Tuberculosis and its Diagnosis: Past, Present and Future

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Abstract

Tuberculosis is an endemic disease in the developing countries worldwide. Early and definitive diagnosis of Active Pulmonary Tuberculosis (TB) is important both at individual and population level for reducing mortality as well as transmission of the disease. Currently available diagnostics can be broadly categorized into microscopy, culture based and molecular method-based approaches. Among the advanced molecular methods, the Nucleic Acid Amplification Test (NAA), Line Probe Assay and Cartridge Based Nucleic Acid Amplification Test (CB NAAT) are mentionable. However, all these diagnostic methods either have compromised sensitivity or require expensive instrumentations that ultimately limit their efficacy as established diagnostic method. Currently used rapid and affordable diagnostic test for TB is sputum microscopy that detects *Mycobacterium tuberculosis* (Mtb) in clinical sample. This test has sub-optimal sensitivity as it depends firstly on the abundance of Mtb in the sample and secondly on operator's accuracy. On the other hand, culture-based diagnostic tests are time-consuming and dependent on expertise and infrastructure. Likewise, molecular tests are also dependent on expensive instrumentation and technical sophistication. Hence, rapid, sensitive and affordable diagnostic method for the detection of Active Pulmonary tuberculosis at an early phase is still a primary requisite.

1 Introduction

Active Pulmonary Tuberculosis (Active TB) is a pathological condition caused by the chronic infection of *Mycobacterium tuberculosis* (Mtb). The infection spreads through air and primarily infects lungs. As per recent worldwide disease monitoring, the country with highest instances of this disease is Swaziland, southern Africa (detected with 1200 cases per 100,000 people in the year 2007) [1]. In developed countries like United Kingdom, this is an urban disease whereas in Western Europe, 0.3 % of the population is TB affected [2, 3]. In India largest incidence of TB was detected in 2014 when 2.2 million new cases were traced with 0.3 million death [4, 5]. Even in current dates, two deaths of Active TB patients occur in every three minutes within India. In rural areas of many States, lack of sufficient health care infrastructure is the major barrier to the control and elimination of this deadly disease [6, 7]. National Tobacco program (NTCP) in collaboration with private sector and Indian Medical Association have taken several steps to

overcome the challenges for managing this disease. The primary step for the management of any pathological condition is the proper and definitive diagnosis of the same in an endemic population [8]. For tuberculosis, several diagnostic tests are available for detecting the latent as well active infection. Latent infection is detected through Tuberculin skin test and Interferon gamma release blood test for long time although these tests do not guarantee accurate results. Chest X-ray was used as a method of diagnosis of the TB infection in earlier days, however the method is not specific. Detection of *Mycobacterium tuberculosis* in sputum samples of patients has carried out through Sputum Smear microscopy and its modified version such as fluorescent microscopy, light emitting diodes (LED) microscopy and Ziehl-Neelsen light microscopy. These tests provide representation of active TB infection. Detecting patient derived antibodies against Mtb infection is performed through serological test, ALS (Antibodies from Lymphocyte Secretion) assay and transdermal patch insertion test.

However these tests are not sensitive enough since production of the antibody depends on individual patient's immune response and it remains in the circulation for a short while only. The latest diagnostic test for Active TB as endorsed by World Health Organisation (WHO) in 2010 uses Nucleic Acid Amplification (NAA) for definitive diagnosis of the Mtb genome in the sputum samples of patients [9]. This test can also detect Multi-drug resistant (MDR) TB infection depending on the

mutation in the Mtb genome. Although the commercially available test cartridges are expensive a concessional price is applied in TB endemic countries. The aim of this review is to cover all types of available diagnostic tests for Tuberculosis and highlighting the pros and cons of each one. This article will also project a future perspective of the current scenario that will help to develop ideas for detection of tuberculosis in more rapid, definitive, affordable and field adaptable manner [10].

