Original article

CONTINUING MENACE OF RIFAMPICIN RESISTANT TUBERCULOSIS IN RIMS HOSPITAL, IMPHAL, MANIPUR

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Abstract

Background:
India has set an ambitious goal of tuberculosis elimination by 2025 which is going to be limited by a large burden of multidrug resistant tuberculosis (MDR-TB). Resistance to rifampicin is a surrogate marker of MDR-TB. Detection of rpoB gene mutation by Cartridge Based Nucleic Acid Amplification Test (CB-NAAT) is used to detect rifampicin resistance.

Objective:
To determine the burden of rifampicin resistant tuberculosis in Regional Institute of Medical Sciences (RIMS) Hospital, Imphal, a tertiary care hospital in Manipur.

Method:
The study was a cross sectional study conducted on 100 cases of tuberculosis between the age group of 14-80 years visiting Regional Institute of Medical Sciences (RIMS) Hospital, Imphal, a tertiary care centre from September 2018 to December 2019. Samples were subjected to Ziehl-Neelsen (ZN) stain and smear positive samples were then sent for CB-NAAT analysis at Intermediate Reference Laboratory (IRL), Lamphel, Imphal and a part of it was inoculated in Lowenstein Jensen (LJ) medium for culture.

Results:
Of 100 samples (M=71, F=29), 42 were of grade 1+, 29 were grade 2+ and 29 were grade 3+ on ZN staining. 87(M=64, F=23) showed positive culture growth. Maximum cases of tuberculosis as well as rifampicin resistance were seen in the age group of 41-50 years. 5 males (7%) and 2 females (6.9%) were resistant to rifampicin respectively. 6(6.4%) of newly diagnosed and 1(16.7%) of old cases were rifampicin resistant tuberculosis (RR-TB). The total number of rifampicin resistance in our study was 7(7%).

Conclusion:
Although MDR-TB/RR-TB is emerging as a significant threat to tuberculosis control, limited data is available. Hence, the present research work is undertaken to highlight the continuing menace of rifampicin resistant tuberculosis in RIMS Hospital, a tertiary care centre in Manipur. In our study, 7% rifampicin resistance was observed, which is significantly high. This indicates the importance of strengthening the awareness programmes, establishing early diagnosis and administration of correct and prompt treatment.

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Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis, is among the oldest diseases known to humanity and one of the top ten causes of death worldwide.[1] It is transmitted from person to person via droplet from respiratory tract of people with the active pulmonary disease. Even two decades after introduction of directly observed treatment, short-course (DOTS) strategy, it still is a major cause of morbidity and mortality worldwide.[2]

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Key Words:
Tuberculosis, RR-TB, CB-NAAT, rpoB gene

Multi drug resistant tuberculosis (MDR-TB) is a form of tuberculosis caused by the organisms that do not respond to, at least isoniazid and rifampicin. MDR-TB arises from a mixture of physician error and patient non-compliance during the treatment of a susceptible TB.[3] Rifampicin and isoniazid remain the two most important drugs for treatment of tuberculosis. Resistance to either drug poses a serious impediment to successful therapy.[4] Resistance in about 95% of rifampicin resistant isolates is due to mutations in 69-bp region of the rpo B gene. This makes it a good target for molecular genotypic diagnostic methods.[5] Although 40 distinct point mutations and in-frame insertions and deletions in rpo B have been identified, point mutations in two codons, those coding for Ser_{531} and His_{526}, are seen in almost 70% of rifampicin resistant clinical isolates, with Ser531-to-Leu (TCG-to-TGG) mutations being the most common.[6] Resistance to rifampicin is a surrogate marker of MDR-TB which is detected by using CB-NAAT based on detection of rpo B gene mutation.[7]

Manipur, a north-eastern state of India has been having high prevalence of MDR-TB. In 2017 the prevalence was 11.2% and 7.9% in 2018 contributing to the high burden of MDR-TB of the country and creating a hindrance to the TB control activities.[8] This high burden of DR-TB should be managed based on the laboratory confirmation with a clear understanding of the drug resistance and its sensitivity.

