



CBNAAT Based Identification and Demographic Pattern of Drug Resistant Mycobacterium Tuberculosis in South Kashmir, India- a One Year Retrospective Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RH and SR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JAB managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

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ABSTRACT

India is the highest TB burden country in the world having an estimated incidence of 26.9 lakh cases in 2019. With a population of 1.32 billion, India has the highest burden of drug resistant TB (DR-TB) in the world. North zone of India is the second highest MDR-TB prevalent zone after the West zone of India. MDR TB treatment involves prolonged treatment with injectable second-line drugs, associated with more adverse effects, suboptimal treatment outcomes and higher risks of mortality compared to patients with drug-sensitive TB and those with lesser resistant forms of TB.

Materials methods: This retrospective study was conducted in the department of Microbiology Government Medical College Anantnag, data was analyzed from March 2017 to February 2018. Non-sterile specimens were processed by Modified Petroff Method. Sterile specimens were concentrated by centrifugation and smear and cultures was inoculated from the sediment. CBNAAT assay was performed by Gene Xpert (Cepheid) 4 system according to the manufacturers' recommendations.

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Results: Of the total 1497 clinically suspected tuberculosis specimens collected, 1370 (91.5%) were pulmonary and 127 (8.5%) were presumptive extra pulmonary tuberculosis received from different anatomical sites. Maximum clustering of cases was seen in 10-20 years age group. Out of the total 1497 samples 200 were CBNAAT confirmed Mycobacterium Tuberculosis positive samples. In which 155 were pulmonary and 45 were extra pulmonary. The average percentage positivity rate (i.e. percentage of MTB positive samples out of total samples tested) was 13.3% (200/1497). Rifampicin resistance (RR-TB) was seen in 5.5% (11/200) samples. Out of the samples detected positive (200): 155 were pulmonary samples and out of these 155 pulmonary samples 8 were found to be RR MTB 5.1% (8/155). Also out of the 200 positive samples 45 were extra pulmonary and out of these 45 extra pulmonary samples 3 were found to be RR MTB 6.6% (3/45).

Conclusion: In this study we found that in our region 5.5% cases of TB were RR-TB, 3.2% were new cases and 13% RR-TB was seen in previously treated cases of MTB. The screening of drug resistance has to be expanded to offer universal DST including expanded DST. The second and most important activity is to strengthen drug resistance surveillance under the various national programs with inclusion of laboratories in the private sector as well. The state level regional studies also give us the opportunity to plan and execute intervention prioritization, based on the drug resistance trends observed.

Keywords: CBNAAT; drug resistant tuberculosis; rifampicin resistant tuberculosis in South Kashmir; RR-TB; MDR-TB; MTB.

1. INTRODUCTION

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV / AIDS). Globally, an estimated 10.0 million (range, 8.9–11.0 million) people fell ill with TB in 2019, a number that has been declining very slowly in recent years. There were an estimated 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2019. Geographically, most people who developed TB in 2019 were in the WHO regions of South-East Asia (44%) [1].

India is the highest TB burden country in the world having an estimated incidence of 26.9 lakh cases in 2019. Overall the age distribution of TB cases shows a predominance in the adolescent and young adult age groups between 15 to 30, indicating ongoing disease transmission [1]. India has more new tuberculosis (TB) patients annually than any other country globally, contributing to 27% of the world's TB burden [2].

MDR i.e. TB resistant to at least two of the first-line drugs – isoniazid and rifampicin. Extensively drug resistant TB (XDR-TB), defined as MDR-TB with additional resistance to at least one fluoroquinolone and one second line injectable drug. Drug-resistant TB continues to be a public health threat. Worldwide in 2019, close to half a million people developed rifampicin-resistant TB

(RR-TB), of which 78% had multidrug-resistant TB (MDR-TB) 9. Globally in 2019, 3.3% of new TB cases and 17.7% of previously treated cases had MDR/RR-TB [1]. The proportion of XDR-TB among MDR-TB patients is 6.2% worldwide [3]. This clearly indicates that drug resistance is present in all settings, and the wide range of resistance patterns, needs to be addressed with strengthening of drug resistance surveillance, universal DST and appropriate DST guided treatment strategies.