Fig 1: Shows estimated number of incident TB cases (In Millions) across the world^[38]

Fig 2: Shows an estimated number of incident TB cases (In Millions) from 2000 to 2018^[38]

2 Available Diagnostics for Tuberculosis

All the currently available diagnostic tests for tuberculosis detect the presence of *Mycobacterium tuberculosis* in patient samples. The tests only vary in mode of detection of the bacteria and/ or accuracy, sensitivity etc. The following section will describe about various available methods for the diagnosis of tuberculosis in detail.

2.1 Tuberculin skin test

Tuberculin skin test or montoux tuberculin test is use for the diagnosis of the bacteria mycobacterium tuberculosis inpatient sample [11]. Tuberculin protein or purified protein derivative is applied under the top layer of the skin on the inner forearm. If the patient is TB infected then the tuberculin antigen reacts with it and a red bump arises at the site within two days and the high or low risk is diagnosed by comparing the size of the red bump.

The above test cannot differentiate between active or latent TB infection. As per US recommendation, the BCG (Bacillus Calmette Guérin) vaccine is not a protection for TB infection and

a positive tuberculin test in a person who received the BCG vaccine is interpreted as latent TB infection.

Fig 3: Shows an estimated number of incident TB deaths (In Thousands) from 2000 to 2015^[38]

Hypersensitivity, Allergic Reactions, etc, can also lead to false-positive outcomes. In the UK, however, no testing for tuberculin skin in persons vaccinated against BCG can be recommended.

This leads to a diverse false diagnosis in the United Kingdom of latent TB and diversity of latent TB infections failing to be diagnosed^[12]. This test is not exact for the screening of the infection with latent TB. In HIV-infected persons or who have a transplant that has been in close contact with a person with active TB, a small reaction (5 mm of firm swelling at the site) has been considered as positive. In the case of persons with diabetes, kidney failure and/or other disorders, the potential for active TB is increased (larger than or equivalent to 10 mm), health workers, injection drug users, immigrants having moved from a high TB country over the past five years, young people under the age of 4, adult-risks exposed, students employed and exposed to the risk of TB. For those without known TB risks, an attractive reaction is shown by the 15 mm or more company swelling at the site^[13].

2.2 Interferon gamma release blood test

This test is fundamentally utilized for the finding of dormant TB contamination and it by and large deliveries explicit interferon-gamma to respond with various antigens. Two sorts of tests, the QuantiFERON Gold Test and TB Spot test is accessible under this test head ^[14]. The QuantiFERON-TB Gold test (QFT-G) is an entire blood test for use as a finding of Mycobacterium tuberculosis disease ^[15] This test was endorsed by the U.S. Food and Drug Administration (FDA) in 2005. Blood tests are blended in with antigens and controls. For QFT-G, the antigens incorporate blends of manufactured peptides speaking to two M.

tuberculosis proteins, ESAT-6 and CFP-10. After brooding of the blood with antigens for 16 to 24 hours, the measure of interferon-gamma (IFN-gamma) is estimated. If the patient is contaminated with M. tuberculosis, their white platelets will deliver IFN-gamma because of contact with the TB antigens. The QFT-G results depend on the measure of IFN-gamma that is delivered in light of the antigens.

Clinical assessment and extra tests are expected to separate between an analysis of dormant TB or dynamic TB. The focal points of the above test incorporate shorter announcing the time for patients and less time length for assessing the outcome. This test isn't influenced by earlier BCG (bacilli Calmette-Guérin) inoculation. Burdens and constraints of the test incorporate impulse for blood test handling inside 16 hours after assortment to accomplish feasible white platelets ^[16].

The T-SPOT BLOOD TEST checks the quantity of against mycobacterial effector T cells, white platelets that produce interferon-gamma, in an example of blood. This gives a general estimation of the host resistant reaction against mycobacteria, which can uncover the nearness of contamination with Mycobacterium tuberculosis, the causative specialist of tuberculosis (TB).

Since this doesn't depend on the creation of a solid immunizer reaction or recoverable microbe, the strategy can be utilized to identify inert tuberculosis ^[17]. This test isn't impacted by BCG immunization and gives quick outcomes. Both these tests utilize various antigens for incitement. In light of the test standards, it is felt that the T-SPOT test is more explicit than the tuberculin skin test.

