ejpmr, 2020,7(1), 401-406

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THE PERFORMANCE OF NUCLEIC ACID AMPLIFICATION TESTS FOR THE DIAGNOSIS OF TUBERCULOSIS AND RESISTANCE TO RIFAMPICIN IN DOTS CENTRES OF A DISTRICT IN SOUTH INDIA

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Article Received on 13/11/2019 **Article Revised on 04/12/2019** **Article Accepted on 25/12/2019**

ABSTRACT

Title of Study: The Performance of Nucleic Acid Amplification Tests for the Diagnosis of Tuberculosis and resistance to rifampicin in DOTS centres of a district in South India. **Background and Objectives:** According to WHO the number of new tuberculosis (TB) cases reported in India in 2017 was 2740 and the rate of incidence of MDR-TB was 10/100,000 population. Early diagnosis facilitates treatment initiation and can limit the spread of the disease. The Revised National Tuberculosis Programme (RNTCP) has scaled up the use of the Cartridge Based Nucleic Acid Amplification Test (CBNAAT), also known as GeneXpert, and provides it free of cost to the patient. In addition to the existing 628 Machines, 507 machines have been deployed to cover all districts of the entire country. The CBNAAT is provided free of cost This study was undertaken to assess the performance of the CB-NAAT test in peripheral DOTS centres of this predominantly rural district. **Methods**: The diagnostic accuracy of the CB-NAAT test in diagnosing tuberculosis in patients presenting at the various DOTS centres of this district was assessed. After approval from ethics committee and written permission from the district tuberculosis officer, patients suspected to have tuberculosis, registered at the DOTS clinic for CB-NAAT 178 participants were serially recruited. **Results:** Of the 178 samples studied, 81 (45.5%) had a positive CBNAAT, while 71 (39.9%) had positive smear microscopy. The remaining 107 smear samples and 97 CBNAAT samples were negative. Of the 81 mycobacteria positive samples diagnosed by CBNAAT, 62 were also diagnosed by smear microscopy, giving a sensitivity of 87.3% while 19 cases identified by CBNAAT were sputum smear negative. Of the 107 smear microscopy negative samples, CBNAAT was negative in 91 subjects giving a specificity of 82.2%. The high positive predictive value (85.2%) indicates the strong probability that a person with a positive test has tuberculosis. The high negative predictive value (95.7%) shows that a negative test in effect rules out tuberculosis. The high likelihood ratio of nearly 5 with a confidence interval of 3.24 to 7.46 tells us that the test has a high probability to identify the presence of mycobacteria. **Interpretation & Conclusions:** The sensitivity of the CB-NAAT test obtained in our study was 87.3% and specificity was 82.20%. Rifampicin resistance was present in less than one-tenth of the samples (7.3%). The study showed that CB-NAAT is an equally sensitive and specific rapid molecular diagnostic test to detect TB and rifampicin resistance in biological specimens in patients suspected to have tuberculosis.

KEYWORDS: Diagnosis of Tuberculosis, Gene expert, CB-NAAT, rifampicin resistance, sputum microscopy.

INTRODUCTION

According to the World Health Organisation (WHO), the number of new cases of tuberculosis reported in India in 2017 was 2740 (rate of 204/100,000 population) with the rate of incidence of drug resistant tuberculosis (DR-TB) / rifampicin resistant tuberculosis (RR-TB) being 10/100,000 population.[1] Early diagnosis facilitates treatment initiation and can limit the spread of this highly contagious disease. The commonly used diagnostic methods are slow, insensitive, cumbersome and inaccessible.[2]

The Revised National Tuberculosis Programme (RNTCP) has scaled up the use of rapid molecular diagnostic tools like the Cartridge Based Nucleic Acid Amplification Test (CBNAAT), also known as GeneXpert, and has over 735 CBNAAT facilities for decentralized DR-TB testing. In addition to the existing

628 Machines, 507 machines have been procured and deployed to cover all districts of the entire country.[3]

The role of CB-NAAT with a potential to diagnose TB and rifampicin resistance within two hours is promising. We will not always arrive at a diagnosis in patients suspected to have tuberculosis with smear microscopy alone and sputum culture and sensitivity is a slow method which takes 4-8 weeks for mycobacterial growth. It is also not widely standardised and not bacterial species tested.[4] Boehme et al, who reported their assessment of the performance of CB-NAAT, in 1730 patients with suspected drug-sensitive or drug-resistant pulmonary tuberculosis, found this test provided sensitive detection of tuberculosis and RR-TB directly from untreated sputum in less than two hours with minimal hands-on time.[5]

