

A Cbnaat based Study on Rifampicin Resistance in Tuberculosis in East Godavari District

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Abstract

Background: Globally 1.2 million Tuberculosis deaths occurred among HIV negative people and an estimated 251000 deaths occurred among HIV positive people in 2018. TB continues to be a public health threat as about half a million news cases were Rifampicin resistant. CBNAAT offer the prospect of very high sensitivity approaching that of liquid culture for TB diagnosis. It also provides information on drug susceptibility to rifampicin, which is a surrogate marker for identification of MDR TB cases. **Objectives-** 1. To know the magnitude of rifampicin resistance among TB cases. 2. To find out the incidence of TB-HIV co-infection. **Materials & Method:** Monthly Data regarding CBNAAT results during the year 2019 January-December was collected from District Tuberculosis Center (DTC), East Godavari district. **Statistical analysis used:** Data was entered and analyzed in MS-EXCEL. **Results:** A total of 20364 cases were screened for tuberculosis using CBNAAT, out of which 9366 patients were PLHIV (people living with HIV/AIDS). Among all the patients who were screened, 1951(9.6%) were positive for mycobacterium tuberculosis. 93(0.45%) cases were resistant to rifampicin. Among the PLHIV patients, 241(2.6%) were positive for tuberculosis. In those PLHIV cases, 20(0.2%) were resistant to rifampicin suggesting that rifampicin resistance was more common in HIV/AIDS. Among previously treated tuberculosis cases prevalence of rifampicin resistance was 5.1% as compared to 1.5% & 1.8% among newly diagnosed cases & non-responders respectively. **Conclusion:** CBNAAT is an effective tool in the diagnosis of tuberculosis and in identifying drug resistant tuberculosis. In our study drug resistance to rifampicin was more common in PLHIV and also in previously treated tuberculosis patients. By increasing the case detection rate of RR-TB and TB-HIV co-infection cases using CBNAAT as the first line of diagnostic procedure we can be able to treat cases effectively in order to achieve the Sustainable Developmental Goal(SDG) of ending TB by 2025 as committed by Government of India.

Keywords: CBNAAT, Rifampicin resistance, TB-HIV co-infection.

Introduction

Globally eight countries accounted for 2/3rds of TB burden, among which India accounts for 27% followed

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by China 9%. TB continues to be a public health threat. Globally, an estimated 10 million people fell ill with TB in 2018. There were estimated 1.2 million TB deaths among HIV negative people in 2018 and 251000 deaths among HIV positive people. TB affects people of both sexes in all age groups but the highest burden is in men, aged >15 years who accounted for 57% of all TB cases in 2018. Among all TB cases, 8.6% were people living with HIV. In 2018, about half a million news cases were Rifampicin resistant. Globally, 3.4% of new TB cases & 18% of previously treated cases had MDR TB/RR TB. The overall goal of WHO End TB strategy is to end the

global TB epidemic by 2035.¹

Sputum microscopy in TB diagnosis was developed more than 100 years ago, and WHO endorsed rapid molecular tests in the year 2010 for the diagnosis of TB. RNTCP adapted CBNAAT in India in April 2012. It was launched in 2012 as a pilot project in Maharashtra by state Tuberculosis unit. By the end of 2012, under EXPAND_x-TB project, 12 CBNAAT labs were established all over India across different states.² CBNAAT test is now being made available throughout India. CBNAAT is a semi-quantitative nested nucleic acid amplification test based on molecular detection of mutated gene. It is simple, rapid, and cost effective and doesn't require technical expertise. It can be carried out in an automated manner including bacterial lysis, nucleic acid extraction, and amplification and amplicon detection. It can diagnose TB within 2 hours and gives accurate results due to use of disposable closed cartridges preventing cross contamination.³ In settings where resources are limited for facilities like culture DST, CBNAAT is extremely useful, simple and reliable test. Unlike conventional nucleic acid amplification tests (NAATs), CBNAAT MTB/RIF is unique because sample processing and PCR amplification and detection are integrated into a single self-enclosed test unit, the CBNAAT cartridge.⁴ CBNAAT offer the prospect of very high sensitivity approaching that of liquid culture -the current gold standard for TB diagnosis. It also provides information on drug susceptibility to rifampicin, which is a surrogate marker for identification of MDR TB cases. It is fully integrated and automated amplification and detection using real time PCR. It is a highly specific test as it uses 3 specific primers & 5 unique molecular probes to target the *rpo* gene of *M. Tuberculi*, which is the critical gene associated with rifampicin resistance. Being a PCR based method, clinical validation trails done in four distinctly diverse setting have shown that 92.2% of culture positive patients were detected by a single CBNAAT test with a specificity of 99% as compared to sensitivity of a single direct sputum smear of 59.5%.⁵