The X pert-MTB/Rif assay (Cepheid) is one of the most frequently used molecular screening test for TB and MDR-TB in both resource-poor and resource rich countries.[9] It had been shown that the X pert-MTB/Rif assay detected pulmonary tuberculosis in all TB patients, including 90% of smear negative patient with a high sensitivity of 97%.[10] Cartridge Based Nucleic Acid Amplification Test (CB-NAAT)/Gene X pert, endorsed by the WHO for use in endemic countries, is an automated, semi-quantitative real time PCR assay designed for the rapid and simultaneous detection of Mycobacterium tuberculosis and rifampicin resistance within 2 hours.[11]

Aims and objectives

To determine the burden of rifampicin resistant tuberculosis in Regional Institute of Medical Sciences (RIMS) Hospital, Imphal, a tertiary care hospital in Manipur.

Materials and methods

The study was a cross sectional study conducted in the Department of Microbiology and Intermediate Reference Laboratory (IRL), Lamphel, Imphal. The samples were collected during September 2018 to December 2019.

Subjects aged between 14-80 years old, who were contacts of active tuberculosis, known drug resistant tuberculosis cases, failure to treatment, HIV-TB co-infected cases at diagnosis, relapse and defaulter were included. In the data analysis, newly diagnosed cases were considered as new cases whereas failure to treatment, relapse and defaulters were grouped under old cases.

Paediatric age group, patients already diagnosed with MDR/XDR-TB by means of CB-NAAT or line probe assay, active or suspected malignancy, terminal disease with poor prognosis were excluded from the study.

The samples were collected from participants suspected of tuberculosis visiting the Departments of Respiratory and Chest Medicine, General Medicine, RIMS hospital, Imphal, fulfilling the inclusion criteria and were processed immediately at the Mycobacteriology laboratory, Department of Microbiology, RIMS, Imphal for direct microscopic examination by Ziehl Neelsen (ZN) stain and culture in Lowenstein Jensen (LJ) media. Further, the smear positive samples were then sent to IRL, Imphal for molecular analysis by CB-NAAT to detect the rpo B gene.

100 samples which were positive for AFB were processed. Out of these, 94 were sputa, 3 broncho alveolar lavage (BAL), 2 pleural fluids and 1 cerebrospinal fluid (CSF). After acid fast staining by Ziehl Neelsen (ZN) technique, grades were given according to the RNTCP guidelines as 0, 1+, 2+, and 3+. All positive samples for AFB were subjected to molecular analysis by CB-NAAT testing for simultaneous detection of Mycobacterium tuberculosis and rifampicin resistance at the IRL, Imphal. Cultures on LJ media were put up simultaneously (Figure 1). Colony growths were studied every week and counter checked by performing acid fast staining from the colonies. Pure strains are being preserved in LJ media in deep freezer for future references.

Approval of research ethics board

The study was started after getting the approval of the Research Ethics Board of the Institute, RIMS. All the participants fulfilling the inclusion criteria were informed about the nature of the study and informed written consents were taken.

Statistical analysis

Statistical analysis was carried out using SPSS version 21 (IBM). Descriptive data were presented using frequency, percentage, mean and standard deviation. Chi square test was used to see association between proportions. The level of statistical significance was set at $p\text{-value}<0.05$.

Results

A total of 100 samples from clinically presumed tuberculosis patients who were positive for AFB were collected. Among these, 94 were sputa, 3 BAL, 2 pleural fluids and 1 was CSF.