In 2009, the Centres for Disease Control and Prevention updated its guidelines to recommend CB-NAAT testing on specimens obtained from the respiratory tract of patients suspected to have pulmonary tuberculosis.[6]

India has scaled up basic TB services in the public health system, treating more than 19 million TB patients under RNTCP, to meet the 2030 Sustainable Development Goals (SDG) and 2035 End TB targets. The requirements for moving towards TB elimination have been integrated into the four strategic pillars of "Detect – Treat – Prevent – Build" (DTPB).[3]

India achieved complete geographical coverage for diagnostic and treatment services for multi-drug resistant tuberculosis (MDR-TB) in 2013 with a remarkable number of 93000 persons with MDR-TB being diagnosed and put on treatment till 2015. However, India continues to be the country with the highest burden of tuberculosis in the world with an incidence of 2.8 million cases in 2015. Within the public sector, there is a heavy dependence on insensitive diagnostic tests and sputum microscopy and there is no way of detecting drug resistance.[7]

The failure of early detection and early initiation of treatment leads to increased emergence of DR-TB and ongoing transmission. In order to tackle this urgent need for a rapid diagnostic tool, a fully automated molecular test for case identification as well as detection of drug resistance was developed called Gene X-pert or Cartridge based Nucleic acid Amplification Test (CBNAAT). CBNAAT uses polymerase chain reaction assay to amplify a sequence of the "rpoB" gene specific to *mycobacterium tuberculosis.* This gene is probed with molecular beacons for mutations within the rifampicin resistance detection region.[5] According to the present guidelines, all presumptive TB patients belonging to vulnerable populations such as children, people living with HIV, extra-pulmonary TB and smear negative cases with X-ray suggestive of TB undergo CBNAAT. In addition all non-responders to treatment, drug-resistant TB contacts, previously treated TB, TB-HIV co-infection and new TB cases will also undergo CBNAAT testing.[3,8]

The CBNAAT is an integrated, automated, closed system, contamination-controlled NAAT platform, which enables the diagnosis of tuberculosis and identification of rifampin resistance in less than three hours. The test can be performed by low-skilled users. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected."[4]

This study was done to assess the specificity and sensitivity of the CBNAAT to diagnose Tuberculosis and identify rifampicin resistance in this rural setting."

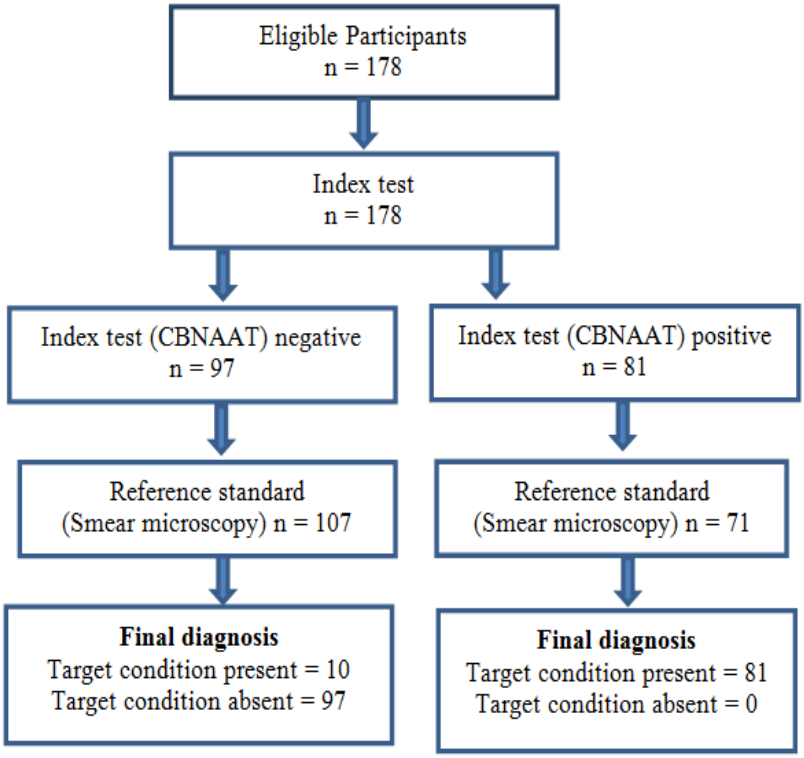
MATERIAL AND METHODS

Ethical approval for this study on the performance of a diagnostic test was obtained from the institutional ethics committee. The medical records of 178 patients with suspected pulmonary tuberculosis, visiting the DOTS centres of a district in Kerala, South India during the period May 2017 – June 2017, were accessed for collecting data after obtaining written permission from the institutional RNTCP clinic and the district tuberculosis officer. All data was maintained confidentially and no patient identifying features were retained in the data collection sheet.

