Study On Prevalence Of Drug Resistance And Genetic Mutation Pattern Among Suspected Drug Resistant Pulmonary Tuberculosis Cases In Jamnagar District.

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Abstract: Background: Drug resistant tuberculosis is a growing public health problem. Diagnosis of drug resistant tuberculosis is challenging due to equivocal disease presentation of drug sensitive and drug resistant tuberculosis. Isoniazide is one of the oldest and most potent anti- tuberculosis drugs and consistent component of time treated Standard Short Course Chemotherapy. Resistance to first line drugs can pose a significant challenge to efforts of TB control especially in developing countries like India. Objective: The present study aims to determine prevalence of isoniazid and rifampicin mono resistance and multidrug resistance among suspected pulmonary tuberculosis cases and study pattern of their genotypic mutation. Methods: We screened 1332 suspected drug resistant pulmonary tuberculosis patients registered from January 1st, 2013 to December 31st, 2014 under Revised National TB Control Program in Jamnagar district. Their sputum samples were subjected drug sensitivity for isoniazid and rifampicin. Out of 1332 patients, valid reports were obtained for 1005 patients. Results: We observed that the prevalence of isoniazid mono resistance, rifampicin mono resistance and multi-drug resistance was 9.25%, 2.29% and 5.57% respectively. Proportion of mono or multi-drug resistance was as high as 85% among category-II patients. Kat-G gene mutation (bad mutation) was found in three fourth of total patients studied. Conclusion: The prevalence of isoniazid mono resistance was higher, followed by multi-drug resistance and least for rifampicin mono resistance. Drug resistance was observed to be higher in retreatment cases than new cases. The prevalence of Kat-G mutation was also very high compared to Inh-A mutation with about three fourth of isoniazid mono resistant cases having Kat-G genotypic pattern.[Rami K Natl J Integr Res Med, 2019; 10(4):6-9]

Key Words: tuberculosis, Isoniazide, rifampicin, resistance, mutation, genotype

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Introduction: India is one of the highest TB burden countries in the world with second highest number of estimated drug resistant TB cases after china[1]. Drug resistant surveys indicate that prevalence of MDR TB in India is as high as 2-3% among new cases and 12-17% among retreatment cases [2].

Drug resistant TB cases are rapidly growing in our country. It has microbial, clinical and programmatic causes. From a microbiological perspective, resistance is caused by a genetic mutation that makes a drug ineffective against mutant bacilli[3].

Currently, throughout the world, isoniazid and rifampicin together represent the backbone of short-course chemotherapy treatment for *Mycobacterium tuberculosis* infections. The rise of multidrug-resistant tuberculosis (MDR-TB), defined as TB showing resistance to at least isoniazid and rifampicin, is a serious threat to TB control[4]. A convergence of data indicates that isoniazid resistant clinical isolates of M. tuberculosis have distinct mutation frequencies in the genes *katG*, *kasA*, and *inhA* (regulatory and structural regions)[5-7]. The *katG* and *inhA* mutations arise before all other drug resistance mutations so frequently that they have been deemed "harbinger mutations", and early detection may be helpful in preventing the evolution and spread of multidrug resistant and extensively drug resistant (MDR and XDR) strains[8].

The present study aims to identify the mutations associated with isoniazid resistance in *M. tuberculosis* apart from estimating prevalence of isoniazid and rifampicin mono resistance and multi-drug resistance among suspected pulmonary tuberculosis patients.

Material and Methods: This analytic study was conducted in the outpatient department of Pulmonary Medicine of M.P. Shah Government Medical College, Jamnagar. We screened 1332 suspected drug resistant pulmonary tuberculosis cases (both smear positive and negative) registered under Revised National TB Control Program between January 1st, 2013 to December

31st 2014. These suspected patients were subjected to two sputum smear examinations.
Sputum smear positive samples were directly studied by LPA (line probe assay) method for DST (drugs sensitivity tests) at IRL (intermediate reference laboratory) at M.P. Shah Government Medical College, Jamnagar while sputum smear negative samples were subjected to liquid culture and subsequently studied by LPA method for DST. Out of 1332 patients, we found 1005 patients with valid reports of DST/LPA. So, our final sample size would be 1005 suspected tuberculosis patients. We included all suspected patients except confirmed mono or multi-drug resistance cases, those unwilling to participant and terminally ill patients.

Study Period: January 1st , 2013 to December 31st 2014.

Ethical clearance was obtained before beginning the study from the institutional ethical committee of M.P. Shah Government Medical College, Jamnagar. Data were entered and analysed using Microsoft Excel.

Results: Out of total suspected drug resistant TB patients, 91.34% were retreatment cases. (table No. 1). We observed that about 82.89% patients were sensitive to first line anti-tuberculosis drugs, 9.25% had resistance to isoniazid alone, 2.29% had resistance to rifampicin alone, and about 5.57% were resistant to both isoniazid and rifampicin.