2.3 Chest X-Ray

A back front (PA) chest X-beam is the standard view utilized; CT outputs might be important in dynamic pneumonic TB, penetrates the cavities are regularly found in the upper lungs with or without mediastinal or hilar lymphadenopathy [18]. In any case, sores may show up anyplace in the lungs. In HIV and other immunosuppressed people, any irregularity may show TB or the chest X-beam may even show up altogether typical [1]. Old mended tuberculosis normally presents as pneumonic knobs in the hilar region or upper flaps, with or without fibrotic scars and volume misfortune [19]. Bronchiectasis and pleural scarring might be available. Knobs and fibrotic scars may contain gradually increasing tubercle bacilli with the potential for future movement to dynamic tuberculosis. People with these discoveries, on the off chance that they have a positive tuberculin skin test response, ought to be viewed as a high-need possibility for treatment of dormant contamination paying little heed to age. Then again, calcified nodular sores (calcified granuloma) represent an okay for future movement to dynamic tuberculosis. Irregularities on chest radiographs might be reminiscent of, yet are never indicative of, TB [19]. Be that as it may, chest radiographs might be utilized to preclude the chance of pneumonic TB in an individual who has a positive response to the tuberculin skin test and no indications of infection.

2.4 Sputum smear test

Smear microscopy of sputum is regularly the first TB test to be utilized in quite a while with a high pace of TB contamination [20]. Sputum is a thick liquid that is delivered in the lungs and the aviation routes prompting the lungs. The individual hacking for the most part gathers an example of sputum and a few examples of sputum will regularly be gathered [21]. In 2012, it was proposed that two examples could be gathered around the same time with no loss of precision [22]. To do the TB test an exceptionally dainty layer of the example is put on a glass slide, and this is known as a smear. A progression of uncommon stains are then applied to the example, and the recolored slide is inspected under a magnifying instrument for indications of the TB microorganisms [23]. Sputum smear microscopy is reasonable and basic, and individuals can be prepared to do it generally rapidly and without any problem. Furthermore, the outcomes are accessible inside hours. The affectability however is just around 50-60%. In nations with a high predominance of both aspiratory TB and HIV disease, the discovery rate can be even lower, the same number of individuals with HIV and TB co-contamination have low degrees of TB microbes in their sputum, and are in this way recorded as sputum negative.

2.5 ALS test

The antibodies from lymphocyte discharges (ALS) measure is an immunological test to identify dynamic infections like tuberculosis, cholera, typhoid and so on. As of late, ALS test

gestures established researchers as it is quickly utilized for analysis of Tuberculosis. The guideline depends on the discharge of neutralizer from in vivo enacted plasma B cells found in blood course for a brief period in light of TB-antigens during dynamic TB disease as opposed to dormant TB contamination. PBMCs were isolated from blood on Ficoll-Paque by differential centrifugation and were suspended in 24-well tissue culture plate's way of life medium. Various weakenings of PBMCs were hatched at 37 °C with 5% CO₂. Culture supernatants were gathered at 24, 48, 72, and 96 h after hatching and the supernatants were test against BCG or PPD by ELISA. The ELISA titer demonstrates the positive or negative outcome [24]. The determination of TB is generally convoluted. The standard finding for TB tolerant is sputum culture based. All things considered, culture need 6-8week with 10-20% bogus positive outcomes [25]. A fast serological test for conclusion, follow-up of infection action, and reaction to treatment would be valuable to clinicians [26-27]. The sanitized protein subordinate (PPD) skin test (Mantoux test) is a significant apparatus for analysis of dormant TB contamination and sickness in the created world, yet it has low prescient incentive in bacilli Calmette-Gue'rin (BCG)– immunized people, just as in people living in zones where TB is endemic. The low prescient worth is a consequence of cross-reactivity with BCG and atypical mycobacteria, just as bogus negative responses in malnourished kids [28-29]. BCG has been utilized as an antigen in EIAs in vitro investigations to decide illness movement, yet its utilization was suspended due to troubles in understanding, issues separating between dynamic or past sickness, and low affectability and particularity [30-32]. The antibodies from lymphocyte discharge (ALS) examine was prior used to distinguish explicit immunizer reaction after oral inoculation with an executed cholera immunization in solid grown-ups with no necessity for in vitro antigen incitement [33]. The principle preferred position of this technique incorporate a High Sensitivity >93 % [34]. This strategy doesn't need an example taken from the site of ailment, it likewise might be valuable in determination of paucibacillary youth TB. Emitted neutralizer might be safeguarded for long an ideal opportunity for additional investigation.

