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Role of CBNAAT in Detecting MDR- Tuberculosis in Northern India

Priya Srivastava[1], Sarvesh Gautam[1] , Dr. Anju Tyagi*[1]
[1]*Institute of Applied Medicines and Research, Ghaziabad, India*

Abstract: ***Tuberculosis (TB) is one of the deadliest infections responsible for millions of deaths yearly over the world from ancient time to till date. India is one of the high tuberculosis (TB) burden nations in the world accounting for nearly 20% of the worldwide rate constituting 9.4 million TB cases. India secures second position in harboring multi drug resistant (MDR) TB cases, i.e., around 99,000 cases. Standard sputum based strategies to detect pulmonary tuberculosis include sputum microscopy and culture. However, these traditional methods results in a delay in the detection of TB and in consequent beginning of treatment. To overcome such delays, in 2010, WHO endorsed a new molecular techniques CBNAAT (Xpert MTB/ RIF) which is a fully automated diagnostic test that simultaneously detects tuberculosis and rifampicin drug resistance associated with mutation of rpoB gene within few hours. In this review we present a general overview of TB including the pathogenesis, diagnosis as well as ability of CBNAAT to detect Pulmonary TB in humans.***
Keywords: Tuberculosis, Northern India, CBNAAT, Molecular Techniques, Standard Methods

I. INTRODUCTION

Tuberculosis (TB) is the foremost old illness of manhood and has co-exists with peoplefor numerous thousands of years [1].It is a long-lasting mycobacterial infection, often found with latent period following primary infection. In humans, primary cause of Tuberculosis is Mycobacterium tuberculosis, but other species, such asM. microti, M. bovis, M. africanum, M. canettii, M. pinnipedii as well asM. caprae are also the infectious agents of TB. All these species combines togetherto shape the Mycobacterium tuberculosis complex (MTBC) [2]. Mycobacterium tuberculosis is a highly pathogenicbacteria belonging to the family Mycobacteriaceae. Mycobacteria are small (0.5 µm - 3 µm), slow-growing, aerobic bacilli. They are distinguished by a complex, lipid-rich cell envelope responsible for their classification as acid-fast and resistivity for Gram stain. It was discovered by Robert Koch in 1882 and for this discovery, Nobel prize in physiology or medicine was granted to him in 1905 [3].

Tuberculosis is a principalcause of disease and deathglobally, with death rate of about 1.7 million people in 2016, mostly in low and middle income countries. HIV/AIDS is the most significant factor to TB infection and deaths in parts of the world where both infections are predominant. The most important cause is false-negative results and default identification of TB suspects in developing countries, as most programmes of TB control use Ziehl-Neelsen (ZN) staining, which has lowaffectability and few visits are required which leads to complex default. On the other hand, mycobacterial culture generally needs 2-6 weeks time to yield a final result and requires proper arrangement and technical knowledge [4].

To overcome these shortcomings, a new molecular technique, Cartridge-based nucleic acid amplification test (CBNAAT) is recently introduced by WHO in 2010, which is relies on Polymerase Chain Reaction (PCR) method for TB detection and resistance to rifampicin. Its role in identifying TB in people having HIV has not been studied widely in India.

Gene Xpert test is based on nested real-time PCR with two uses:

1) The identification of Mycobacterium tuberculosis complex DNA in sputum specimensthat are either acid-fast bacilli (AFB) smear positive or negative.
2) The detection of Rifampicin resistance related mutations of the rpoB gene insamples from patients suspected of MDR-TB.

II. REVIEW OF LITERATURE

Mycobacterium tuberculosis, the infectiouscause of tuberculosis, was recognized by Robert Koch in 1882. It is an ancient disease with vast global health challenge and high burden on worldwide epidemiology among other contagious diseases. TB has ranked second as a main reason for death after human immunodeficiency virus (HIV), and has been found that one-third of the total world population is infected. In spite of fact that, contamination does not leads to active disease, 5-10% of the infected individuals develop active disease each year [5].The rest of the 90% people stay asymptomatic and can have inactive disease, but reactivation may happen any time amid life [6].

