DIAGNOSIS OF MDR TUBERCULOSIS AT A TERTIARY CARE CENTRE WITH CBNAAT TECHNOLOGY- A TWO YEAR STUDY

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ABSTRACT

BACKGROUND

Tuberculosis is a chronic multi system granulomatous disease with a predilection for the respiratory system. Traditional diagnostic protocols include history, chest x-ray and sputum collection and staining for acid fast bacilli along with culture in conventional media. These processes are time consuming and labour intensive. WHO recommends the use of Cartridge Based Nucleic Acid Amplification Testing (CBNAAT) using the Gene Xpert MTB/RIF apparatus which is a comparatively rapid PCR based test.

MATERIALS AND METHODS

In the present study, sputum and tissue samples from 1299 patients were taken and smear preparations were stained for acid fast bacilli. Subsequently, the samples were subjected to CBNAAT testing for both the presence of mycobacterium tuberculosis as well as rifampicin resistance, which is a marker for INH resistance as well. Rifampicin resistant tuberculosis is thus labelled as multi drug resistant tuberculosis (MDR TB).

RESULTS

In the results of the study, mycobacterium tuberculosis (MTB) was found in 1275 cases, Rifampicin sensitivity was found in 1095 cases, rifampicin resistance in 176 cases and indeterminate results in 4 cases. In 122 cases of the present study, CBNAAT has detected mycobacterium tuberculosis (MTB) where the cases were smear negative. On the other hand, 109 smear positive cases have been declared MTB negative by CBNAAT testing. 26 cases showed error, invalid test and no results. In 4 cases though MTB was detected, rifampicin resistance testing showed indeterminate results.

CONCLUSION

CBNAAT technology may be used as the standard diagnostic tool for tuberculosis. However for disputed cases, referral systems must be in place for mycobacterial culture and drug testing at reference laboratories.

KEYWORDS

Mycobacterium tuberculosis, Polymerase Chain Reaction, Acid Fast Staining, Cartridge Based Nucleic Acid Amplification Test, Rifampicin, Multi-Drug Resistance.

HOW TO CITE THIS ARTICLE: Saha P, Saha R. Diagnosis of MDR tuberculosis at a tertiary care centre with CBNAAT technology - a two year study. J. Evid. Based Med. Healthc. 2018; 5(53), 3648-3653. DOI: 10.18410/jebmh/2018/743

BACKGROUND

Tuberculosis (TB) is a disease which has its roots in antiquity. The organism has its only natural reservoir in man and is difficult to culture in animal models. The resurgence of tuberculosis in recent years in India is a result of several factors. The most important cause of the surge in TB cases is the lack of drug compliance of the existing TB patients, partly due to the lack of education and explanation of the course of the disease and its complications. The important point is that patients do not realize that treatment of TB is a long drawn out process and strict adherence to the drug regime is of paramount importance. It is not by any means sufficient to discontinue and resume treatment as and when convenient. The other aspect is lack of proper reporting of cases and contacts and accurate documentation and information sharing. All these factors have resulted in a huge load of multi drug resistant TB (MDR TB) in India, some of which are also extensively drug resistant (XDR TB).

The cornerstone for the management of tuberculosis today remains early and rapid diagnosis, drug susceptibility testing, contact and high risk group screening and timely and effective treatment particularly cases of TB associated with concurrent immunosuppressive conditions like HIV infection.

District level tuberculosis programs are under the supervision of the District Tuberculosis Officer in the RNTCP Scheme (Revised National Tuberculosis Control Program). Under this scheme, sputum is collected and tested for all suspicious cases of cough, haemoptysis, fever, loss of weight and other suggestive cases and tested for acid fast

Financial or Other, Competing Interest: None.
Submission 11-12-2018, Peer Review 18-12-2018,
Acceptance 25-12-2018, Published 31-12-2018.
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DOI: 10.18410/jebmh/2018/743

bacilli with the Ziehl Neelsen stain, followed by culture in Lowenstein Jensen or liquid media. The testing facilities are provided at the district level in district hospitals, sub divisional hospitals, rural hospitals and medical colleges.