On grading the AFB stain microscopically, 42 were of grade 1+, 29 were grade 2+ and 29 were grade 3+ (Table 1). 87 (Male =64, Female =23) out of the 100 samples showed positive culture growth and the remaining 13 (Male =7, Female =6) showed no growth (Table 2). Again, of these 87 positive cultures, 81 were new cases and 6 were of old cases (Table 3). Culture positivity among different grading of AFB staining is statistically significant (p-value<0.05) and can be established that greater the number of acid fast bacilli in the sample, more is the chance of culture positivity (Table 1). Among the 87 culture positive, 7 were RR-TB (Table 4).

In the age wise distribution, maximum cases of tuberculosis as well as rifampicin resistance was seen in the age group of 41-50 (Figure 2). In this age group, 3 RR-TB were seen, all of which were of grade 3+, males and newly diagnosed.

It was observed that out of the 7 RR-TB, 5 of them were AFB grade 3+ and 2 were of grade 2+. This was statistically significant (p-value < 0.05) (Table 5).

In the present study, 71 cases were males and 29 were females. Out of these 71 males, 5 (7%) were resistant to rifampicin whereas of the 29 females, 2 (6.9%) were resistant to rifampicin. More number of males was affected by rifampicin resistant tuberculosis (RR-TB) but these findings are statistically not significant (p-value>0.05) as shown in Table 2. There is no correlation between rifampicin resistance and sex. Therefore, further study is mandated using some other variable to define their association to rifampicin resistance.

Out of the 94 newly diagnosed cases, 68 were males and 26 were females. 81 were culture positive and the remaining 13 were culture negative (Table 3). Again in these 94 new cases, 6 (6.4%) consisting of 5 males and 1 female were RR-TB. Whereas among the 6 old cases which consist of 3 males and 3 females, 1 (16.7%) was RR-TB who is a female participant (Figure 3). Comparing the percentages of the above new and old TB cases, rifampicin resistance is more among old cases. Overall, the total number of rifampicin resistance in the present study was found to be 7 (7%) as shown in Table 4.

Discussions

In 2019, the World Health Organization (WHO) estimated 3.4% new and 18% previously treated multidrug resistant TB cases globally. India accounts for one fourth of the total number of TB cases

Table 1: Association between culture and Acid Fast Bacilli (AFB) grading of the participants (n =100)

AFB	Culture		Total	p-value
	positive	negative		
1+	34	8	42	0.03
2+	24	5	29	
3+	29	0	29	
Total	87	13	100	

Table 2: Association of sex and culture status with rifampicin sensitivity pattern (n=100)

Sl. No.	Sex	Culture		Total	Rifampicin		p-value
		Positive	negative		Sensitive (%)	Resistant (%)	
1	Male	64	07	71	66(93)	5(7)	0.67
2	Female	23	06	29	27(93.1)	2(6.9)	
	Total	87	13	100	93((93)	7(7)	

Table 3: Treatment status and culture cross tabulation count for RR-TB (n=100)

Sl. No.	Treatment status	Culture		Sex		Total
		Positive	negative	Male	Female	
1	New	81	13	68	26	94
2	Old	06	00	3	3	06
	Total	87	13	71	29	100

Table 4: Association between rifampicin sensitivity among the study participants (n=100)

Parameter	Variable of interest	Rifampicin		p-value
		Sensitive (%)	Resistant (%)	
Culture on LJ medium	Positive(n=87)	80(91.9)	7(8.0)	0.59
	Negative(n=13)	13(100)	0(0)	
Treatment status	Old (n=6)	5 (83.3)	1 (16.7)	0.36
	New(n=94)	88 (93.6)	6 (6.4)	

Table 5: AFB grading and rifampicin sensitivity pattern

AFB	Rifampicin		Total	p-value
	sensitive	resistant		
1+	42	0	42	0.01
2+	27	2	29	
3+	24	5	29	
Total	93	7	100	

Figure 1: Buffy colonies of Mycobacterium tuberculosis on LJ medium (red arrows)