2.6 Nucleic acid amplification test

On September 30, 1999, the Food and Drug Administration endorsed a reformulated Amplified Mycobacterium Tuberculosis Direct Test* (MTD) (Gen-Probe®, San Diego, California) for location of Mycobacterium tuberculosis in corrosive quick bacilli (AFB) smear-positive and smear-negative respiratory examples from patients associated with having tuberculosis (TB). MTD and one other nucleic corrosive enhancement (NAA) test, the Amplicor® Mycobacterium Tuberculosis Test (Amplicor) (Roche® Diagnostic Systems, Inc., Branchburg, New Jersey), recently had been endorsed for the immediate location of M. tuberculosis in respiratory examples that have positive AFB

spreads. This notification refreshes the first rundown distributed in 1996 [35] and gives recommendations to utilizing and deciphering NAA test results for overseeing patients associated with having TB. The fitting number of examples to test with NAA will fluctuate contingent upon the clinical circumstance, the pervasiveness of TB, the commonness of nontuberculous mycobacteria (NTM), and research center capability [36,37]. In view of accessible data, the accompanying calculation is a sensible way to deal with NAA testing of respiratory examples from patients with signs or indications of dynamic pneumonic TB for whom an assumed finding has not been built up. Gather sputum examples on 3 distinct days for AFB smear and mycobacterial culture. Perform NAA test on the principal sputum example gathered, the main smear-positive sputum example, and extra sputum examples as demonstrated underneath. In the event that the primary sputum example is smear-positive and NAA-positive, the patient can be dared to have TB without extra NAA testing. Be that as it may, except if concern exists about the nearness of NTM, the NAA test adds little to the indicative work-up. In the event that the principal sputum is smear-positive and NAA-negative, a test for inhibitors ought to be finished. The inhibitor test should be possible as a choice with Amplicor. To test for inhibitors of MTD, spike an aliquot of the lysated sputum test with lysed *M. tuberculosis* (around 10 creatures for every response, or an identical measure of *M. tuberculosis* rRNA) and rehash the test beginning with amplification. If inhibitors are not identified, extra examples (not to surpass a sum of three) ought to be tried.

The patient can be dared to have NTM if a second sputum example is smear-positive, NAA-negative, and has no inhibitors distinguished. On the off chance that inhibitors are identified, the NAA test is of no demonstrative assistance. Extra examples (not to surpass a sum of three) can be tried with NAA. If sputum is smear-negative and MTD-positive†, extra examples (not to surpass three) ought to be tried with MTD. The patient can be ventured to have TB if a resulting example is MTD-positive. On the off chance that sputum is smear-negative and MTD-negative†, an extra example ought to be tried with MTD. The patient can be assumed not to be irresistible if all smear and MTD results are negative. The clinician must depend on clinical judgment in choices with respect to the requirement for antituberculous treatment and further analytic stir up in light of the fact that negative NAA results don't prohibit the chance of dynamic pneumonic TB. If the demonstrated rehash NAA testing neglects to confirm introductory NAA test results, the clinician must depend on clinical judgment in choices in regards to the requirement for antituberculous treatment, further symptomatic work-up, and detachment. Eventually, the patient's reaction to treatment and culture results are utilized to affirm or disprove an analysis of TB. NAA tests can upgrade symptomatic sureness, yet they don't supplant AFB smear or mycobacterial culture, and they don't supplant clinical judgment. Clinicians ought to