It should be noted that culture of TB bacteria is not a simple or rapid procedure. Growth of mycobacterium tuberculosis takes an average of 4-6 weeks in conventional culture media. This is followed by testing of the colonies to look for the presence of atypical mycobacteria. At present, presence of mycobacterium tuberculosis DNA is detected more rapidly by using gene amplification through PCR technology, commonly known as CBNAAT (Cartridge Based Nucleic Acid Amplification Test).

The modern CBNAAT testing equipment has been made available in many district tuberculosis centers. Drug testing facilities are provided in regional laboratories scattered in different zones of the states. Samples are sent to these reference laboratories from a number of districts and results are dispatched to the source hospitals. The equipment is distributed to government institutions and testing is free of cost. However, the price for performing this test is quite high in the private sector and may be out of reach of much of the population.[1]

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Malda Medical College over a period of 2 years from March 2016 to February 2018. Sputum samples were collected from the cases referred by the departments of General Medicine, General Out Patient Department, ENT department and any other department which suspected the presence of tuberculosis in a patient. Cases were also referred to the TB treatment centre by outlying health centers, rural hospitals and private practitioners. Some cases reported to the TB unit on their own, being contacts and relatives of existing TB patients.

Sputum and occasional tissue samples from 2499 patients was taken for smear preparation and acid fast staining along with PCR based identification using CBNAAT technology. The commercial set up used was Gene Xpert MTB / RIF (Figure 1), which detected both presence of Mycobacterium tuberculosis DNA and drug resistance to rifampicin. This is the diagnostic process recommended by the World Health Organization since December 2010. It is to be used for the initial diagnosis of pulmonary, meningeal, lymph nodes and all forms of extra pulmonary tuberculosis.[2] The selection of CBNAAT testing protocols has been further validated as regards it scientific accuracy by several other studies.[3]

In conventional testing, rifampicin (RIF) resistance is used as a surrogate marker for multidrug resistant (MDR) tuberculosis, resistant to both rifampicin and isonicotinic acid hydrazide (INH). RIF resistance is a taken to be a predictor of MDR TB because resistance to RIF, in most instances, co-exists with resistance to INH. The process identifies most of the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the Mycobacterium tuberculosis genome in a real time format using fluorescent probes called molecular beacons. Apart from sputum, in selected cases extrapulmonary tissue material was also used in this assay.

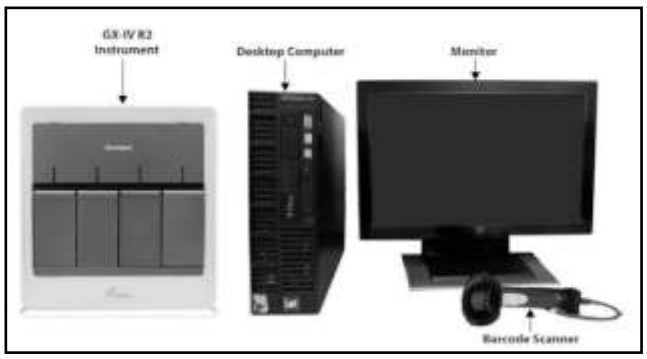


Figure 1. Cepheid GeneXpert CBNAAT Diagnostic System

Gene Xpert MTB / RIF is a real time PCR based assay developed by Cepheid Inc. The setup consists of a disposable cartridge assembly with chambers for sputum or other tissue samples and appropriate reagents. The loaded cartridge is then fed into the Gene Xpert machine.

The Gene Xpert MTB/RIF machine accepts the Gene Xpert cartridges that are loaded into the instrument, lyses the bacterial and human cell samples in the cartridges with the help of ultrasound, releases the nucleic acids, and amplifies the target sequences. Because the system allows us to control the multiple cartridge modules independently in a single machine, we can process different samples using different types of PCR based tests in the same instrument at the same time. The machine houses the thermal recycler system for heating and cooling resulting in sequential denaturation, annealing and extension of native mycobacterial DNA and the specific sequence primers which act as molecular probes. The PCR program is usually set for 45 cycles.