The Sample size was estimated to be 188 subjects using the sensitivity of the new test obtained from the study by Laraque et al.[5] using the formula for estimating the sensitivity of a new test for a confidence interval of 95% and precision of 2.8 % using nMaster sample size calculation software.[9]

Figure1 gives the Study Flow Diagram using STARD guidelines.

Figure 1: Flow Diagram Using STARD Guidelines.



RESULTS

Most of the 178 subjects included in this study were men (70.2%) and coming from a rural background (65.2%). Of the 178 participants seven (3.9%) had co-existing HIV infection. A good number of patients 42 (23.6%) had diabetes mellitus, while 6 (3%) were dependent on substance abuse (alcohol, smoking). The baseline characteristics of the participants are given in Table I.

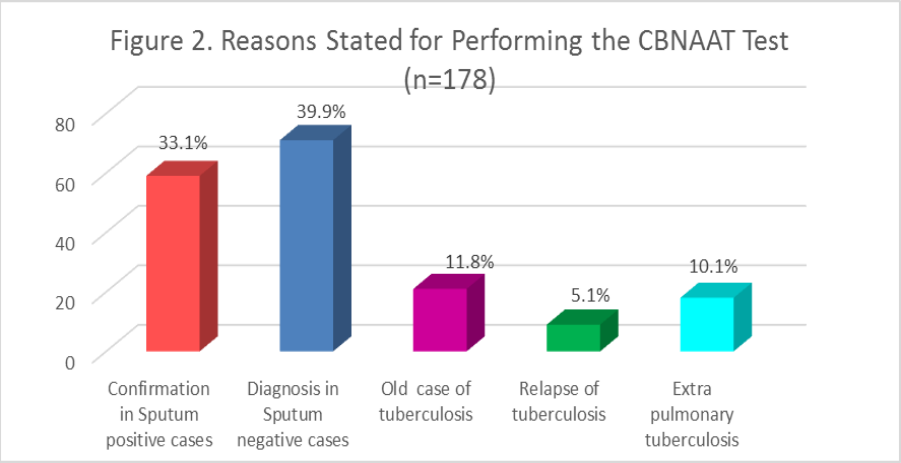
Table I: Baseline Characteristics of The Study Sample (n=178).

Variable		Frequency
Gender	Male	125 (70.2%)
	Female	53 (29.8%)
Rural/urban	Rural	116 (65.2%)
	Urban	62 (34.8%)
Fluid/tissue sample	Sputum	162 (91%)
	Others	16 (9%)
HIV status	HIV +	7 (3.9%)
	HIV -	171 (96.1%)
Comorbidities	Diabetes	42 (23.6%)
	Substance abuse	6 (3%)
	None of the above	130 (73%)

The reasons stated for performing the CBNAAT test were for confirmation of tuberculosis in sputum positive cases, diagnosis in sputum negative cases, previously treated cases of tuberculosis, relapse of tuberculosis and extra-pulmonary tuberculosis as shown in figure 2.