decipher these tests dependent on the clinical circumstance, and research facilities ought to perform NAA testing just in line with the doctor and just on chose examples. Laboratorians ought not save material from clinical examples for NAA testing if this trade offs the capacity to play out the other built up tests that have better-characterized indicative utility and suggestions. Explicitness of NAA tests shifts between research facilities in light of unrecognized procedural contrasts and contrasts in cross-pollution rates [38]. Different examples from a similar patient ought not be tried together to lessen dangers of methodologic blunders. Research center chiefs ought to give to clinicians data on the presentation of NAA tests in the neighborhood setting, including affectability and explicitness contrasted and culture for both smear-positive and smear-negative respiratory examples. Substantial discrepancies can indicate problems with either culture or NAA technique. The number of NAA tests repeated because of failure of negative and positive controls also should be reported. Clinicians should understand the impact that changes in sensitivity, specificity, prevalence of TB, and prevalence of other mycobacterial diseases can have on the predictive value of the NAA test. Information is limited regarding NAA test performance for no respiratory specimens, or specimens from treated patients. NAA tests often remain positive after cultures become negative during therapy and can remain positive even after completion of therapy.

2.7 The line probe assay

The line probe assay could be a fast diagnostic methodology that has intended to spot active *m. TB* complex and rifampicin resistance alone or combined with INH. This test has no specific marker, its supported detection of *Mycobacterium tuberculosis* in an exceedingly patient is phlegm. The line probe assay methodology is dole out in following steps. DNA extraction from positive culture or directly from biological samples. Amplification of specific region of the gene by exploitation the polymers chain reaction methodology, then reverse coupling of amplified factor to specific DNA probe immobilized on strip development that shows bands .wherever the tests are vested. Line test measure for smear positive example indicates high explicitness (>99%) and affectability (>97 %) for rifampicin resistance, together with INH it appears (99%) particularity and (90%) affectability.

2.8 CBNAAT

CBNAAT test is also known as Genexpert in outside Asian country. The test was available in the market in 2004. Moreover, the development of test completed in 2008. In 2009 first clinical trial was carried out. In Gregorian calendar month 2010 world health organization recommended Genexpert to incorporate into their health checkup system, Genexpert is a molecular test which diagnoses T.B. by sleuthing TB microorganism exploitation sputum sample. It gives stages to expedient and easy to use and investigate amplification. It takes less than two

hours. CBNAAT moreover recognizes the hereditary transformations included with rifampicin medicate obstruction by polymerase chain reaction. As per WHO guidelines Genexpert is also upheld for MDR-TB or HIV associated irresistible and infectious disease.

3 Future perspective

To counter the problems associated with the complete eradication of TB, a better and more efficient therapeutic approach is required. Understanding the bacterial structure, different aspects of its survival, biosynthesis of essential components and metabolism, and then designing such novel therapeutic agents that target these vital aspects of bacteria will help, or cannot persist, in bacterial death.

Fig 4: Represents the steps involved in Line Probe Assay

The availability of mycobacterium genome sequence, mycobacterial genetic resources such as transposon mutagenesis, gene transfer, and knockout genes, greatly contributes to target identification. Besides, targets should also be considered for drug development involving the biosynthesis of essential components of the bacterial cell wall as well as the pathogenesis of the disease. The use of multiple drug formulations that reach multiple targets in various pathways involves a system biology approach to achieve the desired effect, in addition to finding new targets.

4 Conclusion

The anti-TB drugs currently available have been developed 40 years ago, and a great need for a new generation of TB drugs that can meet the challenges of drug-resistance tuberculosis and persistence of mycobacterium. Current data on TB epidemiology suggest that there is still a need for targeted sensitization initiatives to raise awareness among clinicians about the importance of this disease. Considering TB is the first step towards combating TB, early detection and early commencement of therapy are critical to ensuring the best outcome and reducing the risk of transmission to others. Particular attention should also be focused on so-called high-risk populations that are most likely to become infected by

tuberculosis and develop active diseases so that a vicious cycle of community transmission is maintained. Care providers are the key to both control and elimination strategies that ultimately are based on the proper diagnosis and treatment of patients and individuals with latent TB.

5 Conflict of Interest

The authors declare no conflict of interest.

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