At the start of 2020 the Central Government of India has renamed the RNTCP as NTEP (National Tuberculosis Elimination Programme)⁶. This implies targeting an 80% reduction in incidence and 90% reduction in mortality (baseline 2015) Union government is committed to achieving the Sustainable Developmental Goal (SDG)

of ending TB by 2025, five years ahead of the global targets.

So, this study was aimed to know the magnitude of rifampicin resistant tuberculosis and TB-HIV co-infection in the East Godavari district based on the CBNAAT investigation results.

Objectives-

1. To know the magnitude of rifampicin resistance among TB cases.
2. To find out the incidence of TB-HIV coinfection.

Materials & Method

CBNAAT results during year January 2019-December 2019 were collected from District Tuberculosis Center (DTC), East Godavari district monthly with prior permission from District Tuberculosis Officer (DTCO). Six CBNAAT machines manufactured by Cepheid, endorsed by WHO are present in 4 different areas of the district (2 at Kakinada, 2 in Rajahmundry, 1 in Amalapuram and 1 in Rampachodavaram area). All presumptive TB cases, presumptive DR TB, paediatric cases, PLHIV, extra pulmonary TB cases were subjected to CBNAAT as first line of diagnostic investigation. Results of CBNAAT are interpreted as a) MTB not detected, b) MTB detected RIF sensitive, c) MTB detected RIF resistant, d) MTB detected & RIF indeterminate, e) invalid and f) errors among various categories of patients. When the test result is RIF resistance positive then the sample is sent to Regional DST (Drug susceptibility testing centre) at Visakhapatnam, for repeating CBNAAT and also line probe assay (LPA). The data for the above categories was entered and analyzed in MS-EXCEL. Ethical approval was taken from the Institutional Ethics Committee.

Results

A total of 20,364 patients were tested with CBNAAT from 1st January, 2019 to 31st December, 2019 in the East Godavari district, Andhra Pradesh. Out of 20,364 cases 15857 (77.9%) were presumptive TB cases, 801 (4%) of extra pulmonary cases and 678 (3.3%) were presumptive drug resistant cases. 1929 (9.4%) of cases were referred from private hospitals (table 1).

Among the patients who are being treated for tuberculosis under DTC, 89.4% are above 18 years of age and 65.4% of them are males. 10.6% are below 18 years of age and 54.2% of them are females. (figure 1)

Place wise distribution shows that 41.8% of patients belong to Rajahmundry area followed by Kakinada(25.6%), Amalapuram(24.2%) and Rampachodavaram(8.4%). Mycobacterium tuberculosis was detected among 1951(9.6%) cases who were tested in the entire district. Out of those, the drug sensitive tuberculosis cases accounts for 1822(93.5%) followed by 93(4.7%) Rifampicin resistant cases and 36(1.8%) indeterminate rifampicin resistance cases. Among those 93 drug resistant cases, more than half of the cases(47) were from Rajahmundry area followed by 26 cases from Kakinada, 10 cases from Amalapuram and 10 cases from Rampachodavaram.(table 2)