A. History

In 1819, the French Theophile Laennac detects the presence of consolidation, pleurisy and pulmonary cavitation as pathogenic signs of pulmonary or extrapulmonary TB [7]. In 1843, the German doctor Philipp Friedrich Hermann Klencke succeeded tentatively within the generation of human and bovine shapes of TB by immunizing tubercle materials into liver and lungs of rabbits [8]. Some years later, in 1867, Theodor Albrecht Edwin Klebs tried to isolate the TB bacillus by sowing the tubercle material on egg white, stored in sterile flasks. In his tests, the culture was rapidly sloppy and it was simple to recognize portable bacilli, which causes the illness in guinea pigs after vaccination into the peritoneal depression [9]. Later on, the renowned scientist Robert Koch was isolates the tubercle bacillus using the methylene blue staining. He recognized, isolated and cultured the bacillus in animal serum. At long last, by vaccinating the bacillus into research facility animals, he reproduced the infection [10]. Robert Koch displayed this result to the Society of Physiology in Berlin on 24 March 1882, forming a breakthrough within the battle against TB disease [3].

B. Transmission

Tuberculosis (TB) is transmitted to a vulnerable person from an infected individual by airborne particles, termedas droplet nuclei. These are 1–5 microns in diameter. These communicable droplet nuclei are minordroplets containing tubercle bacteria which are released when person having pulmonary tuberculosis cough, sneeze, laugh, shout etc. These droplet nuclei stay in the surrounding for few hours. Transmission takes place when an individual breathes in droplet nuclei containing tuberculosis bacteria. These droplet nuclei voyage into the upper respiratory tract via mouth and nasal openings. Thereafter, they move to the bronchi and at last to the alveoli and the lungs[11].

C. Pathogenesis

TB is an airborne bacterial infection caused by *M. Tuberculosis* which infects other body organs and most commonly occurs in the lungs [12]. *M. Tuberculosis* is exposed to the air as droplet nuclei from sneezing, coughing or shouting of a person having pulmonary TB. Once the bacilli reach the alveoli, they are engulfed by alveolar macrophages resulting in the elimination of a greater number of breathe in tubercle bacilli [13]. The unaffected proportion increases in the macrophages and are released when the macrophages destruct. These tubercle bacilli move via the blood flow or lymphatic passages to different body tissues or organs, most commonly to the lungs [14].

There are five stages of pulmonary tuberculosis ([15]-[18]) as mentioned below.

1) *Stage I:* This stage contains no significant growth of bacilli. The bacilli are usually eliminated by alveolar macrophages. However, if the bacilli are not destroyed, they reproduce and alveolar macrophages are generally destroyed due to their multiplication.
2) *Stage II:* This is commonly known as symbiotic stage where the bacilli multiply logarithmically within non-activated macrophages of developing abrasion, called tubercle. These non-activated macrophages, further, enters the tubercle from main blood stream and are referred as monocytes [18].
3) *Stage III:* In this, caseous necrosis occurs and the bacilli become stationary. The growth of *M. tuberculosis* is inhibited by immune reaction to tuberculin like antigens that are released from bacilli [16]. At this stage, the immune reaction is mainly tissue damaging, delayed type hypersensitivity, which destroys the bacilli laden macrophages of symbiotic stage. At the lesion, which contains a soildcaseouscentre,bacillidonotmultiplybecauseofbeingsurroundedbybothnon-activated macrophages (which permit intracellular multiplication of *M. tb*) as well as partly activated macrophages and immature epitheloid cells produced by cell mediated immunity.

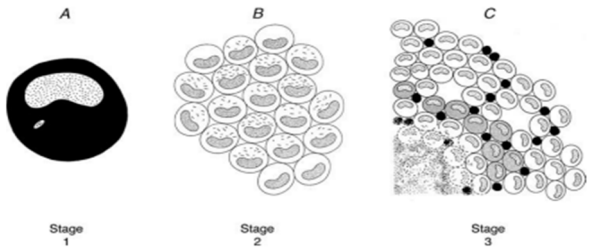

Figure 2(a): Pathogenesis of Tuberculosis [19]

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4) *Stage IV:* This is the stage where the cell mediated immunity plays an important role to determine whether the disease turn out to be clinically recognizable. If the cell mediated immunity is not properly developed, *M. tb* escape from the caseous necrosis, multiply again in non-activated macrophages and partly activated macrophages. The cytotoxic DTHimmune response destructs these macrophages, causing expansion of the caseousc entre and development of disease. If a good cell mediated immune response is developed, a layer of stimulated macrophages surrounds the caseous necrosis. These activated macrophages ingest and inhibits the growth of *M. tb*, often by arresting the development of lesion at a sub clinical stage.

