REVIEW ARTICLE

Cartridge Based Nucleic Acid Amplification Test: The Sherlock of Tuberculosis

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Abstract

Emergence of Tuberculosis and Multi drug resistance tuberculosis at higher pace in the population needs rapid diagnosis with accuracy to reduce the transmission of disease and suffering of the patients. The technique of GenexpertMTb/Rif by Cepheid rolled out by WHO has given high support to the humanity by rapid and accurate detection of Tuberculosis and resistance to rifampicin. The technique has less turnaround time, can be performed in simple lab settings, fully automated processing and result interpretation and the professional needs a hands on training of less than thirty minutes as compared to other sophisticated techniques for TB and drug resistance diagnosis. The article here discussed the role of CBNAAT in different groups of patients with accordance to the researches already published in support of high and promising performance of GenexpertMTb.

Keywords: GenexpertMTb, CBNAAT, Tuberculosis, Diagnosis, Rifampicin, Resistance.

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INTRODUCTION

Tuberculosis is a major public health problem existing since decades. It is considered to be the major reason of morbidity and mortality in developing countries where India ranks on the top most of the list followed by China. According to the WHO global report 2017 in India the patient suffering from TB was 211 per lakh and globally 140 per lakh population in 2016. The incidence of MDR TB in India is 11 per lakh population.[1] Diagnosis of TB has been evolving since 1982 and still evolving. Tuberculosis is a disease of weakness, co morbidities and poor. The TB diagnosis through the conventional techniques was tough as the techniques have a threshold number to produce a desired result. There was a need to design a test which could overdo the limitations of the other conventional technique and the question was answered by CBNAAT (Cartridge based Nucleic acid amplification test).The limitation of low number was diminished by the technique.

Tuberculosis

Mycobacterium tuberculosis spreads due to small droplets nuclei generated from sneezing, coughing of a person infected with pulmonary tuberculosis. Inhalation of the Mycobacterium tuberculosis present in droplet can cause respiratory disease. The infection can spread from lungs and other parts of the body except nails and hair such as lymph nodes, pleura, meningitis and bones. The spread of tuberculosis develops as latent TB primarily not an infection, primary Tuberculosis is often asymptomatic and exists subclinically[2]. Secondary tuberculosis, when patients develop infection with serious symptoms such as weight loss, loss of appetite and fever. The pulmonary tuberculosis accounts for 80% total infected and 20% of extra pulmonary in the left of total.

Conventional Diagnosis of Tuberculosis

The diagnosis of tuberculosis started from smear microscopy invented by Robert Koch. Zeihl -neelson and fluorescent microscopy are the two techniques helping the technicians in diagnosing TB with full expertise but the number always challenged the technique with a threshold number i.e. 103 -105 bacilli/ml of sputum. It is insensitive, non specific. Microscopy is a technical hand expertise, observation dependent.

The sensitivity of microscopy is 60 %.[3] It utilizes cheap equipment and materials. Most of the developing countries used sputum microscopy as the basic toll for the diagnosis. Microscopy is a technique highly specific for *M. tuberculosis* which looked curved, long and beaded. The sample collection site must be precise with the site of infection.

In pulmonary tuberculosis the samples must be collected and must originate from respiratory tract, such as Induce sputum, BAL or a lung biopsy. The WHO introduced three first morning sputum to produce a good quality result. Specimen for extra pulmonary tuberculosis is also very much site dependent. The most common sites of infection are biopsies, aspirates, pus, urine and sterile body fluids including CSF, Synovial, Pleural, Pericardial or peritoneal fluids. Microscopy is of less help for the diagnosis of TB when the sample is collected from an extra pulmonary site or from immune comprised patient due paucibacillary condition.

The culture techniques present for the growth of tuberculosis were promising and more of a gold standard. The standardized procedures were conventional methods which took 8-12 weeks known as Lowenstein-Jenesson medium for growth of *M.tuberculosis.* But LJ could not help the growing burden of tuberculosis. LJ would take weeks and months which challenges diagnosis and treatment and results in delay leading to death of the patients. These challenges lead to loss of life with increasing burden of XDR and HIV. Then a research media by middle brook in form of liquid culture came to the field which decreased the turnaround time for 4 to 8 weeks to 2-4.[3-4] To grow a culture of tuberculosis it require 10-100 of tuberculosis to detect in culture which ranges from 80-95% and specificity as high as 98%.. Though traditional methods were but not enough as they demanded time of the patients and their life. MDR TB is defined as resistance to rifampicin and Isoniazid the two most important drugs in first line for the treatment of tuberculosis.

The rapid technique developed to cover up the 8 weeks to 72 hours. The two drugs most important in treatment of tuberculosis were Rifampicin and Isoniazid and the targets are RpoB, katG and InhA respectively.[5]The diagnosis of TB in clinical set up was based on the clinical investigation before the rise of technology.

The diagnosis was on basis of latent TB infection and the test to support the presence of *M. tuberculosis* in the patient was based on the reactions thus associated. Though TST was a well relied technique but the reporting and recording of the test was a stone in the way of correct diagnosis. One issue that the specificity of serum test is diminishing basically if the person is sensitized to BCG Vaccine earlier in life.[6]

Resistance to rifampicin

The treatment of tuberculosis depends on multi drugs with a combination of both bactericidal and bacteriostatic drugs given for duration of not less than 6 months previously intermittent but was changed to daily fixed dose.

