Original Article

Evaluation of rapid immuno-chromatographic test to differentiate between mycobacterium tuberculosis complex and nontuberculous mycobacteria

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Abstract

Background: Strategies used for the clinical management of patients with Mycobacterium tuberculosis complex (MTBC) and Nontuberculous Mycobacteria (NTM) are different, therefore, prompt detection, isolation, and discrimination is necessary for suitable management. MPT64 TB Ag test was found to be a rapid immunochromatographic test for their differentiation. The aim of the present was to evaluate a commercial assay, SD TB Ag MPT64 Rapid for characterization of Mycobacteria isolated on Lowenstein Jensen (L-J) medium. **Material and Methods:** A total of100 isolates recovered from both pulmonary (50 isolates) as well as extra pulmonary specimens (50 isolates) were characterized as MTBC or NTM based on standard phenotypic characteristics and biochemical tests. These results were compared with commercial SD TB Ag MPT64 Rapid test. **Results:** Among 100 mycobacterial isolates, 98 isolates were identified as MTBC and the two isolates from extra pulmonary cases were identified as NTM on the basis of biochemical tests. There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test. **Conclusion:** The sensitivity and specificity of TB Antigen MPT64 rapid test was 100% as compared to biochemical methods. The MPT64 TB Ag test was found to be a simple, rapid and reliable test to differentiate MTBCfrom NTM.

Keywords: Tuberculosis, MPT 64, Mycobacterial characterization, sensitivity, specificity.

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INTRODUCTION

Tuberculosis remains a major public health problem despite noteworthy socio-economic development, advances and availability of technology[1]. The capacity of the laboratories to perform a rapid culture, identification and differentiation of *M. tuberculosis*complex (MTBC) from non-tuberculous mycobacteria (NTM) is vital in the management of tuberculosis patients.NTM are in herently resistant to conventional anti-tuberculosis drugs, require modified treatment regimens and are often misdiagnosed as multidrug-resistant tuberculosis[2]. Strategies used for the clinical management of patients with MTBC and NTM are different, therefore, prompt detection, isolation, and discrimination is necessary for suitable management. Conventional biochemical methods can identify mycobacterial species; but these are laborious, time-consuming and error-prone. Although molecular methods of identification are reliable, but require a specialized set up, sophisticated and expensive equipment, trained laboratory personnel and are expensive for resource-poor countries. The *M. tuberculosis* protein 64 (MPT64) is a *M. tuberculosis*

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complex specific antigen secreted during the bacterial growth, and is an excellent antigen for the identification of MTBC[3]. Studies have revealed that the MPT 64 is specific for MTBC[4]. This study was carried out to evaluate clinical usefulness of immunochromatographic test (ICT) kit based on mouse monoclonal anti-MPT 64 for discrimination between MTBC and NTM in clinical isolates from patients with pulmonary and extra-pulmonary tuberculosis.

MATERIAL AND METHODS

In this prospective study, a total of 100 mycobacterial isolates on Lowenstein-Jensen (L-J) medium recovered from both pulmonary (50 isolates) as well as extra pulmonary specimens (50 isolates) from adult patients were included. The isolates were confirmed by Ziehl - Neelsen (ZN) staining. The positive cultures were screened to differentiate the growth of mycobacteria into MTBC and NTM by niacin accumulation test, nitrate reduction test, heat stable catalase at 68^0C/pH7 and Para-nitrobenzonic acid (PNB) susceptibility test[5]. The cultures which were not identified as MTBC and suspected to be NTM were further identified by rate of growth, pigment production, Urease test, Tween – 80 hydrolysis test, Arylsulfatase test, Mac Conkey agar test and Sodium Chloride tolerance test[5]. All the cultures were also subjected to TB Antigen MPT64 rapid test for differentiation into MTBC and NTM. Briefly, 3-4 colonies from L-J were emulsified in 200 µl of extraction buffer. 100 µl of suspended solid culture in buffer was added into the sample well. The inoculated cassettes were kept undisturbed atroom temperature and were examined at the end of15 minutes for presence of pink band in "Control" and "Test" region[6]. The appearance of control band confirmed the validity of the test. If the control band was not visible in 15 minutes, the result was considered invalid and the sample was retested. The presence of only control band in the absence of test band was considered a negative test. Presence of both control and test band indicated a positive test.