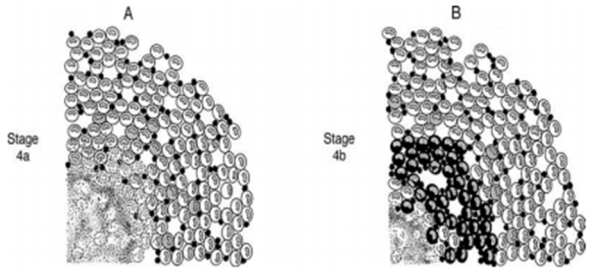

Figure 2(b): Pathogenesis of Tuberculosis [19]

5) *Stage V:* This is called liquefaction stage, where the bacilli escape the host's defense mechanisms. The bacillus multiplies extracellular when liquefaction of the caseouscentre occurs. Even well-developed cell mediated immune response is totally ineffective to control at this stage. The high concentration of tuberculin like products released by bacilli itself causes a tissue damaging DTH response, wears away the bronchial wall and forms a cavity. The bacilli further enterthebronchialtree,spreadtootherpartsofthelungsandtotheexternal environment.

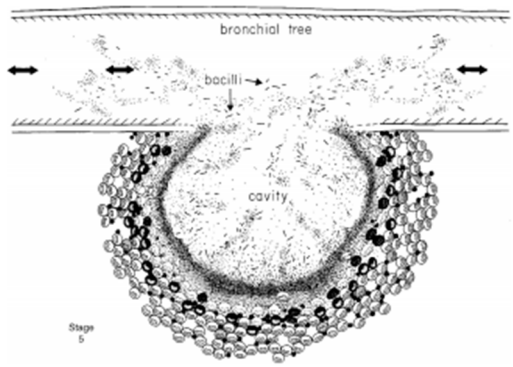

Figure 2(c): Pathogenesis of Tuberculosis [19]

D. Tuberculosis Diagnosis

The identification of tuberculosis from clinical samples is mainly based on clinical characterization, demonstration of acid fast bacilli (AFB), histopathology and isolation of *M. tuberculosis*. Early diagnosis of tuberculosis has always remained a challenging problem especially in case of pauci-bacillary and extra-pulmonary forms. The foremost common strategy for TB diagnosis is smear microscopy which was developed around 100 years ago. Smear microscopy requires 10,000 to 1,00,000 organisms/ml and acid fast bacilli (AFB). It could be saprophytic or pathogenic mycobacteria [20]. The bacilli are examined in sputum smear under a microscope. It is classified as an acid-fast bacillus as *Mycobacterium tuberculosis* retains stains after being treated with acidic solution[21]. The Ziehl–Neelsen stain and the Kinyoun stain are the most commonly used acid-fast staining techniques. Auramine-rhodamine staining [22] and fluorescence microscopy are also used.

Smear microscopy plays an important role in early diagnosis of Mycobacterial infections as the method is highly specific, rapid and cheapest method used for detection of AFB in sputum. Recent advancement in smear microscopy is the arrival of fluorescence microscopy [23]. This alternate technique is known to have increased sensitivity (10% higher) when compared with ZiehlNeelsen (ZN) microscopy methods [24]. Fluorescent acid fast bacilli (AFB) can be visualized at lower magnification and smears can be observed in a lesser time (about 25% less) as compared to ZN smears. The sensitivity of microscopy is influenced by the quality of

collected specimen, the quantity of mycobacterium present in the specimen, the processing method, the staining technique, and the quality of the observation [25].