With a population of 1.32 billion, India has the highest burden of drug resistant TB (DR-TB) in the world [2]. North zone of India is the second highest MDR-TB prevalent zone after the West zone of India. As per the RNTCP status report, a total of 25652 MDR-TB cases were detected in 2014, of which 6184 were reported in North India. In 2013, significantly increasing MDR-TB cases were found in North India [4].

MDR TB involves prolonged treatment with injectable second-line drugs, associated with more adverse effects, suboptimal treatment outcomes and a higher risks of mortality compared to patients with drug-sensitive TB and those with lesser resistant forms of TB. The increase in the burden of multidrug-resistant tuberculosis (MDR-TB) is a matter of grave concern. The present study was undertaken to describe trends in DR tuberculosis in South Kashmir India.

2. MATERIALS AND METHODS

This retrospective study, conducted in the department of microbiology government medical college anantnag, drug susceptibility data was analyzed from March 2017 to February 2018. The study population included all consecutive patients who had submitted sample for mycobacterial microscopy, CBNAAT and culture. Both admitted patients as well as patients attending outpatient department were included. Repeat samples of the same patient, were excluded. The data collected was the patients' demographics (age, sex, site of disease, region / district / village to which patient belonged) and past history of TB treatment. The specimens processed included pulmonary and extra pulmonary samples. The data was recorded from the patients when they visited the RNTCP centre (Revised National TB Control Program) at Government medical college anantnag and then the data was retrospectively obtained from RNTCP patient records and analyzed for this study. Most of the patients belonged to South Kashmir.

Sample Processing: Analysis of samples by Xpert MTB/RIF assay:

The assay was performed by Gene Xpert (Cepheid) 4 system according to the manufacturers' recommendations. Briefly, the sample reagent (containing NaOH and isopropyl alcohol) was added at a 2:1 ratio to clinical specimen to kill the mycobacteria and liquefy the samples. For biopsy specimen, a 2:1 volume of sample reagent (SR) buffer was added to biopsy specimens after they had been chopped into very small pieces with a sterile blade in a sterile petri dish. Fluids were processed directly by the addition of a 2:1 volume of SR buffer, except for CSF (usually <1ml), which was raised to 2ml by

the addition of SR buffer. The sample-SR mixture was shaken vigorously and incubated for 10 minutes before being shaken again and kept at room temperature for another 10 minutes. Two ml of the digested material was transferred to the cartridge. The cartridge was subsequently loaded in the Gene Xpert instrument where all subsequent steps occurred automatically. In case the results were reported as invalid, error or no result, the sample was reprocessed and rerun, if sufficient material was available [5].

3. RESULTS AND DISCUSSION

Of the total 1497 clinically suspected tuberculosis specimens collected, 1370 (91.5%) were pulmonary and 127 (8.48%) were extra pulmonary samples received from different anatomical sites (tissue biopsies or fine-needle aspirates, pus (6/127, 4.7%, pleural fluid (28/127, 22.0%) and other body fluids. 882 samples were from males. Maximum clustering of cases was seen in 11-20 years age group. Out of the total 1497 samples, 200 were Mycobacterium tuberculosis CBNAAT confirmed Positive samples. Out of the samples detected positive (200): 155 were pulmonary samples and out of these 155 pulmonary samples 8 were found to be RR MTB 5.1% (8/155). Also out of the 200 positive samples 45 were extra pulmonary and out of these 45 extra pulmonary samples 3 were found to be RR MTB 6.6% (3/45) (Table 1). The average percentage positivity rate (i.e. percentage of MTB positive samples out of total samples tested) was 13.3% (200/1497) (Fig. 1). A higher number of the MTB positive cases came from Kukernag, Pahalgam And Dooru Areas of South Kashmir. Rifampicin Resistance (RR-TB) was seen in 5.5% (11/200) of all the positive samples.

