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REVIEW ARTICLE

Common Challenges in Laboratory Diagnosis and Management of Tuberculosis

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Abstract

The history of tuberculosis (TB) traces back to antiquity. Despite significant progress of various diagnostic methods and introduction of anti-tuberculosis drugs in past decades, TB is still a major worldwide health concern which leads annually to two million deaths, especially after the emergence of multidrug-resistant *Mycobacterium* TB and HIV co-infection. Presented here is a brief review of conventional and new TB diagnostic laboratory methods including their advantages and disadvantages as well as common challenges in diagnosis and management of TB.

Keywords: Tuberculosis; Laboratory; Diagnosis; Management

Introduction

Since earlier millennia ,tuberculosis (TB) was regarded as a serious human health threat and the paleopathological studies confirmed its evidence in ancient times.[1] In 1882, the identification of infective agent of TB by Robert Koch (1843–1910) and the succeeding advances in diagnostic and therapeutic methods were very influential in TB controlling, nevertheless it is not yet eradicated and is a major worldwide health problem, particularly in developing countries, where leads to high morbidity and mortality. Current challenging issues are the emergence of multidrug-resistant TB (MDR-TB) and HIV co-infection which made the combat against TB more difficult. A glance at available health data shows that in 2008, around 1.8 million people worldwide died of TB. Of these victims, 500,000 had HIV co-infection and in 2009 as the World Health Organization (WHO) reported, over 2 billion people in the world were infected with TB bacilli, and 1 out of 10 eventually developed active infection.

Resistant cases are also significantly increasing. The health information of more than 100 countries in 2009 revealed that around 5% of all TB cases were MDR-TB[2] and according to the 2010 report of WHO, treatment success rate of the MDR-TB cases has been 60%.[3] In Iran, in 2010, 10485 old and new cases of TB have been detected; of which 326 patients (around 2% of cases) were HIV positive.[4]

TB Classification

TB is generally classified as pulmonary and extrapulmonary forms and definite diagnosis of extra- pulmonary TB is more challenging than pulmonary type. The most common presentation of extra-pulmonary TB is lymphadenitis.[5] Clinically, TB manifests as ATBI (Acute TB infection) or dormant disease (Latent TB infection or LTBI). Diagnosis of LTBI is often more difficult than ATBI and it is seen in children, immunocompermised hosts such as HIV positive people, diabetics and those people who receive chemotherapy. LTBI may involve a single body organ or appear as a disseminated disease with multiple organ involvement.

Laboratory Diagnosis of TB

In suspected cases, diagnosis of TB mainly depends on taking a thorough medical history, performing an accurate physical examination and appropriate radiological, microbiological, immunological, molecular-biological and histological assessments.[6] Laboratory

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diagnostics are helpful in definite diagnosis of TB and evaluation of sensitivity of TB bacilli to available anti-tuberculosis drugs. However, conventional laboratory methods may be inadequate especially in children. Thus, in order to improve management of TB patients, more novel and cost-effective laboratory diagnostic tests are required, particularly in the era of MDR-TB, XDR-TB (Extensively Drug Resistant) and TB–AIDS co-morbidity. TB laboratory diagnostic methods may classify as follows:

A. Conventional Methods

1. Tuberculin Skin Test (TST)

TST or purified protein derivative (PPD) test was suggested initially by Charles Mantoux (1874-1944) and he won the Noble Prize in medicine in 1909.[7] In due course, TST has been widely used up to present time as a confirmatory diagnostic method. The result of TST is considered positive when 48 to 72 hours after PPD intradermal injection, the skin induration diameter is ≥10 mm. Since in developing countries, TB is highly prevalent, bacillus Calmette-Guerin (BCG) vaccination of the infants at birth is still continued as a preventive public health measure, the false-positive results may occur in those individuals who were previously vaccinated by BCG. In addition, the false-negative results may also happen in children,[8] elderly, AIDS patients and those who recently infected with TB bacilli.[9]

