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Role of CBNAAT for Early Diagnosis of Pulmonary Tuberculosis and Percentage of Rifampicin Resistance among the Patients Admitted in Government General Hospital, Siddipet

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Abstract: ***India is the highest tuberculosis (TB) burden country in the world. The global incidence of multidrug-resistant TB (MDR-TB) is 630,000 cases. India constitutes one tenth of the global burden with 64, 000 cases, presently. Though the conventional drug susceptibility testing (DST) is con standard" for the detection of drug-resistant TB, it is time-consuming taking about 6–8 weeks. Hence, there is need of introduction of rapid diagnostic tools to detect MDR-TB. Cartridge-based nucleic[1]. Aims and objective of the study: To assess diagnostic usefulness of Gene Xpert MTB/RIF assay technique in comparison with routine smear microscopy, so that early detection is essential to reduce the death rate and interrupt transmission and detection of percentage of rifampicin resistance among the positive samples, it helps to avoid injudicious use of anti tubercular drugs. Material and methods: This is a retrospective study conducted in the department of Microbiology, Government General hospital, Siddipet, between January 2018 to December 2018. Early morning sputum sample was collected from the patients in a clean sterile container. Results: Among the total 2129 samples, 474 were detected tuberculosis positive by CBNAAT method and 1568 were negative. And among 474 positive cases, 439 were Rifampicin sensitive and 35 were Rifampicin resistant. Conclusion: CBNAAT detects pulmonary TB with greater efficacy than sputum microscopy also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so that early therapy can be started thus decreasing the incidence of MDR-TB. WHO recommends CBNAAT for early diagnosis of pulmonary tuberculosis and detection of rifampicin resistance and retreatment cases, who are at risk of MDR-TB.***

1. Introduction

Tuberculosis remains the most common opportunistic infection among PLHIV and HIV-TB co-injected individuals are at high risk of death. Standard sputum based methods to detect pulmonary tuberculosis include sputum microcopy and culture. However, in PLHIV, there is scantly sputum production, lack of caseous necrosis leading to decreased number of bacilli in sputum, and high incidence of non-tuberculer mycobacterial infection. These factors decrease the sensitivity and specificity of sputum microscopy as diagnostic tool.

To overcome these short comings, sputum culture and sensitivity for Mycobacteria can be used. But it is a slow test usually taking 4-8 weeks, not widely standardized, and not economical. This delays initiation of anti-tubercular treatment, especially for drug resistant forons of TB[6].

Catridge-based nucleic acid amplification test (CBNAAT) is a recently introduced polymerase chain reaction (PCR) based methods for detection of TB. It also detects rifampicin resistance, as it targets the rpo B gene of mycobacreria. CBNAAT is a mycobacterium tuberculosis specific automated, catridge based nucleic acid amplification essay, having fully integrated and automated amplification and detection using real time PCR, Providing result within 100 minutes. It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the rpo B gene of mycobacterium tuberculosis which is the critical gene associated with rifampicin resistance[2].

The global burden of TB remains enormous. More than 9 million new M tuberculosis (MTB) cases and 1.7 million deaths occur annually worldwide. Most of them occur in resource-limited settings.1 Smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive.

Smear microscopy is the cornerstone for the diagnosis of TB in resource-limited settings but it has only modest (35-80%) sensitivity and a poor positive predictive value (PPV). Culture is the "gold standard" for final determination, and also permits drug susceptibility testing. However, it remains largely inaccessible in resource limited settings as a result of infrastructure and financial limitations. Even where accessible, culture results are typically not available for 2-6 weeks. Diagnosis through either smear or culture requires multiple steps that significantly impede program effectiveness. The need is for accurate, feasible, rapid, affordable, and if possible, near-point-of-case TB diagnostic tests for use in resource limited settings.

Drug resistance is a major issue in the treatment of tuberculosis. Though rare for Rifampicin (RIF), drug resistance is common in other 1st line drugs Isoniazid (INH), Ethambutol (EMB), and Pyrazinamide (PZA). Multi drug resistance is a reflection of either mismanagement of tuberculous patient's wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and mistreatment of both first and second line drugs. Injudicious use of drugs is to be avoided in the better interest of patients. Thus, for rapid identification, which is essential for earlier initiation of treatment and improved outcomes, more effective public

health interventions and newer methods of detection are required. Multiple approaches to improve diagnosis of TB are in development. One test, Gene Xpert® MTB/RIF, which was recently endorsed by the World Health Organization (WHO), has the potential to lead a revolution in the diagnosis of active TB disease and multidrug-resistant (MDR) TB[3].

2. Materials and Methods

A retrospective study of all the known HIV positive patients, who were suspected to have pulmonary tuberculosis, attended to government general hospital siddipet from July to December 2018 a total of sputum samples was sent as well as for CBNAAT.

Early morning deep coughed sputum specimens in sterile containers were included in the study. Specimens were stored at 2-8 degree c in freezer till further processing and stored at 35 degree c for three days[3].

