore it is often not considered with priorities in national tuberculosis control program[7]. As the burden of tuberculosis in India is highest which accounts for 21% of total global incidence this uncommon form of this tuberculosis cannot be overlooked and due attention on the patients of extra pulmonary tuberculosis should be given[13].

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Competing interests

The authors declare that they have no competing interests.

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