2. Looking for TB bacilli

Sputum smear microscopy: To confirm pulmonary TB, usually three consecutive sputum samples are examined by Ziel Neelsen (ZN) staining technique to detect the acid-fast bacilli (AFB). It is considered positive when at least 5000-10000 AFB are available in each ml of the examined sputum. Added to its diagnostic application, the sputum sample may be evaluated for detection of resistant *Mycobacrtium* TB in the patients with history of unresponsiveness to proper anti-TB medications. Sputum smear microscopy is a simple and cheap laboratory diagnostic test which is especially useful in adults; nevertheless the false negative or positive results lessen its sensitivity. The sensitivity of direct sputum smear microscopic examination for the first sputum sample is between 80 and 82% and for the second and third samples, the sensitivity will be increased up to 10-14% and 5-8% respectively.[10] The specificity of the sputum smear microscopy is around 98%.[11] The false positive results may be observed in the cases of atypical ones caused by TB non-tuberculous *Mycobacteria* (NTM).[12] Before the global HIV outbreak, it was estimated that for each TB smear-positive case, 1.22 smear-negative and extra-pulmonary TB cases would be detected. Some of the features of smear-negative pulmonary TB are different from smear-positive cases. Smear-negative pulmonary TB is less infective and has lower mortality, however; between 50 and 71% of these patients finally develop ATI. Additionally, most anti-TB medications are effective in the cases of smear-negative pulmonary TB. In a study, sodium hypochlorite concentration technique (bleach method) was used for detection of AFB and the results were compared to routine ZN staining technique. This study showed that bleach technique enhanced the sensitivity of TB diagnosis in the patients with history of cough for 3 months duration.[13]

Bronchial washing (BAL): It may be applied for diagnosis of suspected cases of pulmonary TB with negative sputum smears and it is probably helpful especially in young children. The investigator of a study reported that in suspected childhood pulmonary TB, for isolation of *Mycobacrtium* TB, the outcome of samples obtained from bronchoalveolar lavage was more favorable than those of gastric lavage.[14,15]

Gastric lavage (GL): For diagnosis of suspected childhood pulmonary TB, GL can be carried out as an outpatient procedure and as Maciel and colleagues reported its accuracy to be similar to that of inpatient GL.[16] Dickson *et al.* found that bronchoscopy was superior to gastric washing in smear–negative pulmonary TB, nevertheless, they advocated concurrent application of both methods for the optimal diagnosis.[17]

Culture: The culture of TB bacilli is the golden standard test for precise diagnosis of TB and its sensitivity is more than direct smear microscopy; however, it is a time consuming procedure and takes between 6 and 8 weeks.[18] Moreover; an equipped diagnostic center and expert staff are required. In older children, either gastric aspirate or sputum sample may be used as specimens for culture. In a study by Han and co-authors, they explained the positive AFB culture and negative results of T-SPOT. TB test may be caused by bacterial organisms belonging to NTM.[19]

B. New methods

In recent years, WHO has been approved some of the novel diagnostic TB tests.[20] New laboratory diagnostic assays for TB are:

Polymerase chain reaction (PCR) technique: PCR is a rapid and sensitive test which detects DNA of *Mycobacterium* TB in sputum sample as well as in other body fluids such as blood, pleural fluid, CSF and urine. According to a recent study in 2011, the sensitivity and specificity of commercial real-time PCR for evaluating respiratory and non-respiratory samples were studied and the authors found that the sensitivity and specificity of real-time PCR were 100% for respiratory samples and for non-respiratory samples were 85.7% and 97.3% respectively.[21] It is expected that TB bacilli or their nucleic acids to be detected in renal excretion and based on this idea, Gopinath and colleagues evaluated the spot urine for diagnosing pulmonary TB and they found the specific PCR and culture examination of spot urine samples can be utilized as a supplementary diagnostic method which considerably enhances the detection rate of pulmonary TB.[22] PCR may be used for detection of TB bacilli in paraffin blocks with good results.

Molecular diagnostic tests: There are two important commercially available molecular tests:

a. The Enzyme-Linked Immunosorbent Assay (ELISA), QuantiFERON-TB Gold In-Tube (QFT-IT)

b. The Enzyme-Linked Immunospot Assay (ELISPOT) ,T-SPOT.TB

These new biological tests have been used for the diagnosis of LTBI. In these tests, production of interferon gamma by mononuclear cells of the blood in response to specific antigens (ESAT-6 and CFP10) of *Mycobacrtium* TB is measured in-vitro. According to Lagrange and colleagues, the published studies showed that their sensitivity was at least equal to that of TST and in some studies, its sensitivity had been superior to that of T-SPOT-TB. [23] In addition, molecular tests are highly specific.[24] In a recent study in Indonesia on known adult TB cases, the sensitivity of QFT-IT for detection of latent TB infection was compared to the TST and the investigator reported that these tests were highly sensitivity, however; both QFT-IT and TST were unable to differentiate ATBI from LTBI.[25] Further advancements in molecular TB diagnostic methods are hopeful, nevertheless according to Chegu *et al.*" no tests are available which are universally applicable to all patients."[26,27]