CBNAAT is an automated, rapid catridge based method for TB case detection and rifampicin resistance. It was launched in 2004, by cepheid and was partnered with FIND (Foundation for innovative new diagnostics)[3].

Components
Modules- Thermal and Optical systems
Cartridge- Self contained disposable kit
Computer system- Software, barcode scanner

Parts of the machine
Plunger motor, plunger drive shaft, mother board, I core, Cartridge insertor, valve drive motor, ultrasonic horn.

Parts of the Cartridge
Processing chambers, Reaction tube, valve body

- Processing chambers – contains beads, reagents, primers and probes, buffers

Reaction tube – performs a rapid thermal cycling and optical excitation and detection.

- Valve body- by turning it directs fluids into different chambers and the PCR tube.

CBNAAT
CBNAAT is a rapid cartridge based fully automated NAAT (Nucleic acid amplification test) for TB case detection and Rifampicin resistance testing suitable for use in disease endemic countries. It was launched in 2004 by Cepheid and was partnered with FIND (Foundation for Innovative New Diagnostics)

CBNAAT system

3. Procedure

Collect the sample in a falcon tube or universal container. Add sample reagent to the sample in a ratio 2:1. Cap and shake it vigorously for 20 times. Keep the tube in a rack and let it stand still for 10 min. Reshake contents for 20 times and leave for 5 more min. Affix sample in to the side of cartridge. Pipette 2ml of sample and carefully transfer to open port of cartridge. Close the lid till it snap closed. Scan the cartridge bar code. Open the module door where the light is blinking. Insert the loaded cartridge into the bay and close the module door properly[4].

Working of the machine
Sample is automatically filtered and washed. This concentrates bacilli and removes inhibitors. Ultrasonic lysis of filter captured bacilli occurs to release DNA. DNA is mixed with dry PCR reagents. The solution is then pimped into the reaction tube. Here seminested real time amplification and detection accurs. Test results can be displayed on the monitor which can be printed.

Detection of Rifampicin resistance
CBNAAT uses molecular beacon technology to detect Rif resistance. Molecular beacons are probes that recognize and report the presence or absence of the normal Rif sensitive wild type sequence of the rpo-B gene of MTB. rpo-B gene has a 81 bp Rif resistance determining region (core region).5 different coloured beacons are used covering a separate nucleic acid sequence (wild type) within the amplified rpo-B gene. When a beacon binds to the matching sequence it fluoresces which is characteristic of Rif sensitivity. If beacon binds fails to bind to the mutant sequence or if binding is delayed, the sample is potentially resistant to Rifampicin[4].

4. Result

A total of 2129 samples were tested by CBNAAT in a period of one year that is from January 2018 to December 2018, at GMC siddipet. Among these, 474 were TB positive by CBNAAT and 1568 were negative. And among the TB positive cases, 35 were Rifampicin resistance.

Table 1

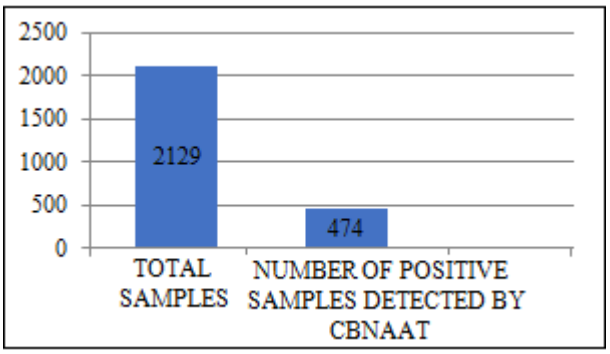


Table 2

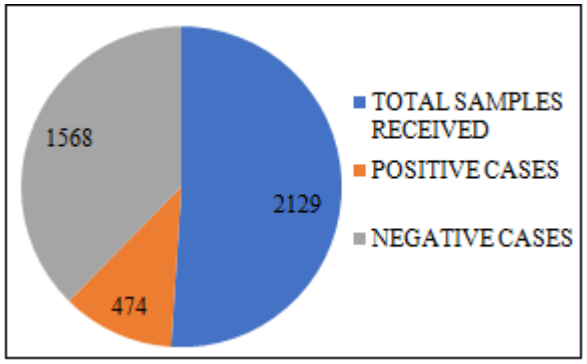
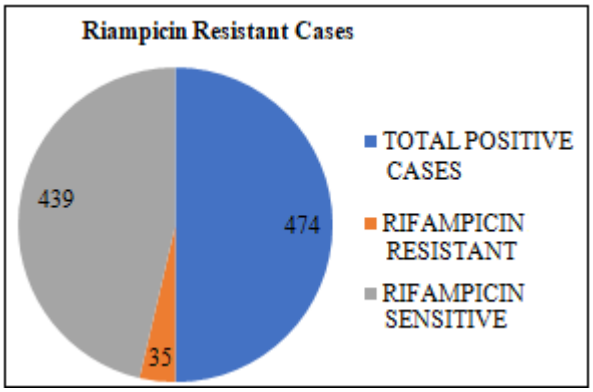


Table 3



5. Discussion

This study was conducted to evaluate the role of CBNAAT and detection of RIF resistance among pts suspects to be TB positive at GMC Siddipet a total number of 2129 sputum samples were tested, which were suspected to have tuberculosis out of which 474 (22.26%) were tuberculosis positive and 1568 (73.64%) were non tuberculosis samples, 35(7.38%) were Rifampicin resistance and 439(92.6%) were Rifampicin sensitive.

