Original Research Paper

Volume - 11 | Issue - 05 | May - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

ISONIAZID MONORESISTANCE : THE NEW CHALLENGE IN DRUG RESISTANT TUBERCULOSIS

Paulami Palchowdhury	Junior Resident, Dept of Microbiology, Calcutta School of Tropical Medicine.
Anindita Rakshit	Assistant Professor, Dept of Microbiology, Calcutta School of Tropical Medicine.
Aitihya Chakraborty	Junior Resident, Dept of Microbiology, Calcutta School of Tropical Medicine.
Banya Chakraborty*	Professor and Head, Dept of Microbiology, Calcutta School of Tropical Medicine. *Corresponding Author

ABSTRACT

Background: Drug resistant tuberculosis is a global threat to overall tuberculosis control. Isoniazid is a critically important first line drug. Isoniazid monoresistance increases the incidence of treatment failure and relapse.

Method: A total of 100 sputum samples positive for acid fast bacilli by Ziehl-Neelsen staining were collected from pulmonary tuberculosis patients between September 2019 to January 2020 (four months) and subjected to Line probe assay (LPA).

Result: Mycobacterium tuberculosis complex was detected in all the 100 samples by LPA. It was found that 82 (82%) samples were sensitive to both Isoniazid and Rifampicin, 9(9%) of them were multidrug resistant ,8(8%) were Isoniazid monoresistant and 1(1%) was Rifampicin monoresistant.Out of the Isoniazid monoresistance cases mutation in katG was seen in 87% cases and inhA mutation in 13%.

Conclusion: Isoniazid resistance is surprisingly increasing and is missed out by rapid tests.

KEYWORDS : Line probe assay, Tuberculosis, Isoniazid monoresistance

INTRODUCTION:

Tuberculosis is a disease that affects mainly the lungs(pulmonary tuberculosis) and can also affect other parts of the body like bones,intestine,meninges,lymph nodes (extrapulmonary tuberculosis). It is caused by the bacteria Mycobacterium tuberculosis . Most infections do not have symptoms, in which case it is known as latent tuberculosis.About 10% of latent infections progress to active disease. If left untreated, it can kill about half of those affected. It is a chronic disease with cardinal symptoms being persistent chronic cough with blood-tinged mucus, intermittent fever, night sweats, and unexplained weight loss.

Tuberculosis is one of the top three infectious diseases in the world along with HIV, malaria where tuberculosis alone kills 2 million people every year.[1] Recent estimates suggest that one fourth of the world's population is infected with tuberculosis and maximum of them ie around 95% of all newly infected patients live in developing countries like India. With this there has been a significant rise in the multidrug and extensive drug resistant cases that has become a threat to the global tuberculosis control. The culture methods and drug-susceptibility tests (DST) using solid media take up to 10–12 weeks for results and liquid-based culture techniques still take 4–6 weeks. Such prolonged time taken by these conventional methods delays the treatment procedure and increases the need for rapid diagnostic methods. The spread of primary drug resistance calls for immediate action in order to prevent a massive spread of multidrug resistant tuberculosis.[2] New strategies and diagnostic advancement is needed for early diagnosis, to shorten the duration of therapy and improve treatment outcomes.[3]

Line probe assay (LPA) is a promising rapid diagnostic tool that detects mutation in rpoB , katG, and inhA genes. It targets the mutations in the 81 bp core region of rpoB gene. By this it can detect more than 95% rifampicin resistant strains [4]. It can be used for testing direct smear microscopy specimens. Several molecular assays are available for diagnosis of tuberculosis and among them promising results have been shown by LPA in many studies.[5] According to world stastistics data, isoniazid monoresistant TB without MDR-TB is estimated to be 9.5% of all cases [6]. LPAs are more difficult to perform and even takes longer time to complete but they have the ability to detect Isoniazid resistance along with Rifampicin resistance unlike Xpert MTB/RIF [7]. In an urban populations with a significant prevalence of MDR-TB and HIV coinfection a rapid identification test like LPA will be very useful and cost effective.[8] In many studies, LPA has shown good performance even in samples that came as contamination in culture methods or even in smear-negative samples. [9]

MATERIAL AND METHODS:

The study was conducted in the Bacteriology department of School of Tropical Medicine, Kolkata over a span of four months from September 2019 to January 2020 on 100 sputum samples that were positive for acid fast bacilli by Ziehl Neelsen staining. The smears were then graded depending upon the bacilli seen under 1000X magnification according to RNTCP grading[10]. Sputum samples from patients who are diagnosed as smear positive for acid fast bacilli in the Direct Smear Microscopy centre (DSM) of our hospital were collected in 50ml falcons and transported to laboratory at its earliest. According to WHO protocols the whole procedure was done in three separate rooms with unidirectional flow of work using GenoType® MTBD Rplus version 2.0 (Hain Lifescience GmbH, Nehren, Germany) .First the samples were decontaminated with sodium hydroxide-citrate-Nacetyl-L-cysteine. DNA extraction was done using GenoLyse® kit (Hain Lifescience GmbH, Nehren, Germany). Amplification was done by multiplex PCR using biotinylated primers using the amplification profile: denaturation of 15 min at 95°C, followed by 20 cycles of 30 sec at 95°C and 2 min at 65°C, and 30 cycles of 25 sec at 95°C, 40 sec at 50°C and 40 sec at 70°C and the extension step of 8 min at 70°C.Reverse hybridisation was performed using a pre-programmed TwinCubator (Hain Lifescience GmbH, Nehren, Germany)where probes (reaction zone or bands) on the nitrocellulose strips are used to interrogate mycobacterium tuberculosis target DNA .After denaturation, the biotin-labelled amplicons were hybridised to the single stranded membrane-bound probes. After a stringent washing, as streptavidin-alkaline phosphate conjugate was added to the strips, an alkaline phosphate mediated staining reaction was observed as bands where the amplicon and the probe had hybridized with rifampicin and isoniazid .Result were interpreted following manufacturer's protocol and WHO guidelines[11]

RESULT:

Mycobacterium tuberculosis complex was detected in all the 100 samples by LPA. It was found that 82 (82%) samples were sensitive to both Isoniazid (INH) and Rifampicin (RIF) , 9 (9%) of them were multidrug resistant (MDR) ie. resistant to both, 8 (8%) were Isoniazid monoresistant and 1 (1%) was Rifampicin monoresistant.Out of the Isoniazid monoresistance cases mutation in katG was seen in 87% cases which confers “high level” resistance and inhA mutation in 13%. Sputum samples were with 3+ grading in most cases (40%).Patients were maximum in the age group of 30-51 years with more male participants (62%) than female (38%).13% of them were HIV positive.

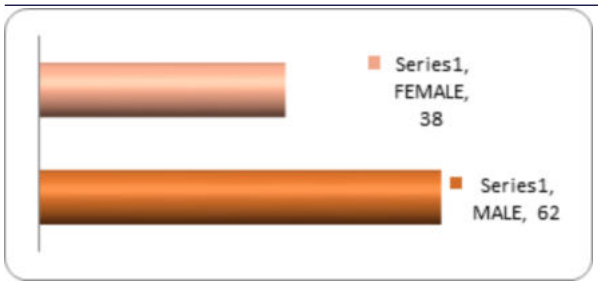

Fig1: Male female ratio of the cases under study

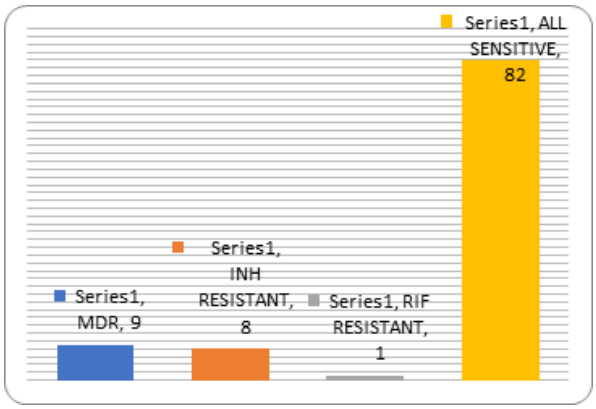

Fig 2:Resistance pattern to the first line antitubercular drugs

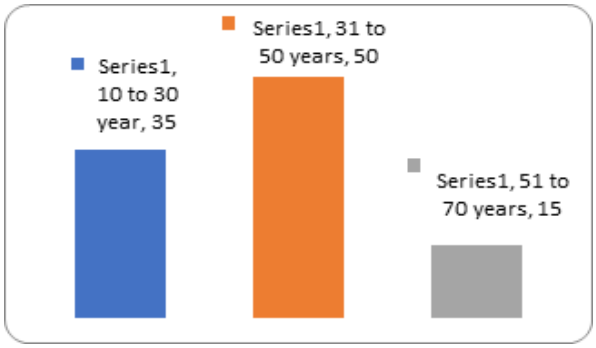

Fig 3: Age distribution of the cases under study

Table 1:Sputum grading of cases under study

SPUTUM GRADING	PERCENTAGE
1+	**31%**
2+	**26%**
3+	**40%**
4+	**3%**

DISCUSSION :

Most of the laboratories in India uses Xpert/RifAssay as a rapid method. Larger studies are required to call Rifampicin resistance as surrogate marker of multidrug resistant tuberculosis. In a study conducted by Deepak Arora et al in New Delhi it was seen that 5% of the resistant cases were missed by Xpert/RifAssay but diagnosed by LPA. [12] India TB report 2019 showed that the incidence of Isoniazid monoresistance is 7.3% whereas that of multidrug resistance is 6% by Line probe assay [13].Isoniazid monoresistant tuberculosis patients who are treated with 2HREZ/4HR regimen show a much higher risk of developing treatment failure or relapse, or acquiring additional resistance than than those who have drug-susceptible Tuberculosis . [14]