The molecular probes are labelled with fluorescent dyes and bind with specific target regions on the mycobacterial DNA. Light from high intensity LEDs are focused on the reaction tube causing the fluorescent dyes to become excited and radiate emission spectra which are captured and analysed by a six colour detection module.

The third part of the installation is a desktop or laptop computer which allows us to run the GeneXpert Dx System software and hosts the GeneXpert Dx System results database. The software allows us to select assay definitions, monitor the test process, view the results, and export selected data to other software for additional analysis. The software also allows us to archive and retrieve the results data and manage the database.[4]

The GeneXpert MTB/RIF apparatus detects mycobacterium tuberculosis and rifampicin resistance by PCR amplification of the 81-bp fragment of the MTB rpoB gene and subsequent probing of this region for mutations that are associated with RIF resistance.

Compared to conventional culture (4-6 weeks) and drug susceptibility testing (another 3 weeks) the Xpert MTB/RIF

assay detects the presence of MTB and rifampicin resistance in less than 2 hours.

RESULTS

Data for the present study were taken over a 24 month period from March 2016 to February 2018. Presumptive cases of TB sent to the Dept. of Microbiology, Malda Medical College for CBNAAT testing were part of the study. The data generated by the study is presented in a tabular form in the following section.

Parameter	Number of Cases											
	Mar 2016	**Apr 2016**	**May 2016**	**Jun 2016**	**Jul 2016**	**Aug 2016**	**Sep 2016**	**Oct 2016**	**Nov 2016**	**Dec 2017**	**Jan 2017**	**Feb 2017**
MTB Detected & Rifampicin Sensitive	24	32	33	59	76	72	66	32	53	62	37	30
MTB Detected & Rifampicin Resistant	09	06	08	13	13	10	07	09	07	07	03	04
MTB Detected & Rifampicin Testing Indeterminate	00	00	00	00	00	01	00	00	01	00	00	00
MTB not Detected	35	14	15	36	47	75	49	18	30	30	39	65
Total Number of Errors	01	01	01	01	00	00	01	00	00	00	00	00
Number of Invalid Tests	00	00	01	01	00	01	00	00	00	01	02	01
Number of "No Results"	00	00	00	00	00	02	00	00	00	00	00	00
Total Number of Tests using CBNAAT	**69**	**53**	**58**	**110**	**136**	**161**	**123**	**59**	**91**	**100**	**81**	**100**

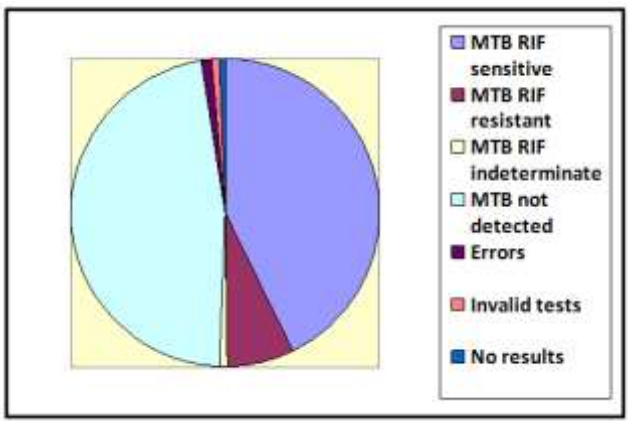
Table 1. Spectrum of CBNAAT Tested Cases of Presumptive TB from March 2016- February 2017

Parameter	Number of Cases											
	Mar 2017	**Apr 2017**	**May 2017**	**Jun 2017**	**Jul 2017**	**Aug 2017**	**Sep 2017**	**Oct 2017**	**Nov 2017**	**Dec 2017**	**Jan 2018**	**Feb 2018**
MTB Detected & Rifampicin Sensitive	48	23	00	28	37	41	45	32	61	67	63	74
MTB Detected & Rifampicin Resistant	07	04	00	03	06	08	09	07	10	10	11	05
MTB Detected & Rifampicin Testing Indeterminate	01	00	00	00	00	00	00	00	00	00	00	01
MTB not Detected	38	28	00	27	70	103	78	50	84	75	78	114
Total Number of Errors	02	00	00	03	00	00	00	00	00	00	00	01
Number of Invalid Tests	03	02	00	00	00	00	00	00	00	00	00	01
Number of "No Results"	00	00	00	00	00	00	00	00	00	00	00	00
Total Number of Tests using CBNAAT	**99**	**57**	**00**	**61**	**113**	**152**	**132**	**89**	**155**	**152**	**152**	**196**