Among 678(3.3%) presumptive drug resistant TB cases, Rifampicin resistance was detected among 12 cases(1.76%), out of which 6(50%) cases were new TB patients at the time of diagnosis, followed by 4 cases of follow up positive & non respondents category and 2 cases were previously treated TB patients. Rifampicin resistance was found to be 1.5% among new tuberculosis cases whereas in previously treated cases and non-

responders it was 5.1% and 1.8% respectively. (table 3)

Out of 15857(77.5%) Presumptive TB cases, 9366(59%) patients were living with HIV/AIDS(PLWHA), 625(4%) were pediatric cases and others includes 5866(37%) of cases. In the PLWHA cases, Mycobacterium tuberculosis was detected among 241(2.6%) cases. Out of which 0.2% were Rifampicin resistant tuberculosis cases. Among 625 paediatric cases tested with CBNAAT, 10(1.6%) cases were positive for mycobacterium tuberculosis and rifampicin sensitive. (table 4)

There were 1929(9.4%) presumptive tuberculosis cases which were referred from private hospitals to District Tuberculosis Center (DTC). Among those 322(16.7%) were positive for Mycobacterium tuberculosis, out of which 309(16%) were Rifampicin sensitive and 13(0.7%) cases were Rifampicin resistant. (figure 2)

Among 801(4%) Extra pulmonary tuberculosis cases tested with CBNAAT, 67(8.4%) cases were positive for Mycobacterium tuberculosis, out of which 64(8%) were Rifampicin sensitive and 3(0.4%) were Rifampicin resistant. (figure 2)

Table 1: Distribution of CBNAAT results among various categories in the East Godavari district

Category	n(%)
Presumptive Tuberculosis cases	15857(77.9%)
Presumptive Drug resistant Tuberculosis cases	678(3.3%)
Referrals from private hospitals	1929(9.4%)
Extra pulmonary TB cases	801(4%)
Indeterminate results	36(0.2%)
Invalid results	26(0.1%)
No results	435(2.1%)
Errors	602(3%)
Total	20364

Table 2: Distribution of CBNAAT results in different areas of East Godavari district

Result	Kakinada	Amalapuram	Rajahmundry	Rampachodavaram	Total
MTB Negative	4280(24.6%)	4445(25.7%)	7413(42.7%)	1212(7%)	17350 (85.2%)
MTB Positive R/F sensitive	558(30.6%)	245(13.5%)	821(45%)	198(10.9%)	1822 (8.95%)
MTB Positive R/F resistant	26(27.9%)	10(10.8%)	47(50.5%)	10(10.8%)	93 (0.45%)
MTB with R/F indeterminate	12(33.3%)	11(30.6%)	13(36.1%)	0	36 (0.2%)
Invalid	9(34.7%)	5(19.2%)	12(46.1%)	0	26 (0.13%)
No results	131(30.1%)	88(20.2%)	23(5.3%)	193(44.4%)	435 (2.13%)
Errors	196(32.5%)	130(21.6%)	201(33.5%)	75(12.4%)	602 (2.94%)
Total	5235(25.6%)	4956(24.2%)	8553(41.8%)	1710(8.4%)	20364

Table 3: Distribution of CBNAAT results of presumptive Drug resistant Tuberculosis cases

Presumptive DR TB	MTB not detected	MTB detected R/F sensitive	MTB detected R/F resistant	Total
New TB patients at the time of Diagnosis	122(29.9%)	280(68.6%)	6(1.5%)	408 (60.2%)
Previous treated TB patients	23(59%)	14(35.9%)	2(5.1%)	39 (5.8%)
Others- follow up positive & non responders	206(89.1%)	21(9.1%)	4(1.8%)	231 (34%)
Total	351(51.8%)	315(46.4%)	12(1.8%)	678