Early morning sputum samples were tested by CBNAAT method the positive have no specific age group and irrespective of sex.

Sputum microscopy for AFB is simple economical and easy to do test for diagnosing pulmonary tuberculosis. However as it needs at least 10, 000 bacili per ml to give a positive result and being a highly subjective (operator dependent) test, its sensitivity has been shown to range from 20% to 60% under different conditions[2].

CBNAAT on the other hand, not only detects tuberculosis positive cases, it also detects rifampicin resistance cases within 2 hours.

Past studies on drug resistance have shown that rifampicin resistance is seldom detected alone and 90% of rifampicin resistance patients turn out to be MDR-TB, Hence CBNAAT can be a useful test for screening for MDR-TB. This is of particular reference to TB endemic area like India where there is high prevalence of MDR-TB of around 3% in new cases and 12-18% in old treated cases(and sputum microscopy is the only screening test used to diagnose tuberculosis).

There are only few studies on CBNAAT from India. A study done in 2011 in Hyderabad showed incremental case detection of 10.8% when CBNAAT was used to diagnose tuberculosis over and above fluorescent microscopy.

The who policy guidance on the use of CBNAAT was issued in December 2010. The recommendations were that it should be used as initial diagnostic test in individuals at risk of having MDR-TB or HIV associated TB and that it could be used as a fellow on test to microscopy in setting were MDR is of lesser concern, especially in smear negative specimens[2].

There are several reasons why the findings of this study might not translate widely into improved care for patients with tuberculosis. First, only reference facilities were used in the study, and it is not certain that our findings would be replicated in microscopy centers, health posts, and other point-of-treatment settings where temperature and electricity supply will be more variable and training issues will be more relevant. However, qualitative questionnaires that were completed during the study suggested that users considered 2 to 3 days a sufficient duration of training for technicians without previous molecular experience (as compared with 2 weeks for Ziehl–Neelsen microscopy). The relative simplicity of the MTB/RIF test, plus its hands-on time of under 15 minutes and its unambiguous readout, is advantageous, whereas the need for annual calibration was identified as a challenge for implementation at peripheral laboratories, especially in rural areas. Large scale projects to show the feasibility and effect of MTB/RIF testing at such sites are under way.

Second, to achieve great simplicity of use, the MTB/RIF test uses sophisticated technology, which is costly to manufacture. Although FIND has negotiated concessionary pricing for public-sector programs in low-income countries and is working to further lower the costs of testing, the costs of instruments and tests will still be considerably higher than those for microscopy, which is all that is currently available in peripheral health care settings in many countries. However, MTB/RIF testing could be less costly than implementation of culture and drug-susceptibility testing.

Globally, ineffective tuberculosis detection and the rise of multidrug resistance and extensively drug-resistant tuberculosis have led to calls for dramatic expansion of culture capability and drug-susceptibility testing in countries in which the disease is endemic. Unfortunately, the

infrastructure and trained personnel required for such testing are not available except in a limited number of reference centers, and results of testing are often not available for at least 4 months, which dramatically reduces its clinical utility. The complexity of standard nucleic acid–amplification tests prevents the expansion of this method. The MTB/RIF test automates DNA extraction, amplification, and detection inside a test cartridge that is never reopened, with little chance of amplicon contamination. Specimen processing is simplified to a single nonprecise step that both liquefies and inactivates sputum, which results in a reduction in viable tubercle bacilli of 6 to 8 logs and eliminates the necessity for a biosafety cabinet. Data from a recent study confirm that the MTB/RIF assay generates no infectious aerosols. These features of simplicity and safety of use could allow for costeffective and highly sensitive detection of tuberculosis and drug resistance outside reference centers, which would increase access to testing and decrease delays in diagnosis, early detection is essential to reduce the death rate and interrupt transmission without the need to build large numbers of laboratories equipped for advanced biosafety.

6. Conclusion

CBNAAT detects pulmonary TB with greater efficacy than sputum microscopy also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so that early therapy can be started thus to interrupt transmission, decreasing the incidence of MDR-TB and to decrease the death rate. WHO recommends CBNAAT for early diagnosis of pulmonary tuberculosis and detection of rifampicin resistance and retreatment cases, who are at risk of MDR-TB.

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