Table 2. Spectrum of CBNAAT tested cases of presumptive TB from March 2017 - February 2018

Testing Parameter	No. of Cases
MTB RIF Sensitive	1095 (43.82%)
MTB RIF Resistant	176 (7.04%)
MTB RIF Indeterminate	04 (0.16%)
MTB not Detected	1198 (47.94%)
Errors	11 (0.44%)
Invalid Tests	13 (0.52%)
No Results	02 (0.08%)
Total	**2499**

Table. 3. Summary of CBNAAT Test Results



Graph 1

Parameter	Total No. of Cases
Smear -ve & CBNAAT +ve	122
Smear +ve & CBNAAT -ve	107

Table 4. Comparison between Smear and CBNAAT Results

DISCUSSION

Accurate diagnosis of tuberculosis and determination of drug resistance has been a major challenge for clinicians and microbiologists worldwide for the last few decades. The most common case finding procedure has been a constellation of signs and symptoms suggestive of respiratory disease coupled with general ill health, loss of weight and occasional haemoptysis. "Presumptive TB" refers to cases having cough or fever for > 2 weeks, weight loss, haemoptysis or abnormal chest x-rays.[5] This was followed up with sputum smears stained for acid fast bacilli and culture of sputum and other body fluids and tissues in appropriate media. The emergence of CBNAAT testing protocols has revolutionized the diagnosis and treatment of tuberculosis in recent times with an MTB/RIF test sensitivity of 88% and specificity of 99%.[6] These results are further reinforced by independent studies.[7] WHO recommends the use of CBNAAT Xpert MTB/RIF as the initial diagnostic test in adults and children with a probability of MDR TB. However, the procedure is not without its problems and pitfalls. The accuracy of testing is however greater in pulmonary as compared to extra pulmonary tuberculosis.[8]

In the present study 2499 cases had been sent to the Dept. of Microbiology for CBNAAT testing for tuberculosis. Out of this number mycobacterium tuberculosis (MTB) was found in a total of 1275 cases. Of these cases, Rifampicin sensitivity was found in 1095 cases, rifampicin resistance in 176 cases and indeterminate results in 4 cases. Thus, about 14% of the detected cases of MTB were rifampicin (and possibly multi drug) resistant (Tables 1, 2 & 3). Rifampicin resistance has been reported in wide variations in different studies in India ranging from around 2.5% in newly diagnosed cases to 16% in old treated cases.[9] The figures of MDR TB soar to 56% in crowded metropolitan cities like Mumbai.[10]

In 122 cases of the present study, CBNAAT has detected mycobacterium tuberculosis (MTB) where the cases were smear negative (Table 4). This proves the ability of CBNAAT to detect cases of TB which would be missed by conventional testing.[11] These cases were referred for CBNAAT testing based on clinical examination and a high index of suspicion. The smear negativity may arise because of faulty smear preparation and staining for acid fast bacilli. This may be due to improper sputum collection, bad quality stains or improper procedure. Low smear positivity in spite of MTB infection may naturally occur in regions of low MTB prevalence.

On the other hand, 109 smear positive cases have been declared MTB negative by CBNAAT testing (Table 4). It is these cases which have now captured our attention and created a diagnostic dilemma for the clinician. The possible cause of this situation is twofold - improper sputum sample collection, prolonged storage in hot environment and secondly incorrect use of the CBNAAT apparatus. Studies by various authors have shown a high degree of sensitivity of CBNAAT in smear positive cases.[12]

A very important finding of the present study is that in almost half of the sputum samples sent for CBNAAT testing, MTB was not detected. This reflects the lack of proper screening from the primary healthcare level. The threat and terror of tuberculosis is such that almost all cases of prolonged cough and cold are being referred for CBNAAT testing in most cases without sputum smear staining for acid fast bacillus. AFB staining requires considerable time, energy, experience and expertise as well as trained personnel.[13] The lack of proper laboratory facilities and technicians in the peripheral health setups has forced the health workers and doctors in the periphery to refer large number of cases of various respiratory ailments and chronic ill health for CBNAAT testing to tertiary care centers. On the whole, CBNAAT testing is still more efficient than attempts to diagnose tuberculosis at the primary care level in spite of being a burden on tertiary care centers.