Table 1 : **Distribution of suspected TB patients according to category of treatment.**

Category	**No. (%)**
Category-I (New cases)	87 (8.66)
Category-II (Retreatment cases)	918 (91.34)
Total	1005 (100)

Thus, most common type of drug resistance observed was H-mono resistance, followed by multi-drug resistance. Rifampicin resistance was least commonly observed among study participants (Table 1 to 4).

Table 2 : Drug resistant Pattern observed among suspected TB patients.

Drug resistant pattern observed	**No. (%)**
Isoniazid (H) monoresistance	93 (9.25)
Rifampicin (R) monoresistance	23 (2.29)
Multi-drug (H and R) resistance	56 (5.57)
Drug sensitive patients	833 (82.89)
Total	1005 (100)

As table no. 3 shows, proportion of drug resistance (either mono or multi) is as high as 85% among patients receiving category-II treatment. On the contrary, proportion of drug sensitive patients are as high as 92.44% among those receiving category-II treatment.

Table No.3. Pattern of drug resistance among different category of patients.

Drug resistant pattern observed	Category-I (New cases)	Category-II (Previously treated cases)	Total
Isoniazid (H) monoresistance	14 (15.05)	79(84.95)	93
Rifampicin (R) monoresistance	3 (13.04)	20 (86.96)	23
Multi-drug (H and R) resistance	7 (12.5)	49 (87.5)	56
Drug sensitive patients	63 (7.56)	770 (92.44)	833
Total	87	918	1005
Chi square test: X2=0.2, P<0.05			

Out of total category-I patients screened, majority of them (16.09%) have isoniazid mono resistance pattern, followed by multi-drug resistance pattern (8.05%). Similar findings were observed in patients receiving category-II also, where majority of them (8.60%) were H-mono resistant, followed by multi-drug resistance (5.34%).

Table No.4 Category-wise distribution of drug resistant cases.

Drug resistant pattern observed	H mono resistance	R mono resistance	Multi-drug resistance	Drug sensitive	Total	Chi square Test
Category-I	14 (16.09)	3 (3.45)	7 (8.05)	63	87	X2= 0.20
Category-II	79 (8.60)	20 (2.18)	49 (5.34)	770	918	P<0.05
Total	93	23	56	833	1005	

Table No. 5. Genetic mutation pattern among isoniazid (H) mono resistant cases among different category of treatment.

Genotype pattern	Category-I	Category-II	Total	Chi square test 0.17 P<0.05
Kat-G	11 (15.94)	58 (84.06)	69 (74.20)	
Inh-A	3 (12.5)	21 (87.5)	24 (25.80)	
Total	14 (15.05)	79 (84.95)	93 (100)	

Discussion: After a hundred years of discovery of the tubercle bacilli, TB still remains one of the most challenging issues in global health. An important challenge for TB control is the emergence of strains that are resistant to the most potent anti- TB agents i.e. isoniazid and rifampicin. It commonly results from genetic mutations during inadequate or incomplete therapy[9]. Over the past many years, there has been an emergence and escalated recognition of several patterns of drug resistance in TB strains like mono-resistance, multi-drug resistance (MDR) and extensively drug resistance (XDR) [10].

In our study, about 82.89% cases were sensitive to first line anti-tuberculosis drugs, 9.25% had resistance to isoniazid alone, 2.29% had resistance to rifampicin, and about 5.57% were resistant to both drugs. Thus, most of the cases were resistant to isoniazid, followed by multi drug resistant cases. In a study by C. Thakur et al, about 65.45% patients were sensitive to both drugs, 8.63% and 6.14% were resistant to isoniazid and rifampicin respectively[11]. A study conducted in Gujarat in the past revealed that about 7.86% cases were isoniazid mono-resistant, 0.5% cases were resistant to rifampicin alone, and about 8.37% cases were found to be multi-dug resistant (MDR)[12].

As table no. 3 shows, proportion of drug resistance (either mono or multi) is as high as 85% among patients receiving category-II treatment. On the contrary, proportion of drug sensitive patients are as high as 92.44% among those receiving category-II treatment. A study in South India also observed similar findings, where all forms of drug resistance (mono or multi) was higher in retreatment cases than new cases[13]. In India, resistance to anti-tubercular drugs among previously treated cases was found to be in the range of 40-70% for isoniazid and 20-30% for rifampicin[14].

Kat-G mutation is considered to be a high level mutation in isoniazid mono-resistant cases in which the mono resistance cannot be improved even if we increase concentration of isoniazid drug. On the contrary, Inh-A mutation is said to be a low level mutation, in which we may obtain sensitivity to isoniazid by increasing isoniazid drug concentration. Moreover, in isoniazid mono-resistant cases with Kat-G mutation pattern, we may administer new shorter regime (9 months duration) for drug resistant TB, but it is ineffective in case of Inh-A mutation cases.

