



REVIEW ARTICLE

Latest trends in diagnosis of tuberculosis; A Review.

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ABSTRACT... Tuberculosis is the most ancient and resistant entity in the world of microbes that holds significant attention due to its substantial organ association. A lot has been done in diagnosing it and much is desired to isolate and enfold it in order to eradicate it especially from the third world countries. Presenting as a pulmonary inhabitant, it translocates itself in every organ causing significant morbidity and mortality. Recently, advances have been made in the field of molecular diagnostics to markedly improve the accuracy and point-in-time diagnosis. In addition, more promising methods are under development to further optimize the diagnosis.

Key words: Diagnosis, Methods, Tuberculosis.

INTRODUCTION

Tuberculosis is one of the oldest known human diseases, primarily affecting lungs causing widespread morbidity and mortality.¹ Archeologists discovered a mother and a child buried together with traces of tuberculosis tracing back to ancient times.² “Vampire panics to Phthisis”, tuberculosis has been a dreadful experience of the human age until 24th march, 1882, when Dr. Robert Koch isolated the causal agent, a bacilli associated with this transience.³

Tuberculosis is primarily the disease of lungs initiated by the aerosol droplets landing on the lung alveoli, spreading to lymph nodes, intestine, bones, genital organs, liver, kidneys and skin leaving no organ vulnerable to its contagion.⁴ Ranging from painful nodules around oral cavities to adhesions and strictures, tuberculosis has widespread clinical illustration involving every organ that can have hematogenous access.⁵ The progression of the disease has several outcomes, depending largely upon the response of the host immune system.⁶ This calls upon the availability of fast and reliable screening and diagnostic techniques for effective case management.⁷

Diagnostic approach to this ailment starts from pertinent history, clinical examination, radiography and bacterial cultures.⁸ In addition, recent advances in molecular diagnostic techniques have been used to diagnose early disease and effective treatment.⁹

MATERIAL & METHODS

Here, we review the availability of currently employed methods and strategies for diagnosis of tuberculosis, including conventional to recently implemented techniques used to detect MTB in clinical perspective. Reduction in turnaround time employing these molecular techniques has led to proficient case management.

DISCUSSION

The genus Mycobacterium has more than 100 recognized or anticipated species causing significant morbidity and mortality in humans and animals.¹⁰ The two familiar pathogens causing disease in humans are Mycobacterium tuberculosis (pulmonary and extra pulmonary manifestations) and mycobacterium leprae (leprosy or Hansen’s disease).¹¹

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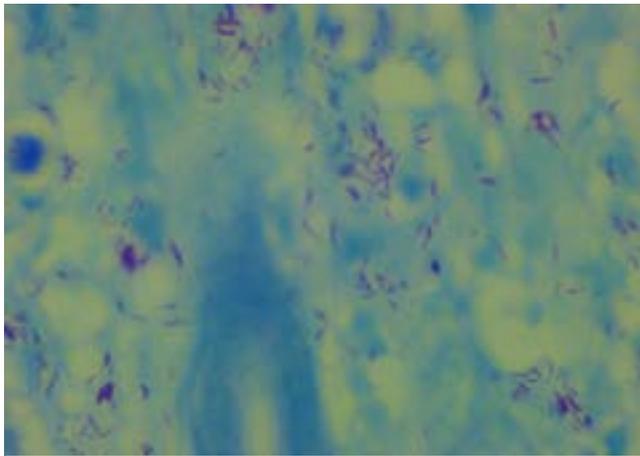


Figure-1. Acid fast bacilli, Zn stain, 100X

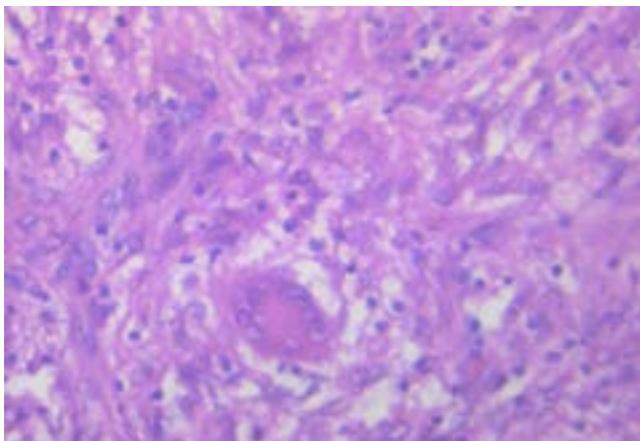


Figure-2. Granuloma H & E Stain, 40X

Mycobacterium species produce a wide spectrum of infections ranging from localized lesions to disseminated disease. An estimated 10 million new cases and 2-3 million deaths occur per year.¹² At the minute, one fourth of the global population estimated to be 2 billion are infected with tuberculosis out of which most are asymptomatic and classified as having latent tuberculosis (LTBI).¹³ Despite the severity of epidemics, most of the carriers are below the tip of iceberg due to under diagnosis as well as under reporting to TB national program. The World Health Organization End TB strategy calls for finding these missing cases in order to meet the goals of eradicating tuberculosis by 2030.¹⁴