Table 4: Distribution of CBNAAT results of presumptive Tuberculosis cases

Presumptive TB cases	MTB not detected	MTB detected R/F sensitive	MTB detected R/F resistant	Total
PLHIV	9125(97.4%)	221(2.4%)	20(0.2%)	9366 (59%)
Paediatric	615(98.4%)	10(1.6%)	0	625 (4%)
Others	4918(83.83%)	903(15.39%)	45(0.76%)	5866 (37%)
Total	14658(92.43%)	1134(7.15%)	65(0.4%)	15857

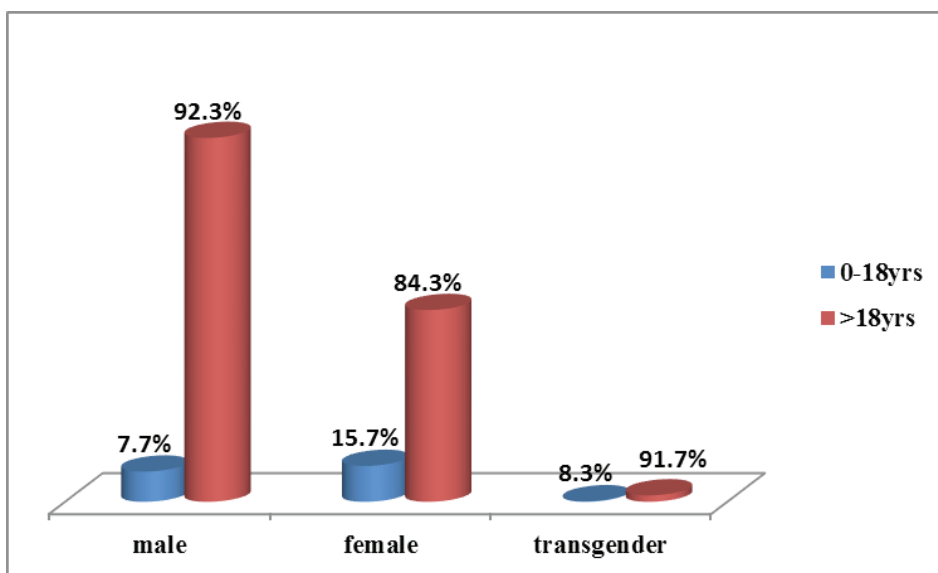


Figure 1: Age wise and gender wise distribution of patients taking tuberculosis treatment from the DTC

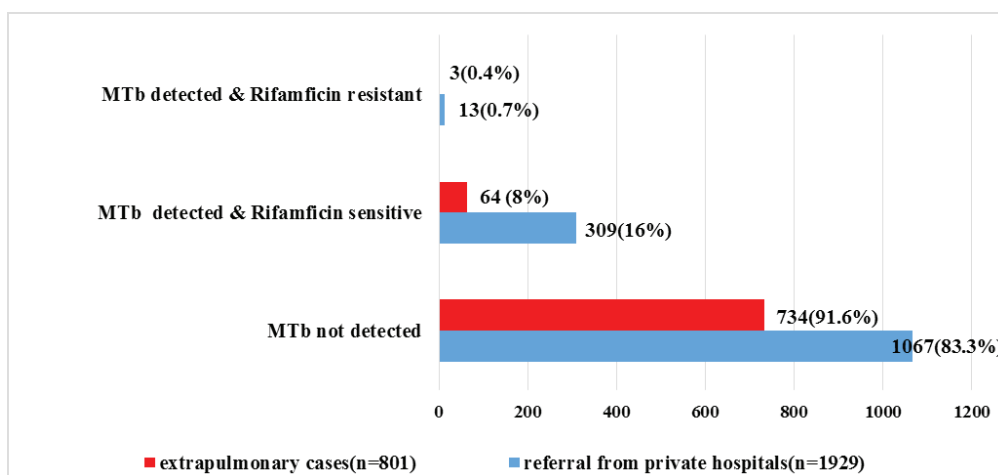


Figure 2: Distribution of CBNAAT results among cases referred from private hospitals (n=1929) and Extrapulmonary Tuberculosis suspect cases(n=801)