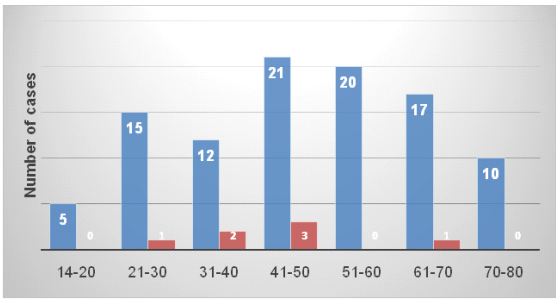

Figure 2: Age wise distribution of TB and RR-TB (n=100)

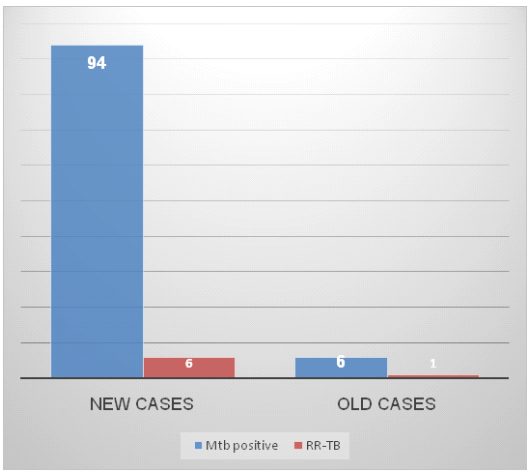

Figure 3: RR-TB in new and old cases (n=100)

worldwide, with 2.8% of the new and 14% retreatment cases being caused by multidrug resistant strain.[12] India has set an ambitious goal of TB elimination by 2025. This large burden of DR-TB will limit progress towards that goal.[8] The primary aim of prompt diagnosis and treatment of pulmonary TB is to cure the individual, as well as rendering him or her non-infectious and thus interrupting the chain of transmission.[5]

The present study shows the burden of rifampicin resistant tuberculosis among patients attending RIMS Hospital, a tertiary care hospital in Manipur in which 6 (6.4%) of the 94 new cases and 1 (16.7%) among 6 old cases were RR-TB. Comparing the old and new cases of TB, burden of RR-TB was much higher among the old cases (16.7%). Furthermore, the burden of RR-TB among new cases as well as old cases are also much higher in the present study than the overall above stated national RR-TB burden data of 2.8% among new and 14% among old cases.[12] Nevertheless, the findings of the present study are similar to those of the studies conducted by Menon et al ., in Mumbai and Rasaki et al., in Nigeria who also found the prevalence of MDR/RR-TB to be 5.9%, 6.0% and 7.2% respectively.[13,14] On the contrary, a study conducted by Saldanha et al., in western India showed lower prevalence of 2.5%.[15]

Regarding the district wise distribution in the present study, it was observed that the maximum cases of TB were concentrated in Imphal west district, which is a thickly populated area in Manipur. But further studies need to be conducted as these findings can be claimed biased due to under reporting from the remote districts and also very few similar studies have been conducted for MDR-TB in this region.

It was also observed that maximum number of the cases were in the working age group, highest being in 41-50 years range. In this age group, high rate of 3 RR-TB among the newly diagnosed male patients were observed. This is comparable to the study by Rasaki et al.[14]All these 3 RR-TB were of grade 3+. The bacterial load in these cases was high which may be alarming.

On examining the association of RR-TB with AFB grading, in our study all the RR-TB were in the AFB grade 2+ and 3+, that is, 5 RR-TB were grade 3+ and 2 RR-TB were grade 2+. This was found to be statistically significant (p-value <0.05). This indicates that there is a direct correlation between drug sensitivity and microscopic grading. This was also shown as a risk factor resulting in poor outcome as conducted by Phu et al.[16] On the other hand, this finding is contradictory to the findings of study conducted by Soedarsono et al., in Indonesia, in which they found that there is no correlation between microscopic grading and 1st line drug resistance.[17]

The present findings of 7% of RR-TB among males, and 6.9% of RR-TB in females were also in conjunction with the study conducted by More et al Maharastra.[18]