The present study observed that majority of isoniazid resistant cases (74.20%) had Kat-G genotypic pattern, while only 25.80% patients had Inh-A genotypic pattern. A study in China observed that 94.3% INH-resistant isolates had mutations in the *katG* gene[4]. Another study in Brazil also observed similar findings in which Mutations in *katG* were found in 83 (85.6%) of the 97 INH-resistant isolates , whereas Mutations in the *inhA* promoter region occurred in 25 (25.8%) of the INH-resistant isolates[15].

Conclusion: The prevalence of isoniazid mono resistance was higher, followed by multi-drug resistance. Drug resistance was observed to be higher in retreatment cases than new cases. The prevalence of Kat-G mutation (bad mutation) was also very high (three fourth) compared to Inh-A mutation among isoniazid mono resistant cases.

Recommendations: The higher prevalence of isoniazid mono resistance warrants screening of all tuberculosis cases especially retreatment cases and for drug resistance to first line drugs. The retreatment cases should be followed up both clinically and microbiologically at regular intervals during treatment for early identification of resistance and retrieval action if needed. Better management of TB cases, establishing advanced diagnostic methods and universal use of standard treatment guidelines are strongly recommended to avoid further emergence of drug resistant cases.

Reference-

1. Who Global TB Control. WHO Report 2010. Available at http://www.who.int/tb/publications/global_r

eport/2010/en/index.html. Last seen on 30th May, 2019.

2. Institute of Medicine (US). Facing the Reality of Drug-Resistant Tuberculosis in India: Challenges and Potential Solutions: Summary of a Joint Workshop by the Institute of Medicine, the Indian National Science Academy, and the Indian Council of Medical Research. Washington (DC): National Academies Press (US); 2012. 2, Drug-Resistant TB in India. Available from: https://www.ncbi.nlm.nih.gov/books/NBK100386/. Last seen on June 12, 2019.
3. K. Park. Text Book of Preventive and Social Medicine. 23rd ed. Banarasidas Bhanot Publications, Jabalpur.2015:205.
4. Zhang M, Yue J, Yang YP, Zhang HM, Lei JQ, Jin RL, Zhang XL, Wang HH. 2005. Detection of mutations associated with isoniazid resistance in Mycobacterium tuberculosis isolates from China. J Clin Microbiol 2005;43: 5477–5482.
5. Garcı´a-Garcı´a, ML, Ponce de Leo´n, Jimenez-Corona ME, Jimenez-Corona A, Palacios-Martinez M, Balandrano-Campos S.et al. Clinical consequences and transmissibility of drug-resistant tuberculosis in southern Mexico. Arch Intern Med 2000;160:630– 636.
6. Hazbon, MH., M. Bobadilla del Valle, M. I. Guerrero et al. Role of *embB* codon 306 mutations in *Mycobacterium tuberculosis* revisited: a novel association with broad drug resistance and IS*6110* clustering rather than ethambutol resistance. Antimicrob. Agents Chemother. 2005;49:3794–3802.
7. Lee AS, Lim IH, Tang LL, Telenti A, Wong SY. Contribution of *kasA* analysis to detection of isoniazid-resistant Mycobacterium tuberculosis in Singapore. Antimicrob. Agents Chemother. 1999;43:2087–2089.
8. Manson AL et al. Genomic analysis of globally diverse Mycobacterium tuberculosis strains provides insights into the emergence and spread of multidrug resistance. Nat Genet. 2017;49:395–402.
9. World Health Organization. Towards Universal Access to Diagnosis and Treatment of Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis by 2015: WHO Progress Report 2011. WHO, Geneva (2011)
10. Ahmed MM, Velayati AA, Mohammed SH. Epidemiology of multidrug-resistant, extensively drug resistant, and totally drug resistant tuberculosis in Middle East Countries. Int J Mycobact.2016;5:249-256.
11. Thakur C, Kumar V, Gupta AK Detecting mutation pattern of drug-resistant Mycobacterium tuberculosis isolates in Himachal Pradesh using GenoType(®) MTB DR plus assay. Ind J Med Microbiol. 2015;33:547-53.
12. Ramachandran R, Nalini S, Chandrasekar V, Dave PV, Sanghvi AS, Wares F et al. Surveillance of drug resistant tuberculosis in the state of Gujarat, India. Int J Tuberc Lung Dis.2009;13(9):1154-1160.
13. Paramsivan CN, Venkataraman P, Chandrasekaran V, Bhat S, Narayanan PR. Surveillance of drug resistance in tuberculosis in two districts of South India. Int J Tuberc Lung Dis. 2002;6(6):479-84.
14. Shah AR, Agarwal SK, Shah KV. Study of drug resistance in previously treated tuberculosis patients in Gujarat, India. Int J Tuberc Lung Dis.2002;6(12):1098-01.
15. Cardoso RF, Cooksey RC, Morlock GP et al. Screening and Characterization of Mutations in Isoniazid-Resistant Mycobacterium tuberculosis Isolates Obtained in Brazil. Antimicrob Agents Chemother. 2004;48:3373-81.

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