The drugs in the combination are Streptomycin, Isoniazid, Rifampicin, Ethambutol, Pyrazinamide and Para amino salicylic acid. More than 90% of drug sensitive Tb can be cured by the regimen mentioned but the story changes when the most important drugs Rifampicin and Isoniazid gets resistant and the patient does not respond to the drugs regimen and so termed as Drug resistant TB.[7] Rifampicin resistant being the most potent marker to term the patient MDR and give a treatment with less effective more toxic second line drugs. Rifampicin being the most potent first line drug inhibits DNA directed RNA synthesis by binding to the RNA polymerase subunit. *Mycobacterium tuberculosis* frames a great escape mechanism by mutating the RNA polymerase . In many phenotypic studies mutations in RpoB gene leads to resistance to action of rifampicin drugs. Mutations in RpoB genes are largely in the region of 81 base pair region commonly read by researchers as hotspot region and RRDR region.[8]The treatment of the patients suffering from TB was a challenge as to rule out to rifampicin resistant at the very first step the conventional techniques were not an answer. The CBNAAT technique was a boom in the field of diagnosis and relief to the patients to get clear status of resistant at very first time diagnosed.

Genexpert role as game changer in diagnosis of tuberculosis

Genexpert Mtb/Rif (Cepheid Inc, Sunnyvale, USA) test is a rapid assay for the diagnosis of TB as well as rifampicin resistance in not less than two hours miraculously. Genexpert is a nucleic acid amplification test which detects tuberculosis by amplification of DNA in a closed and easy to handle set up.[9] It detects TB complex and rifampicin resistance in a most common RRDR hotspot region of 81 base pair. In a very short period of time in comparison to culture and other genotypic techniques compromised with best infrastructure and expert hands. According to the manufactures the test used 3 specific primers and 5 unique molecular probes to assure and guarantee a high degree of specificity The target in the assay is the RpoB gene, critical for identifying mutations associated with rifampicin resistance with sensitivity and specificity of 96.7% and 98.6% respectively. After the introduction of CBNAAT to diagnostic algorithms different studies were conducted which shows usefulness of CBNAAT not only in diagnosis of TB and resistance but major role played while diagnosis paucibacillary samples such as in HIV, Children, and extrapulmonary .The threshold number is 5 genome copies of DNA or 131 CFU of tuberculosis. The procedure is simple and simple to perform For each of the samples first open lid of sputum container and add Sample Reagent in 1:2 (v/v) where one is sample and 2 is Sample reagent, put the lid, tightly and shake 10 - 20 times. Incubate for 15 minutes at room temperature and let aerosol settle. Samples should be liquefied with no clumps present in the sputum.[10] In respect to study performance of CBNAAT in diagnosis of TB in pulmonary tuberculosis Sharma SK et al from AIIMS, India showed overall sensitivity and specificity of 95.7% and 99.3% respectively. In smear negative samples which came out to be culture positive cases, the test had a sensitivity of 77.7%. The sensitivity and specificity for detecting rifampicin resistance was 94.5% and 97.7% respectively with respect to culture and other tests and when the samples were tested by gene sequencing, the sensitivity and specificity was noted as high as 99.0% and 99.3% respectively.[11] This study clearly indicated promising results from CBNAAT with less result uncertainty.

CBNAAT plays an equally important role in diagnosis of TB in smear negative samples as discussed in study shown by K.N Mohan Rao et al.in which 80 clinically significant patients with AFB smear Negative status gave CBNAAT positive in 29 which was 30% positivity.[12]A study by M.singh et al showed the useful ness of Genexpert in

Diagnosis of TB in pediatrics from North Indian medical colleges with promising results in which out of 50 children with age group of 0-14 23 were diagnosed TB the rest were clearly negative for TB when tested by culture and with the gold standard the sensitivity and specificity was 84.6% and 86.4% respectively.[13] A study conducted in South Africa by G Theoron et al in 2011 and in 496 patients from a high HIV prevalence setting in which Xpert MTB/RIF detected 95% of smear-positive culture-positive cases and the specificity was 94%.When In comparison to smear microscopy Xpert MTB/RIF detected an additional 17 cases giving a rise of 18% in the rapid TB case detection.[14] Performance of CBNAAT in FNAC was discussed by Biadglegne F from Ethiopia in which 231 FNAC were tested in which the Xpert test in relation to culture identified 29 from 32 culture positive, 5 from 11 with contaminated results, and 56 from 188 culture negative results. The overall sensitivity of the test was 93.5% and specificity 69.2%.[15] All the studies clearly shows that CBNAAT changed the story of TB diagnosis from days months to hours and with high sensitivity and specificity and to add finding the unknown which would likely get missed.

CONCLUSION

In diagnosis of patients whether pulmonary or extra pulmonary children or PLHIV Genexpert MTb stands with high sensitivity and specificity with respect to smear microscopy. Though culture is still the gold standard but the time duration in culture can cost the life of patients and Genexpert again answers for point of care.

Though smear positive patients are detected within 30 minutes but an added benefit of CBNAAT to rule out Rifampicin resistance cannot be overlooked. The ongoing algorithm by WHO to test all patients on CBNAAT will help and diagnosis of resistance thus decreasing the suffering of patients and cutting the chain of infection of MDR.

In developing countries such as India it is essential to rule out drug resistance to know the true burden of MDR in the population to achieve end TB goal.

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CONFLICT OF INTEREST

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