Serological tests: Serum immunoglobulin G (IgG) antibodies against antigen 85 complex (Ag 85 complex) of *Mycobacterium* TB are detectable. In a recent study on Indian children with pulmonary and central nervous system TB; ELISA method (a biochemical technique used mainly in immunology) was applied to the patients' serum samples to detect IgG antibodies against Ag85 and the investigators found that its specificity rate was 71.9%.[28] In another study, serum IgA, IgG and IgM antibodies against the Mycobacterial A60 were detected for distinction of active TB from other pulmonary diseases and the authors concluded that A60-based serodiagnostic IgG assay was a useful test for the rapid diagnosis and differentiation of active pulmonary TB from other non-TB pulmonary diseases. In addition, the test can also be used to enhance diagnostic accuracy, particularly in smear-negative cases.[29] However, the current serological tests are rapid but without desired sensitivity.[30]

Extra-pulmonary TB: The main laboratory diagnostic methods for recognition of extra-pulmonary TB included bacteriologic assessment, culture and histopathologic examination, but identification of AFB in the tissue specimens usually had no high diagnostic accuracy. By fine needle aspiration cytology, TB caseating granuloma can be differentiated from non-caseating granulomas. In a retrospective study in Pakistan on 194 patients with extra-pulmonary TB, the investigator found that lymph nodes and spine were mostly involved and the cure rate in both males and females was equal, around 40.7%.[31]

Bone marrow culture, aspiration and biopsy for detection of TB in HIV positive patients: In a study, the researchers found that for detection of opportunistic infections including TB in HIV positive patients, diagnostic sensitivity of the bone marrow (BM) cultures had no superiority to that of blood cultures. In addition, the histopathologic assessment of the BM specimens led to relatively rapid recognition of around one third of the patients and additionally, in some culture negative patients, it was diagnostic for detection of opportunistic infections. The authors then suggested the application of BM aspiration, biopsy and culture for the diagnosis of opportunistic infections including TB in HIV positive patients, especially in the cases with pertinent clinical findings.[32] In a study in Taiwan from 1998 to 2007, the clinicopathologic findings of twenty-four patients with confirmed NTM pulmonary disease were studied. The histological features of pulmonary NTM infection showed three distinct pathogenesis including fibrocavitary/tuberculoid, nodular bronchiectatic and sarcoidal types. The fibrocavitary/tuberculoid histopathologic type mostly were observed in the upper lobes of old patients with prior lung disease.[33]

Diagnosis of pediatric TB: Childhood TB is occasionally a diagnostic dilemma because it presents

various confusing manifestations or it can be asymptomatic (latent disease). On the other hand, disseminated childhood TB may be accompanied with severe weight loss and cachexia. Thus, childhood TB may remain unidentified or detected with delay mainly due to the lack of advanced diagnostic facilities, especially in developing countries.[34] New TB diagnostic tests including real-time PCR are highly suggested for early diagnosis of childhood TB.[35] Between July 2008 and January 2009, a study on HIV-infected children less than 14 years old was conducted in Tanzania. Added to physical examination, TST, and chest X-ray, two sputum specimens as well as a blood sample were collected for microscopic examination, culture and CD4 T-lymphocyte count. These researchers found that for prediction of active TB in HIV-positive children, the low count of CD4 T-lymphocyte may be helpful.[36] Definite diagnosis or exclusion of disseminated childhood TB can be difficult. In a study carried out between 2007 and 2008, the investigators addressed the diagnostic role of the BM biopsy and culture in thirty-five children with suspected disseminated TB and they found BM biopsy as a valuable diagnostic method in these patients, preferably before treatment.[37]

Selected comments on TB management and diagnosis: In management and diagnosis of TB the following comments should be remembered:

- Some of the TB patients do not take their medications in a regular base. Hence, Directly Observed Therapy Strategy (DOTS) advised by WHO must be encouraged.[38] DOTS ensures that patients properly take their anti-TB medications; usually in a short course of treatment.
- Education of patients is useful because patients' compliance affects outcome of treatment. In a study in Pakistan, awareness of 203 patients in regard to TB was investigated and the misconceptions were identified. The authors recommended the targeted approach education programs for elimination of the misconceptions about TB.[39]
- Despite introduction of recent therapeutic modalities, drug resistance is a major factor resulted in treatment failure and it is related either to the patients' compliance or the resistance of TB bacilli. In 2008, over 500/000 cases of MDR-TB were globally identified.[40] Drug resistance may either present as MDR-TB or XDR-TB. It was shown that 5.4% of MDR-TB finally appeared as XDR-TB. Therefore, laboratory services for accurate and timely diagnosis of XDR-TB must be reinforced.[41] In developing countries, the majority of TB patients are not screened for detection of drug resistance, because sophisticated laboratory diagnostic facilities are lacking.[42] When feasible, the appropriate novel laboratory diagnostic tests should be used.
- TB is still among the 10 major causes of global mortality.[43] It is highly contagious and according to Nature Magazine "TB can be easily spread by inhalation of few droplets with 2 to 5 microns in diameter contains only 1-3 bacilli".[44] Close contacts of the individuals with active pulmonary TB patients and those people living in TB endemic regions are at highest risk of developing primary infection, while immunocompromised hosts are at maximum risk of reactivation of LTBI.[45] In addition to possibility of exposure to TB bacilli due to close contacts, the health care workers including laboratory personal are potentially at risk of exposure to various pathogens[46] including *Mycobacrtium* TB. Thus, biosafety practices and preventive measures should be applied in TB diagnostic laboratories to protect personnel.
- Lack of proper TB laboratory diagnostics at most general hospitals may lead to misdiagnosis,[47] consequently the required facilities should be accessible at least in specific medical centers of large cities.
- Diagnosis of TB in immunocompromised hosts including AIDS and diabetic patients is more difficult and needs refined diagnostic tests. According to an investigation carried out in Turkey between 1997 and 2003, those diabetics who developed TB needed longer duration of treatment and developed more drug resistance than non-diabetics, but the cure rates were similar.[48]
- Any organ in human body may be involved by TB.[43] Thus, diagnosis should be individualized which necessitates applying multiple diagnostic laboratory tests. No single test is totally diagnostic. For instance, in a prospective study on thirty-three TB pleurisy patients, no single test was completely diagnostic. In this study, the mean Erythrocyte Sedimentation Rate (ESR) in first hour was 97.04 mm and 57.6% of the cases had an ESR level more than 100 mm. In 63.6% of cases, TST was positive but no AFB was detected microscopically in pleural fluids. In 54.5% of the cases, pleural biopsy showed granulomatous lesions with or without caseation and in 24.2% of cases, chronic inflammation was noticed.[49]
- As Mofenson and Laughon stated, the combination of HIV, TB and pregnancy is a deadly condition

which needs prompt diagnosis and proper management. These investigators reported that TST in HIV positive people had a lower sensitivity, particularly in the cases with low CD4 cell count. A newer version of the Quantiferon-TB Gold assay seems to have a higher aspecificity.[50]

- Until now, 311 cases of congenital tuberculosis have been reported.[51] In the neonates who were born by mothers with TB, the risk of morbidity and mortality increased.[52] Neonatal TB may present as LTBI and its diagnosis is difficult, however, specific immunologic diagnostic tests are helpful.[53]
- For achieving a successful TB control, performing further national and international researches on TB are instrumental. In a systemic review, the investigators studied the available data related to TB researches and found that the most common preformed researches on TB were devoted to its treatment and prevention of MDR-TB in patients with HIV.[54]
- Transmission of TB is linked with patient-related risk factors, nonetheless, the investigators found a role for bacteriological factors based on DNA fingerprint analysis and they concluded the spread of TB also correlated to bacteriological factors.[55]
- The basis of TB control program is a diagnostic laboratory. According to an international study on TB control program in Ghana, the laboratory services were the weakest point. As a result, the researchers recommended continuous training and re-training of laboratory personnel and establishing a quality assurance system.[56]
- The final goal of health authorities should not be restricted to TB control, but their attempts should be focused on the global TB eradication (a goal known as the STOP TB Partnership). Obviously it is not an easy task and its achievement is highly correlated to political will and economic support of the governments especially in developing countries.[57] It was shown that poor people were more vulnerable to TB and TB was highly associated with economic poverty. For instance, according to WHO in 2005, the average estimated incidence of TB in high-income countries was 10 per 100,000 i.e. 20 times less than the mean incidence of TB in low-income countries.[58]

In 2008, the researchers of Johns Hopkins reported the results of a study in South Africa, Brazil and Kenya in which the cost-effectiveness of TB diagnostic tests including sputum smear and a novel TB diagnostic assay with 70% sensitivity and 95% specificity were assessed. They found that new diagnostic tests with high-specificity for TB were potentially highly cost-effective.[59] In similar study, Marra *et al.* investigated the cost-effectiveness of QuantiFERON-TB Gold (QFT-G) in comparison to TST. In this study, the tests were used for diagnosis of LTBI as a screening tool in TB close-contacts cases, and the authors finally concluded if the QFT-G assay is used selectively, seems to be cost-effective.[60] Similar results were obtained in a study which was performed in Germany in 2007.[61]

Conflict of interest: None declared.

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