Although, culture is the gold standard

recommended by WHO for the definitive diagnosis of Mycobacterium tuberculosis, it was positive only in 87 (87%) of the samples which maybe mainly because of its low sensitivity compared to Gene expert/CB-NAAT.14 We observed that out of the 87 culture positive cases, 7 of them were RR-TB, but then again, it was statistically not significant (p-value>0.05) and accordingly, there is no association between the rifampicin resistance and culture positivity. If viable bacilli were present in significant amount, culture was seen to be positive irrespective of the drug sensitivity pattern. Here, the finding is similar to the findings of Theron et al wherein they hypothesized that lesser proportions of patients with drug-resistant TB have culturable Mycobacterium tuberculosis from respirable, cough-generated aerosols compared to patients with drug-susceptible TB, and that multiple factors, including mycobacterial genomic variation, would predict culturable cough aerosol production.[19]

Another factor that may decrease the sensitivity of growth in culture may be the over enthusiastic decontamination of sample, which causes decrease load of viable bacilli. This can be correlated with study conducted by Mtafya et al., in which they found that decontamination reduces the viability of the bacilli for culture.[20] Again, our findings of culture sensitivity of 87% is comparable to the findings of study conducted by Villegas et al.[21]

Since culture takes very long period of time to come to a conclusion, CBNAAT, which takes less than 2 hours to detect, can be claimed as a mainstay for decision making for commencement of early diagnosis and prompt treatment which may help in efficient control and timely eradication of the TB disease, for which correlation can be made with the culture positivity later. A study conducted by Dewan et al., concluded that CBNAAT helps in increased case detection in a very short span of time to diagnose pulmonary TB in PLHIV (patients living with HIV) as compared to conventional sputum microscopy.[22]

Conclusion

Although MDR-TB is emerging as a significant threat to tuberculosis control, limited data is available. Hence, the present research work is undertaken to highlight the continuing menace of rifampicin resistant tuberculosis among patients attending RIMS Hospital, a tertiary care centre in Manipur. As far as the state data is concerned, Manipur is observing a decreasing trend of resistance from 11.2% in 2017 to 7.9% in 2018, but it still continues to be a menace, posing as a great obstacle to the fight against this deadly disease. The high prevalence of RR-TB found in the present study indicates the importance of strengthening the awareness programmes, establishing early and accurate diagnosis and administration of correct and prompt treatment. In this regard, the CBNAAT comes as an effective tool to aid proper and early diagnosis.

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References

1. Raviglione MC. Tuberculosis. In: Kasper, Fauci, Hauser, Longo, Jameson, Loscalzo, editors. Harrison's Principles of Internal Medicine. 19th Edition. New York: McGraw- Hill; 2015. p. 1102-3.
2. Kingsley E, Ike Anthony C, Chuku A. Prevalence of tuberculosis and rifampicin resistance among patients seeking medical care in Nasarawa State, north central Nigeria. Sci 2016; 4(3):214-8.
3. Guled AY, Elmi AH, Abdi BM, Rage AM, Ali FM, Abdinur AH et al. Prevalence of Rifampicin Resistance and Associated Risk Factors among Suspected Multidrug Resistant Tuberculosis Cases in TB Centers Mogadishu-Somalia: Descriptive Study. Open J Res Dis 2016;6(02):15
4. Krishnamurthy A, Rodrigues C, Mehta AP. Rapid detection of rifampicin resistance in M. tuberculosis by phage assay. Ind J Med Microbiol 2002; 20(4):211.
5. Watterson SA, Wilson SM, Yates MD, Drobniewski FA. Comparison of three molecular assays for rapid detection of rifampin resistance in Mycobacterium tuberculosis. J ClinMicrobiol 1998;36(7):1969-73.
6. Morlock GP, Plikaytis BB, Crawford JT. Characterization of spontaneous, in vitro-selected, rifampin-resistant mutants of Mycobacterium tuberculosis strain H37Rv. Antimicrob Agents Chemother 2000; 44(12):3298-301.
7. Sahana KS, Prabhu AS, Saldanha PR. Usage of cartridge based nucleic acid amplification test (CB-NAAT/GeneXpert) test as diagnostic modality for pediatric tuberculosis; case series from Mangalore, South India. J Clin Tuberc Other Mycobact Dis 2018;11(1):7-9.
8. India TB report 2019. Ministry of Health and Family Welfare Government of India.
9. Somoskovi A, Deggim V, Ciardo D, Bloemberg