RESULTS

One hundred mycobacterial isolates on L-J media were included in the present study and demographic details of these patients were recorded. Maximum number of patients belonged to 16- 45 years (83%) of age group followed by 46-60 years (16%) of age group in both pulmonary and extrapulmonary cases. There were 53 males and 47 females with male predominance in extra pulmonary cases (M:F=1.27:1). The pus (60%) was most common specimen followed by pleural fluid (14%) and lymph node aspirate (12%) amongst extrapulmonary specimens. Pleural involvement (32%; pleural effusion and empyema thoracis) followed by lymph node involvement (18%) was most commonly seen in extrapulmonary cases. Other sites (16%) included axillary, mastoid and submandibular abscesses. Most common symptom in pulmonary cases was cough (92%) followed by fever (74%) and weight loss (64%) while in extrapulmonary cases most common symptom was fever (44%) followed by lymphyadenopathy (36%). Out of 50 culture positive pulmonary specimens, only 23 (46%) were positive on primary ZN smear. Whereas, among 50 culture positive extrapulmonary specimens, only 18 (36%) were primary ZN smear positive. All the isolates from pulmonary cases (n=50) were positive for niacin and nitrate reduction test while negative for catalase and PNB test. In extrapulmonary isolates, 48 were positive for niacin test, negative for catalase and PNB test while 49 were positive for nitrate reduction test. The two isolates from extrapulmonary cases were positive for PNB test. Therefore, all the 98 isolates were identified as MTBC and the two isolates from extrapulmonary cases identified as NTM on the basis of biochemical tests. These two NTM isolates from extrapulmonary specimens were further identified as *Mycobacterium fortuitum* and *Mycobacterium chelonae* on the basis of biochemical tests. These isolates belong to Group IV (Rapid Growers) of Runyon's Classification. When subjected to TB Ag MPT64 Rapid test, the control band was seen in all the tested cultures (n=100), validating the test. All the 50 isolates from pulmonary cases and 48 isolates from extrapulmonary cases showed positive results (visible band for MPT64 antigen) and the two isolates from extrapulmonary cases gave negative results. There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test. The sensitivity and specificity of TB Antigen MPT64 rapid test was found to be 100% as compared to biochemical methods.

DISCUSSION

In the present study, majority of patients (83%) belonged to 16 – 45 years of age group in both pulmonary and extrapulmonary cases with slight male predominance (M:F = 1.27:1) seen in extrapulmonary cases. Similar demographic profile of patients was found in a study by Sivasankari et al[7]. Pus (60%) was most common specimen and lymph node aspirate (12%) amongst extra-pulmonary specimens in the present study. Previous studies also found pus as the most common specimen followed by lymph node[8-12]. In the present study, most common symptom in pulmonary cases was cough (92%) followed by fever (74%) and weight loss (64%) while extrapulmonary cases showed fever (44%) as most common symptom followed by lymphyadenopathy

(36%).Sharma *et al*[13] also reported fever (98%) and cough (95%) as most common symptom in pulmonary tuberculosis. Whereas Koshti *et al*[14] documented cough (66.6%) as most common symptom in pulmonary cases while fever (86.8%) followed by weight loss (58.1%) in extra-pulmonary cases. In the present study, 46% and 36% of Z-N smear positivity was seen in pulmonary and extrapulmonary cases respectively. A study published by Selvakumar et al, reported ZN smear sensitivity of 47% from liquefied sputum samples[15]. It was found that the sensitivity of extra-pulmonary smears was low as compared to the smears from pulmonary specimens. Paucibacillary nature of disease, inadequate sample or too long storage of sample may result in low smear positivity in extra pulmonary specimens. In the present study, 98% isolates were found *M.tuberculosis* complex while only 2% were NTM. Both the NTM were isolated from extrapulmonary tuberculosis cases. There was no NTM isolated from pulmonary tuberculosis cases. MTBC was found to be the most common isolate than NTM in previous studies. In developing countries like India, more than 90% of tuberculosis infections are still caused by *M. tuberculosis*. In a scenario of NTM disease prevalence and increasing drug resistance in MTB, speciation and drug susceptibility testing havebecome a necessity today for appropriate patient management. There is a need for a rapid, simple yet accurate test for differentiation of mycobacteria. The major attraction for using MPT64 TB Ag test was its claim to characterize mycobacterial isolates accurately in 15 minutes with a low cost(Rs. 125/- per test). This assay was evaluated for rapid characterization of 100 cultureisolates and whether it could replace the tedious conventional phenotypic methods. An important observation of this study was 100% specificity and sensitivity for the assay. Other studies have also demonstrated specificity of 100% and sensitivity ranging from 96.5% to 100%[4,16,17]. To conclude, TB Antigen MPT64 test was found to be rapid, easy to perform and cost effective with high sensitivity and specificity to identify MTBC from culture isolates. It is It can also differentiate MTBC from NTM.

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