Mycobacterium is an acid fast bacillus attributed to its unique cell wall components including peptidoglycan, arabinogalactan and mycolic

acid.¹⁵ These cell wall components render it acid fast properties by making stable complexes with aryl methane dyes (carbol fuchsin, auramine and rhodamine), resistant to host defense, antibiotic attack and also possess anti-inflammatory and immunoregulatory properties¹⁶ (Figure-1). The organism is obligate aerobe with rapid flourishing characteristics in oxygen rich environment making it reside at sub pleural areas having maximum oxygen concentration.¹⁷ The slow generation time of 15-20 hours makes it difficult to cultivate the organism in labs as compared to other bacteria that can be grown in minutes.¹⁸

Culture requirements include collection of samples from non-sterile sites that further require decontamination with NaOH and concentration with centrifugation whereas sterile specimens do not need to be decontaminated; only concentration by centrifugation is required.¹⁹ Inoculation at highly sophisticated biosafety labs is recommended due to increased risk of contamination and infection.²⁰ Moreover; slow generation time requires prolonged incubation for weeks.²¹ After collection, sterile specimen must be inoculated on Lowenstein Jensen media that requires incubation for 6-8 weeks and if available, specialized liquid media BACTEC MICRO MGIT can turn out results within 21 days.²² The use of liquid culture media with radiometric growth detection has simplified the culture scheme.²³ Lipoarabinomannan (LAM); a lipopolysaccharide in cell walls of mycobacteria can be detected in urine, making it noninvasive biomarker.²⁴

The immunological methods of detection of host response to pathogen employ tuberculin skin test and interferon gamma release assays.²⁵ These tests are widely used for diagnosing latent and active tuberculous infection. 0.5 ml of purified protein derivative standardized in terms of tuberculin units is injected subcutaneously and the skin reaction is noticed. However, the probability of false positive and false negative results due to BCG vaccine and immunocompromised individuals respectively make it less popular among the clinical practitioners.²⁶ The infected immune system of the host release INF- γ response to ESAT-6 and CFP-10 in the plasma

that can be determined by ELISA.²⁷ The two commercial assays generally used to determine the interferons are the Quantiferon-Gold in Tube test (QFT-GIT) formally called TB Gold and T-SPOT-TB formally called TB SPOT.²⁸ The test time required for TB GOLD is 16 hours, results interpreted as positive, negative or indeterminate in contrast to TB SPOT that requires 8 hours, gives positive, negative or borderline results.²⁹ Similarly, the MTB antigens can bind to the antibodies coated on latex beads employing latex agglutination assay but cross reactivity of BCG vaccination might interfere with the test adding further to the short falls of immunological assays.³⁰ Flow cytometry in terms of high reproducibility has become a famous technique but due to unavailability of logistic support makes it difficult to implement in developing countries.³¹

The use of biopsy samples for histopathological diagnosis is gold standard in establishing clinical opinion.³² The tissue reaction for MTB consists of granuloma formation with central caseation surrounded by epithelioid histiocytes, lymphocytes, plasma cells, multinucleated giant cells (Langhans type) and rim of fibroblasts³³ (Figure-2). These granulomas are characteristic of pulmonary as well as extra pulmonary tuberculosis. The biopsy samples fixed in 10% formalin, processed and stained with H & E stain can be kept for prolonged period and are reproducible.³⁴ However, the invasive technique used for obtaining biopsy makes this method less appealing.³⁵

Molecular diagnostic techniques used nowadays are highly specific and nonintegrated.³⁶ Use of clinical samples for detecting mycobacterial rRNA and DNA show higher sensitivity and specificity.³⁷ These include PCR, Loop Isothermal Amplification PCR (LAMP), Xpert MTB/RIF assay and line probe assay. PCR-based sequencing for mycobacterial identification consists of PCR amplification of mycobacterial DNA with genus-specific primers and sequencing of the amplicons. The organism is identified by comparison of the nucleotide sequence with reference sequences. The most reliable sequence for the identification of

mycobacteria is approximately 1500 bp 16S rRNA gene.³⁸ Loop isothermal amplification PCR does not require prior purification and detects MTB DNA with high sensitivity and specificity and can produce results in less than 2 hours.³⁹ However, there is still need to make this test easy, cheaper and more efficient to make it competitive against other PCR methods already available.⁴⁰

The Xpert MTB/RIF assay is an automated tool integrated with nucleic acid amplification technique (NAAT) platform isolating MTB and its resistant forms.⁴¹ The WHO endorsed its use in 2010, world's 69 countries recommend this as initial diagnostic test however, maintenance, cost, expertise of the technical staff and training make this test least preferred in developing countries.⁴² Line probe assay (LPA) technique endorsed by WHO in 2013 has the ability to detect smear positive samples as well as resilient cases in terms of genetic mutations or antibiotic resistance however, the sensitivity issue requires additional confirmation by combining another test to this technique.⁴³

The newer techniques in the form of Nano diagnostics and lab-on-chip (LOC) devices have revolutionized the diagnostic world but much has to be done to make these methods useful, cheaper and user friendly to combat this battle of fighting this dreadful disease.⁴⁴

CONCLUSION

Tuberculosis has long been neglected and under diagnosed with conventional tools but the molecular methods have not only revolutionized the medical world but also reduced the time of diagnosis in terms of patient care and case management. Employing these methods, we can not only control but also can eradicate the disease.

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5	Shazia Aslam	Supervisor Final manuscript.	
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