On the other hand, as of late presented, Xpert MTB/RIF test could be a novel, fast, mechanized, and cartridge-based NAA test that can identify TB beside rifampicin resistance straightforwardly from sputum inside 2 hours of collection [26]. The GeneXpert cartridges are pre-loaded with all of the essential reagents for test preparing, DNA extraction, enhancement, and laser discovery of the opened up rpo B quality target. A major advantage of the Xpert MTB/RIF test is that it can be precisely administrated with negligible hands-on specialized time. The affectability and specificity of this test has been detailed to be worthy for TB discovery [27].

In the recent study of execution of Xpert MTB/RIF, among the 561 culture-positive patients (561/1730), a single, direct Xpert MTB/RIF test recognized 98.2% (551 out of 561) of the sputum smear-positive TB cases and 72.5% (124 out of 171) of those with sputum smear-negative TB. The test was particular in 604 of 609 patients (99.2%) not influenced by TB. A second Xpert MTB/RIF test among patients with sputum smear-negative, culture-positive TB increased detection sensitivity by 12.6% and a third by 5.1%, to reach 90.2%.

When compared to phenotypic DST, the Xpert MTB/RIF measure accurately identified 97.6% (200 out of 205) of patients harboring rifampicin-resistant strains and 98.1% (504 out of 514) of those with rifampicin-susceptible strains [28].

The WHO issued starting proposals on Xpert MTB/RIF, particularly for people suspected of having MDR-TB [29]. Xpert MTB/RIF has higher affectability for TB location in smear-positive patients than in smear-negative patients; in any case, this test may be important as an add-on test taking after spread microscopy in patients already found to be smear-negative [30.

III. EPIDEMIOLOGY OF TUBERCULOSIS

Tuberculosis (TB) has been ranked as second leading cause of death among infectious diseases, globally, after HIV [26]. In 2013, approximately, 9 million people developed incident TB, whereas 1.5 million people died due to TB, including 0.4 million death among HIV positive individuals [26]. Though, most of the TB cases and deaths were among men, but the burden of tubercular infection was also observed higher in women and is increasing. In 2013, an approximate number of 3.3 million cases and 510,000 TB deaths occurred among women.

Along with this, estimated number of 550,000 TB cases and 80,000 deaths were reported among children [26]. Since 1993, World Health Organization (WHO) declared TB as a global public health emergency. TB mortality rates have fallen by 45% since 1990 and incidence rates are also falling in developed countries. The estimated number of TB cases in 2013 in Asia and Africa were 56% and 29%, respectively.

India has ranked first among six high burden countries with largest number of incident cases and has accounted for an estimated for 24% of all TB cases worldwide [26]. The annual risk of infection was observed to be 1.5%, while the TB mortality rate per 100,000 population was found to be 24 (15-35) persons. About 4.2% (3.3-5.2) of HIV prevalence in incident cases was recorded [31], [29]. The status of TB in tribal communities is found to be relatively serious as compared to non-tribal population. Various studies in Sahariya have reported variable overall prevalence of TB, e.g., the first report published by Chakma et al (1996) estimated the prevalence as 1270/100,000, which was followed by revised survey by Sharma et al [32](as 29.75/100), Rao et al [33] (as 1518/100,000) and a recent revised estimate by Rao as 3294/100,000. Bhat et al [33] reported the prevalence for TB in various tribes of Madhya Pradesh to be 387/100,000 population. Variable rates of prevalence of pulmonary tuberculosis has been reported for other Indian tribes also, e.g., in Pahadis of Kashmir valley it is 260/100,000 population [34], in tribes of Wardha district, Maharshtra the rate is 133/100,000 in Jawadhu tribe of North Arcot district, Tamil Nadu, it is 840/100,000 [35], in tribes of Car Nicobar, 140/100,000 and in Baiga tribe of M. P. it is reported to be 146/100,000 of population.

IV. CONCLUSION

Diagnosis of tuberculosis in people is challenging indeed with progressive advances, the determination is frequently troublesome to affirm microbiologically in portion due to the paucibacillary nature of the malady. Clinical conclusion needs standardization, and conventional and atomic microbiologic strategies need affectability, especially in people. Most of the tests make strides affectability, but these tests cannot recognize tuberculosis infection from idle contamination and few need specificity. These apparatus have progress our capacity to identify Mycobacterium tuberculosis as well as MDR-TB.

