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Original article

Extrapulmonary tuberculosis: comparative analysis of diagnostic modalities used in a tertiary healthcare setting

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ABSTRACT

Background and Aims - Abstract Introduction: Tuberculosis caused by the Mycobacterium tuberculosis complex is one of the oldest diseases known to affect humans and it is a major cause of death worldwide; it usually affects the lungs, although other organs are involved in up to one-third of cases. The diagnosis of extra-pulmonary tuberculosis is often missed or misdiagnosed due to its atypical presentations. Aim: The present study aims to evaluate the utility of all the available techniques used in the diagnosis of extrapulmonary tuberculosis in the department of microbiology of a tertiary care hospital in Mumbai. Materials and Methods: The study included 331 clinically suspected cases of extrapulmonary tuberculosis from outpatient department and wards of a tertiary care Hospital. Clinical specimens (body fluids and tissue specimens) were subjected to Ziehl-Neelsen (Z-N) staining, Amplified Mycobacterial Tuberculosis Direct Test (AMDT) and culture by conventional method for mycobacteria. Results: Out of 331 samples processed, 5.7% of specimens showed AFB on Z-N staining and 14.5% were positive by AMDT. The overall sensitivity and specificity (taking culture as gold standard) showed by AMDT was 84% and 95% respectively, whereas Z-N staining showed sensitivity of 35% and specificity of 96%. Conclusion: Although AMDT is validated only for pulmonary samples, it can be used as a sensitive tool for rapid diagnosis of extrapulmonary tuberculosis.

Introduction

Tuberculosis is the cause of significant morbidity and mortality worldwide. Tuberculosis can involve any organ system in the body. While pulmonary tuberculosis is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical problem [1].

The extrapulmonary sites most commonly involved in tuberculosis are the lymph nodes, pleura, genitourinary tract, bones and joints, meninges, peritoneum, and pericardium. However, virtually all organ systems may be affected [2,3,4]. The diagnosis of extra-pulmonary tuberculosis is often missed or misdiagnosed due to its atypical presentations [5].

Bacteriological diagnosis of extrapulmonary tuberculosis is currently based on acid-fast staining and culture on solid and/or liquid media. Detection by microscopy is useful as a rapid screening test, but lacks sensitivity[6]. Culture on solid media can take up to 8 weeks to yield a positive result.

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The liquid culture systems has been an important addition to culture methods, although this technique requires an average of 13 to 15 days to detect positive specimens[7,8,9]. More recently, commercially available standardized nucleic acid-based amplification techniques have shown reliable results within 5 to 7 hrs of sample processing [10.]

The Amplified Mycobacterium Tuberculosis Direct test (AMDT) is an isothermal transcription-mediated amplification system based on the reverse transcription of mycobacterial- specific rRNA targets, followed by transcription of the DNA intermediate template. Subsequent detection of RNA amplicons is through a hybridization protection assay using an acridinium ester-labeled M. tuberculosis complex-specific DNA probe.

Hence the present study was undertaken to evaluate the efficacy of Amplified Mycobacterial Tuberculosis Direct Test-AMDT in rapid identification of mycobacterial species from extrapulmonary specimens.

Materials and Methods

This was a prospective study carried out in the microbiology department of a tertiary health care centre. The study protocol was approved by the Institutional Ethics Committee.

Specimens: During the study period (from Jan 2011 to June 2012), 331 specimens of clinically suspected cases of extrapulmonary tuberculosis from outpatient department and wards of tertiary care centre were obtained. Out of these 331 nonrespiratory specimens, 231 were body fluids (comprised of 158 pleural effusions, 14 ascitic fluids, 2 synovial fluids, and 57 pus or miscellaneous cystic fluids), and 46 tissue specimens (including 18 lymph node samples and 28biopsy/FNAC samples).

All these samples were initially subjected to microscopy by Zeihl-Neelson staining, then after digestion-decontamination process by the N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH)method, specimen concentrates were subjected to conventional culture method on Lowenstein-Jenesen medium and Amplified Mycobacterium Tuberculosis Direct Test (AMDT, Gen-Probe Inc.,San Diego, California).