Discussion

Males account for 65.4% of those being treated for TB in above 18 years of age. This is similar to the study done by Patel et al⁷ in Ahmedabad and Global TB Report 2019.¹

According to Indian TB Report 2019, India accounts for 8.4% of Rifampicin resistant TB cases & Andhra Pradesh account for 5.7% Rifampicin resistant TB cases.⁸ However in our study rifampicin resistant mycobacterium tuberculosis in the East Godavari district was 4.7%. In the study conducted by Patel et al on the role of CBNAAT in the diagnosis of new pulmonary TB cases in Ahmedabad,⁷ Rifampicin resistance was found to be 4.5% and in another study done by Bansal SK et al in DRTB centre of Faridkot it was around 6%.⁹ Among the newly diagnosed tuberculosis cases, the rifampicin resistance was found to be 1.5%, whereas it was two times of our finding (3%) in the study done by Chandrasekran et al among the patients attending an Urban District Tuberculosis centre.¹⁰ In our study among the previously treated tuberculosis (relapse) cases rifampicin resistance was found to be 5.1% unlike in Shanta et al study, where 17% of cases of relapse showed resistance to rifampicin.¹¹ Among paediatric cases tested with CBNAAT, 1.6% cases were positive for mycobacterium tuberculosis and rifampicin sensitive. No drug resistant cases were detected among pediatric cases. Country statistics is 9% of the TB cases were pediatric cases among which rifampicin resistance was 14% and in Andhra Pradesh 4% of TB cases were pediatric cases out of which rifampicin resistance was 9%.

In our study TB-HIV co-infection, was 2.5% which was similar to the study done by Deepak Arora et al,¹² whereas according to India TB report 2019,⁷ it was 3.4% in the country and 8% in the state of Andhra Pradesh but, in the study conducted in AIIMS-New Delhi by Sharma SK et al, the TB-HIV co-infection was very high(33.2%).¹³

Among the Extrapulmonary TB cases, Mycobacterium tuberculosis was detected among 8.4% of cases, where as in the study done by Kasat S et al on the effectiveness of CBNAAT in the diagnosis of the Extrapulmonary Tuberculosis it was 15% (25 out of 166 cases).¹⁴ According to Indian TB Report 2019,

out of total cases on treatment for TB 14.7% were extra pulmonary cases. Study done by Sharma et al in diagnosing EPTB by GeneXpert has shown an overall sensitivity of 71%.¹⁵

Conclusion

In this rapidly evolving era of TB and HIV co-infection, updated knowledge and changing research priorities, with respect to new TB diagnostic procedure is the need of the hour. Rifampicin resistance was found to be 1.5% among new tuberculosis case whereas in previously treated cases and non-responders it was 5.1% and 1.8% respectively. As the result is available in less than 2h and hence the screening capacity of healthcare centre can be increased. Use of CBNAAT has significantly increased TB finding and it has also significantly increased MDR case finding and it is cost effective when compared to culture and DST. It can be operated with minimally trained staff and least biosafety concerns. But every packaged deal has its own share of negativities like device maintenance and annual calibration of instrument is required along with constant power supply, which may pose some problems in remote areas. So by increasing the case detection we can treat more number of TB cases effectively with clear distinction between drug sensitive and resistant cases.

Recommendation-

The case detection rate of Tuberculosis and MDR-TB case finding can be increased by providing CBNAAT machines to Designated Microscopy Centers and Primary Health Centres, which is rapid, cost effective and doesn't require technical expertise when compared to smear microscopy.

Limitation of the Study-

The tuberculosis cases which were diagnosed by other investigation methods were not included in the study.

Conflicts of Interest: *None declared*

Source of Funding: *Nil*

Ethical Clearance *was taken from the institutional ethics committee*

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