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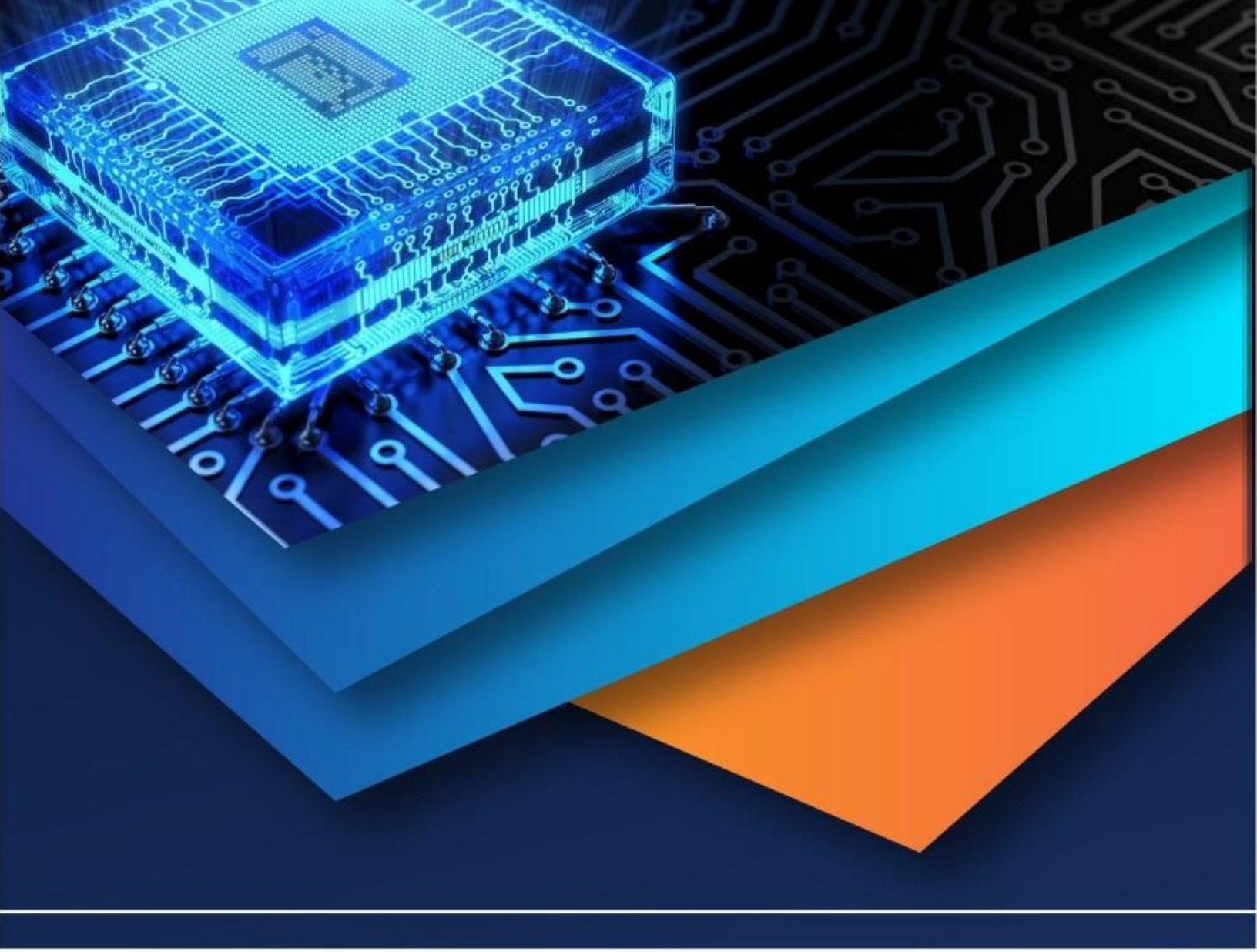

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