For culture, two L-J media were inoculated per specimen and were observed for any growth every week and results were available within 8 to 10 weeks.

The AMDT test was performed according to the manufacturer's instructions. A portion (450 µl) of the sediment from the sodium hydroxide-treated specimen was placed in a tube containing lysing solution, vortexed, and sonicated for 15 min. A 0.25 µl volume of lysate was transferred into an amplification tube containing amplification reagent. Tubes were incubated for 15 min at 95°C and cooled at 42°C for 5 min. Enzyme reagent (25 µl) was added to each tube and mixed, and the mixture was incubated for 45 min at 42°C. Hybridization reagent (100 µl) was added to each tube, vortexed, and incubated for 15 min at 60°C. Tubes were removed, 300 µl of selection reagent was added, and the mixture was vortexed and incubated for 15 min at 60°C. All temperature-controlled incubation steps were carried out in heating blocks. Prior to being read in a luminometer (LEADER 50,Gen-Probe) the tubes were cooled at room temperature for 5 min. All runs included MTD amplification-positive and-negative controls. The cut off value was set at 30,000 relative light units (RLU). Samples with values of> 30,000 RLU were considered positive; samples with values <30,000 RLU were considered negative [11].

Results

Out of 331 samples processed, 19 specimens showed AFB on Z-N staining and 48 were positive by AMDT while 32 specimens were culture positive. Considering culture as gold standard, sensitivity and specificity of both the techniques (Z-N staining and AMDT) are shown in **table 1.**

Test	Sensitivity	Specificity	PPV	NPV
Z-N Staining	35%	96%	55%	93%
AMDT	84%	95%	66%	98%

PPV:Positive predictive value, NPV: Negative predictive value

There were total 19 discrepancies in results of AMDT and Culture (15 false positive and 4 false negative). They are illustrated in **table 2**

Type of specimen	No. of specimen	Smear result	Culture result	AMDT result	Interpretation of AMDT
Pleural fluid	2	Negative	Positive	Negative	FN
Pleural fluid	3	Negative	Negative	Positive	FP
Pus	3	Negative	Negative	Positive	FP
Pus	2	Negative	Positive	Negative	FN
FNAC	1	Positive	Negative	Positive	FP
FNAC	3	Negative	Negative	Positive	FP
Tissue	1	Negative	Negative	Positive	FP
Lymph Node	2	Negative	Negative	Positive	FP
Lymph Node	1	Positive	Negative	Positive	FP
Endometrium	1	Negative	Negative	Positive	FP

FP:False positive, FN: False Negative

Discussion

One feature of the mycobacteria belonging to the M. tuberculosis complex which delays detection in cultures is the slow division time, about 20 h under the best culture conditions. When a clinical specimen is smear negative, it takes several weeks for the culture to become positive, even when the most appropriate culture techniques are used. To provide physicians with accurate and rapid bacteriological results, it is necessary to shorten the delay in reporting the presence of M. tuberculosis in clinical specimens. In the present study the sensitivity and specificity of the AMTD test were assessed relative to positive culture for M. tuberculosis in the setting of a routine tertiary care center.

It was found that there were 19 discrepancies between AMDT and culture. Clinical histories of patients were reviewed, and out of 15 false positive results, 11 patients were on antituberculous drugs. The latter two discrepancies could not be resolved. Perhaps the organisms could have caused the false-positive results. Four false-negative test results were seen during the study. As the AMDT detects mycobacteria belonging to M. tuberculosis complex only, the false negative results were attributed to infection caused by non-tuberculous mycobacteria. This was also confirmed by subsequent identification of mycobacterial isolates.

Conclusion

For the diagnosis of extra pulmonary tuberculosis, Z-N staining can serve as a rapid tool in resource poor settings but it lacks sensitivity whereas AMDT is rapid as well as sensitive technique. Culture remains the gold standard method.

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