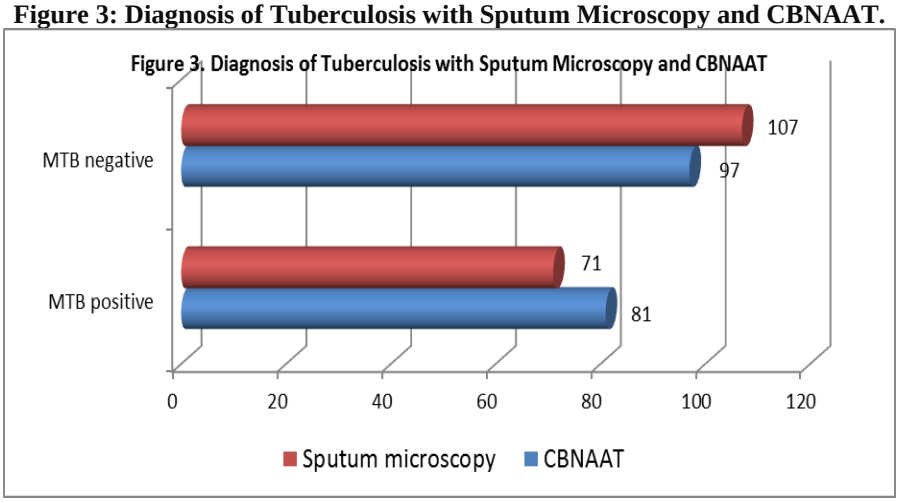
Figure 3 shows that there were 81 positive and 110 negative samples on smear microscopy, while there were 71 positive and 97 negative CBNAAT results.

Figure 2: Reasons Stated For Performing The CBNAAT Test.



Legend Figure 2. 33.1% were done to confirm diagnosis after sputum fluorescein test was positive. The rest were sent for diagnostic purposes to detect the presence of tubercle bacilli in patients suspected to have tuberculosis.

Figure 3: Diagnosis of Tuberculosis with Sputum Microscopy and CBNAAT.



Legend Figure 3: Of the 178 samples studied, 81 (45.5%) had a positive CBNAAT, while 71 (39.9%) had positive sputum smears. The remaining 107 sputum samples and 97 CBNAAT samples were negative.

Of the **81** positive samples diagnosed by CBNAAT, **62** were also diagnosed by smear microscopy, giving us a sensitivity of **87.3%** while **19** samples identified by CBNAAT were negative for mycobacteria on smear microscopy. Of the 110 sputum smear negative subjects CBNAAT was negative in 91 subjects giving a specificity of 82.2%. (Table 2)

Table 2: Diagnosis of Tuberculosis Using the CBNAAT Test and Smear Microscopy (n=178).

	Smear Microscopy Positive	Smear Microscopy Negative	Total
CB NAAT Positive	62	19	81 (45.5%)
CB NAAT Negative	9	88	97 (54.5%)
Total	71 (39.9%)	107 (60.1%)	178 (100%)

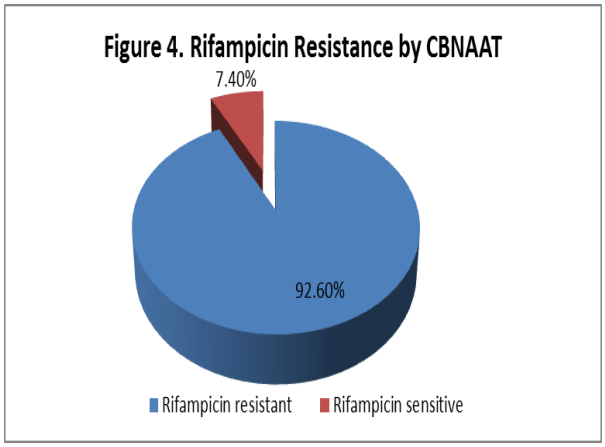
Table 3 gives us the sensitivity (87.3%), specificity (77.3%), positive predictive value (76.5%), negative predictive value (90.7%) and the likelihood ratio (4.92) of the CBNAAT test. The CBNAAT test and sputum microscopy have proximate sensitivity and specificity values. The predictive value reflects the diagnostic power of the test. The high positive predictive value (85.2%) indicates the strong probability that a person with a positive test has tuberculosis. The high negative predictive value (95.7%) means that a negative test in effect rules out tuberculosis. The likelihood ratio is used for assessing the value of performing a test. The high likelihood ratio of nearly 5 with a confidence interval of 3.24 to 7.46 tells us that the test has a high probability to estimate the presence of tuberculosis.

Table 3: Diagnostics of The CBNAAT Test as Compared to Sputum Microscopy (n=178).

Diagnostic	Estimates	95% Confidence Interval	
Sensitivity	87.30%	77.30%	94.0%
Specificity	82.20%	73.30%	89.00%
Likelihood Ratio (+)	4.92	3.24	7.46
Positive predictive value	76.50%	65.80%	85.20%
Negative predictive value	90.70%	83.10%	95.70%

THE CBNAAT Test also detects the presence of Rifampicin resistance in mycobacteria. The detection of rifampicin resistance is manifested by the presence of the "rpo B" gene. Rifampicin resistance was performed for 81 positive CBNAAT samples, 6 of whom showed rifampicin resistances as shown in Figure 4.