GV. Diagnostic implications of inconsistent results obtained with the Xpert MTB/Rif assay in detection of Mycobacterium tuberculosis isolates with an rpoB mutation associated with low-level rifampin resistance. J ClinMicrobiol 2013; 51(9):3127-9.
10. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpertMTB/RIF system. J ClinMicrobiol 2011;49(4):1202-5.
11. Bainara MK, Datta A, Sharma DK. Prevalence of rifampicin resistance by CB-NAAT in previously treated pulmonary tuberculosis cases. EurRespSoc 2016;48:1.
12. World Health Organization. (2018) . Global tuberculosis report 2018. World Health Organization.
https://apps.who.int/iris/handle/10665/274453.
13. Menon S, Dharmshale S, Chande C, Gohil A, Lilani S, Mohammad S et al. Drug resistance profiles of Mycobacterium tuberculosis isolates to first line anti-tuberculous drugs: A five years study. Lung India 2012;29(3):227-8.
14. Rasaki SO, AJibola AI, Musa SA, Moradeyo AK, Odeigah LO, Abdullateef SG, et al. Rifampicin resistant tuberculosis in a secondary health institution in Nigeria, West Africa. J Infect Dis Ther 2014;2(3):1-5.
15. Saldanha N, Runwal K, Ghanekar C, Gaikwad S, Sane S, Pujari S. High prevalence of multi drug resistant tuberculosis in people living with HIV in Western India. BMC infect dis 2019;19(1):391.
16. Phu PT, Vinh DN, Son VT, Hanh NT, Lan NH, Van Vinh T GEet al. Risk factors for poor treatment outcomes of 2266 multidrug-resistant tuberculosis cases in Ho Chi Minh City: a retrospective study. BMC infect dis 2020;20(1):1-0.
17. Soedarsono S, Mertaniasih NM, Sulistyowati T. First line anti-tuberculosis drug resistance pattern in multidrug-resistant pulmonary tuberculosis patients correlate with acid fast bacilli microscopy grading. Indonesian J Tropical InfectDis 2020;8(2):83-9.
18. More SW, Parande MA, Kamble SW, Kamble MS. Profile of drug-resistant tuberculosis in Western Maharashtra. J Family Med Prim Care 2017 ; 6(1):29-30.
19. Theron G, Limberis J, Venter R, Smith L, Pietersen E, Esmail Aet al. Bacterial and host determinants of cough aerosol culture positivity in patients with drug-resistant versus drug-susceptible tuberculosis. Nature Med 2020;1(1):1-9.
20. Mtafya B, Sabiiti W, Sabi I, John J, Sichone E, Ntinginya NE, et al. Molecular bacterial load assay concurs with culture on NaOH-induced loss of Mycobacterium tuberculosis viability. J ClinMicrobiol 2019;57(7):01992-18.
21. Villegas L, Otero L, Sterling TR, Huaman MA, Van der Stuyft P, Gotuzzo E et al. Prevalence, risk factors, and treatment outcomes of isoniazid-and rifampicin-mono-resistant pulmonary tuberculosis in Lima, Peru. PLoS 2016;11(4):1-11.
22. Dewan R, Anuradha S, Khanna A, Garg S, Singla S, Ish P, Agarwal S. Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. J IndAcadClin Med 2015;16 (2):114-7.