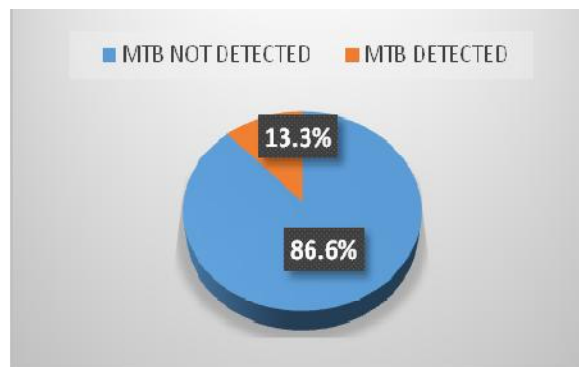


Fig. 1. Positivity distribution of samples

Table 1. Demographics of MTB positive and RR-TB Patients

Characteristics	MTB+(200)		RR-TB(11)
	Number	Number	Percentage (%)
Sex			
Male	120	7	5.8%
Female	80	4	5%
Age group			
below 10 years	0	0	0
11-20 years	60	4	6.6%
21-30 years	31	2	6.4%
31-40 years	15	1	6.6%
41-50 years	10	1	10%
51 -60 years	30	2	6.6%
61 -70 years	20	1	5%
>70 years	34	0	0
Site of disease			
Pulmonary	155	8	5.1%
Extra pulmonary	45	3	6.6%
Treatment history			
New	154	5	3.2%
Previously treated	46	6	13%
Region			
kokernag	39	3	7.6%
phalgam	20	1	5%
dooru	17	1	5.8%
kulgam	8	1	12%
Various other aeras	116	5	4.3%

4. CONCLUSION

The emergence of MDR-TB is a global problem, which is threatening to destabilize the best efforts of TB control and has been attributed to factors such as non-adherence to treatment, inappropriate treatment regimens, drug malabsorption, poor drug quality, and a poor health infrastructure for effective delivery of treatment [6].

In 2009, a study of the profile of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) in tertiary care hospital setting, representing almost the whole affected population of Kashmir valley, India was conducted and in this study 5.7% cases of MDR-TB were identified, among which 15.3% were diagnosed as XDR-TB [6]. Another similar study was conducted in 10 districts of Kashmir valley, India in 2017 where CBNAAT tests were done on individuals with suspicious X-ray findings in this study the prevalence of bacteriologically positive pulmonary tuberculosis was found to be 147 per 100,000 population. They also found that females were affected more than males, and the age of female tuberculosis patients was seen to be less than that of males [7]. This was in contrast to our finding, where in we found more males to be affected with MTB than females.

For people with drug susceptible TB, treatment success rates of at least 85% are regularly being reported to WHO by its 194 member states while as treatment for people with rifampicin-resistant TB (RR-TB) and multidrug resistant TB (MDR-TB) is longer, and requires drugs that are more expensive and more toxic. The latest data reported to WHO show a treatment success rate for MDR TB of only 57% worldwide. Though Globally the burden of multidrug- or rifampicin-resistant TB (MDR/RR-TB) as a share of the number of TB cases remains stable, 3.3% of new TB cases were found to be MDR /RR-TB [1], a similar percentage of 3.2% of new TB cases were seen to be RR-TB in our research. While as in our study 13% of previously treated cases of TB were found to be RR-TB, which is lesser than the global figure of 17.7% of MDR/RR-TB in previously treated patients [1].

The report of the first National Anti-Tuberculosis Drug Resistance Survey (NDRS) from India released on the occasion of World TB Day 24th March 2018 showed a state level analysis of drug resistance indicating that DR-TB is prevalent in all states of India, albeit with wide variations ranging from 18.42% in Himachal Pradesh to 36.84% in Jammu and Kashmir. This national report also presented an incidence of MDR TB in India to be 6.19% among all TB patients, 2.8% in new TB cases and 11.6% in

previously treated cases of TB [2]. Our study found lesser percentage of cases of RR-TB / MDR TB in our region in comparison to national incidence.

The screening of drug resistance has to be expanded to offer universal DST including expanded DST as envisaged in the updated PMDT guidelines [8]. The second and most important activity is to strengthen drug resistance surveillance under the various national programs with inclusion of laboratories in the private sector as well. The state level Regional studies also give us the opportunity to plan and execute intervention prioritization, based on the drug resistance trends observed. This study also underlines the importance of CBNAAT as an important diagnostic modality for rapid and early diagnosis of tuberculosis. As has been endorsed by WHO the use of CBNAAT as a rapid diagnostic test for diagnosis of tuberculosis and its use in prioritized areas (like drug-resistant tuberculosis, paediatric tuberculosis, TB-HIV co-infection, extra pulmonary tuberculosis and sputum smear-negative PTB) is vital [9]. In addition to that estimation of disease burden of tuberculosis at regional level should help in development of more effective and tailored public health policies improving overall tuberculosis control in our country.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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