Figure 4: Rifampicin Resistance as shown by CBNAAT.



Legend Figure 3. The CBNAAT genetic analyser detected the rpo B gene mutations found in rifampicin resistant strains in 6 (7.4%) of these 81 TB-positive CBNAAT samples.

DISCUSSION

Multiple large international retrospective and prospective evaluation studies on single sputum sample GeneXpert MTB/RIF assay has a clinical specificity of approximately 99% for culture-negative samples, and a clinical sensitivity of approximately 98–100% for smear- and culture-positive samples [2, 5, 6] This study was conducted in order to evaluate the role of CBNAAT in diagnosing tuberculosis and rifampin resistance in the peripheral settings. Of all patients who met with inclusion criteria, the CBNAAT identified 87.3% of patients who had positive sputum microscopy. It also identified 19 cases who had negative sputum microscopy. The findings of this study indicate that with the availability of the CBNAAT test there is widely improved case detection for patients with tuberculosis.

Laraque et al reviewed the medical records of over 16000 patients The CB-NAAT reliably and rapidly identified patients with pulmonary tuberculosis. The sensitivity, specificity, and predictive values of the test were high for specimens that tested positive for AFB smear, and acceptable for those that tested negative for AFB on smear.[10] In our study, the CBNAAT test and sputum microscopy have proximate sensitivity and specificity values. The predictive value reflects the diagnostic power of the test. The high positive predictive value (85.2%) indicates the strong probability that a person with a positive test has tuberculosis. The high negative predictive value (95.7%) means that a negative test in effect rules out tuberculosis. The likelihood ratio is used for assessing the value of performing a test. The high likelihood ratio of nearly 5 with a confidence interval of 3.24 to 7.46 tells us that the test has a high probability of identifying the presence of tuberculosis.

Boehme et al reported that MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria.[5] Blackmore et found that rifampicin resistance was correctly identified in all 37 resistant isolates and in none of the 42 susceptible isolates that they studied.[11] In this study only 6 out of the 81 specimens tested for rifampicin resistance were found to be resistant to rifampicin (7.4%). Thus most of the patients diagnosed in these peripheral centres had rifampicin sensitive tuberculosis.

CBNAAT uses a very sophisticated technology which makes it an expensive test which could not be routinely used due to the financial constraints of the rural people. However it is highly commendable that the RNTCP provides this test free of cost through the DOTS centres. Spreading awareness of the value of this test and expansion of its use will increase the early detection of tuberculosis and decrease the incidence of multi drug resistance. In addition the ability to detect rifampicin resistance will prevent the selection of more resistant forms and rational treatment for the patient with agents to which the mycobacteria are susceptible. Initiation of the CBNAAT test provides cost-effective and highly sensitive detection of tuberculosis even outside the reference centres which not only increases the access but also decreases the delay in diagnosis.[5]

This study was done in the context of peripheral DOTS centres, most of which are in rural areas. Therefore it is of value because most cases of tuberculosis can be picked up at the district level.

CONCLUSION

The study showed that CB-NAAT is a equally sensitive and specific rapid molecular diagnostic test to detect tuberculosis and rifampicin resistance in biological specimens in patients suspected to have TB.CBNAAT

identified 62 of the 71 (87.3%) patients with positive sputum microscopy and detected TB in 19 cases that were sputum negative.

The CBNAAT test has a significant relevance as a screening tool, Rifampicin resistance was found in less than one-tenth of the population since occurrence of DR-TB is less in this area. The CBNAAT can act as an excellent tool for handpicking drug resistant tuberculosis and prescribing drugs that the mycobacteria will be susceptible to.

ACKNOWLEDGEMENT

We acknowledge the support of the Dean, Management and Research Department of MOSC Medical College, Kolenchery, Kerala. The RNTCP clinic of this institution was very supportive and we are grateful to Dr Sweety Joy for all the help received. The data was obtained from the RNTCP DOTS centres in this district. We are grateful to Mr Sharath G Rao, District TB Officer, for granting us permission to conduct the study and the details of the study have been submitted to him, prior to publication. We are grateful to the staff of the various regional RNTCP centres for the help and support rendered for this study.

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