CONCLUSIONS:

The present study has evaluated the performance of MTBDRplus molecular assay for rapid detection of multidrug resistant M. tuberculosis as well as isoniazid monoresistance directly from smear positive pulmonary samples. Isoniazid resistance is surprisingly increasing and is missed out by rapid tests like Xpert/RifAssay as it does not detect mutations in katG and inhA genes. Inappropriate diagnosis and incorrect treatment regimen is making the control of drug resistant tuberculosis even more difficult. Therefore, the rapid detection of such isoniazid monoresistance cases is also critical for reducing overall morbidity and mortality. If there is implementation of stable infrastructure and trained laboratory personals, LPA can be a game changer in the diagnostic field. Isoniazid is a critically important first line drug and our aim in diagnosing its resistance is to reduce the incidence of treatment failure and relapse. A prolonged time required by conventional DSTs will lead to patients being treated with an inappropriate drug regimen. This will cause selection of drug resistant mutant strains and their continuous spread in the community that will hamper in the procedure of tuberculosis management and control. Till now, a few studies have documented Isoniazid-monoresistant TB in India.

REFERENCES:

1. Sandhu G. K. (2011). Tuberculosis: current situation, challenges and overview of its control programs in India. Journal of global infectious diseases, 3(2), 143–150. https://doi.org/10.4103/0974-777X.81691
2. Heifets, L. B., & Cangelosi, G. A. (1999). Drug susceptibility testing of Mycobacterium tuberculosis: a neglected problem at the turn of the century. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease, 3(7), 564–581.
3. Schito, M., Migliori, G. B., Fletcher, H. A., McNerney, R., Centis, R., D'Ambrosio, L., Bates, M., Kibiki, G., Kapata, N., Corrah, T., Bomanji, J., Vilaplana, C., Johnson, D., Mwaba, P., Maeurer, M., & Zumla, A. (2015). Perspectives on Advances in Tuberculosis Diagnostics, Drugs, and Vaccines. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 61Suppl 3(Suppl 3), S102–S118. https://doi.org/10.1093/cid/civ609
4 Rkia Eddabra, Mounsef Neffa, "Mutations Associated with Rifampicin Resistance in *Mycobacterium tuberculosis* Isolates from Moroccan Patients: Systematic Review", *Interdisciplinary Perspectives on Infectious Diseases*, vol. 2020, Article ID 5185896, 8 pages, 2020. https://doi.org/10.1155/2020/5185896
5. Ling et al.Rapid diagnosis of drug- resistant TB using line probe Oct2008.:Expert review respiratory medicine 2(5),583-588 (2008)
6. Cornejo Garcia, J. G., Alarcón Guizado, V. A., Mendoza Ticona, A., Alarcon, E., Heldal, E., & Moore, D. (2018). Treatment outcomes for isoniazid-monoresistant tuberculosis in Peru, 2012-2014. PloS one, 13(12), e0206658. https://doi.org/10.1371/ journal.pone. 0206658
7. Nathavitharana, R. R., Cudahy, P. G., Schumacher, S. G., Steingart, K. R., Pai, M., & Denkinger, C. M. (2017). Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and meta-analysis. The European respiratory journal, 49(1), 1601075. https://doi.org/10.1183/ 13993003. 010 75-2016
8. Telenti, A., Honoré, N., Bernasconi, C., March, J., Ortega, A., Heym, B., Takiff, H. E., & Cole, S. T. (1997). Genotypic assessment of isoniazid and rifampin resistance in Mycobacterium tuberculosis: a blind study at reference laboratory level. Journal of clinical microbiology, 35(3), 719–723. https://doi.org/10.1128/JCM.35.3.719-723.1997
9. Barnard, M., Albert, H., Coetzee, G., O'Brien, R., & Bosman, M. E. (2008). Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. American journal of respiratory and critical care medicine, 177(7), 787–792. https://doi.org/10.1164/rccm.200709-1436OC
10. Manual for Sputum Smear Fluorescence Microscopy RNTCP, Central TB Division Directorate General of Health Services
11. GenoType MTBDRplus VER 2.0 Instruction for use. Hain Lifesciences
12. Arora, D., Jindal, N., Bansal, R., & Arora, S. (2015). Rapid Detection of Mycobacterium tuberculosis in Sputum Samples by Cepheid Xpert Assay: A Clinical Study. Journal of clinical and diagnostic research : JCDR, 9(5),DC03–DC5. https://doi.org/ 10.7860/ JCDR/2015/11352.5935
13. India Tb Report 2019 ,Central TB Division https:// tbcindia.gov.in/index1. php?lang= 1& level=2&sublinkid=5358&lid=345
14. WHO treatment guidelines for isoniazid-resistant tuberculosis Version: 24 April 2018)