The diagnostic pitfalls of MTB testing are further compounded by a few "indeterminate" test results. Indeterminate results in this context mean that the sample is MTB positive but the CBNAAT apparatus is unable to determine RIF resistance. In such cases, as the patient is MTB positive, antitubercular drugs have to be started but the drug combination cannot be decided upon. These cases may be re-tested on CBNAAT and if the confusion persists, have to be forwarded to a reference laboratory for additional testing using line probe assays and liquid culture.[14] In fact, further testing using other modalities after an Xpert MTB/RIF negative report is considered to be better than an immediate repeat Xpert test.[15]

Errors arose in a number of samples due to various reasons - some were due to high sputum viscosity and excess pressure, incorrect sample volume, bubbles or incorrect filling of cartridge reaction tube. Invalid results occur due to internal control failure arising because of sample contamination with pus or food particles causing inhibition of PCR. The CBNAAT apparatus may show a "no result" when the test could not be completed or insufficient

data was collected. This is usually due to power failure or accidental activation of "stop test" function. In all such cases, retesting is desirable, if possible with a fresh sample. If faulty results persist, the samples have to be forwarded to a reference laboratory. In the present study errors, invalid study and "no result" were the outcome in 15 samples.

On the whole it appears to be a good policy to confirm rifampicin resistant MTB cases by culture and additional drug sensitivity testing for increased accuracy in diagnosis.[16]

CONCLUSION

Tuberculosis is a raging public health problem faced by clinicians today. The spectrum of infectious cases of pulmonary tuberculosis is compounded by tuberculosis of other organs systems like genitourinary, bone and joint, gastrointestinal and neurological cases of tuberculosis. The traditional method of radiology, sputum and tissue smear testing and conventional culture for acid fast bacilli is cumbersome, technically difficult and time consuming. WHO now recommends rapid testing with the use of PCR technology with the CBNAAT apparatus. In the present study, samples from 2499 cases were tested using the Gene Xpert MTB/RIF machine for presence of MTB genes as well as multidrug resistance using rifampicin resistance as a surrogate. Overall, MTB was detected in 51.02% cases of which 43.82% cases were rifampicin sensitive, 7.04% cases were rifampicin resistant and 0.16% gave indeterminate results for RIF sensitivity. MTB was not detected in 47.94% cases in spite of being clinically suspicious and 1.04% cases gave error and invalid test results.

The study clearly demonstrates that in spite of our best efforts, CBNAAT testing is not the final solution to all diagnostic problems of MTB detection and drug resistance. Poor performance in this testing procedure is mainly due to technical errors and non-adherence to standard operational procedure.[17] Disputed cases need repeat-testing and frequent transfer to referral laboratories for further tests and culture. There also remains a case for clinical suspicion of TB and therapeutic trial of anti TB drugs. However, all things considered, CBNAAT testing still remains the most accurate and time saving diagnostic test with a high level of standardization and reproducibility. It is hoped that the continued use of this technology will minimize procedural errors and bring about a revolution in the diagnosis and treatment of this dreaded disease.

Abbreviations

TB- Tuberculosis, MTB- Mycobacterium tuberculosis, RIF- rifampicin, PCR- Polymerase Chain Reaction, CBNAAT- Cartridge Based Nucleic Acid Amplification Test, MDRTB- Multidrug Resistant Tuberculosis, XDRTB- Extensively Drug Resistant Tuberculosis, HIV- Human Immunodeficiency Virus, WHO- World Health Organization, LED- Light Emitting Diode.

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