Original Article

Evaluation of the frequency of resistance to 2 drugs (Isoniazid and Rifampin) by molecular investigation and it's risk factors in new cases of smear positive pulmonary tuberculosis in health centers under the cover of Jundishapur University of Medical Sciences in 2017

Shokrollah Salmanzadeh[1], Masoumeh Karamian[2], Seyed Mohammad Alavi[1], Roohangiz Nashibi[1]

[1]Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, [2]Infectious Diseases and Tropical Medicine, Ahvaz Jundishapur University of Medical Sciences, Medical School, Razi Teaching Hospital, Ahvaz, Iran

Abstract

Introduction: Despite the great efforts to control tuberculosis (TB), the disease is still one of the major health challenges throughout the world. The basic treatment for TB is drug therapy. Currently, the main anti-tuberculosis drugs with major use in the treatment and control of the disease are isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. One of the serious crises in controlling TB epidemic is diagnosis and treatment of patients with Multidrug Resistant Tuberculosis (MDR-TB MDR). The purpose of the study was to examine and evaluate the resistance of mycobacterium TB strains isolated from specimens of newly diagnosed smear positive pulmonary TB to isoniazid and rifampin using molecular methods and their risk factors. **Methods:** Sputum samples of newly diagnosed smear positive pulmonary TB patients were prepared, collected, and sent to Reference Laboratory in Ahvaz. DNA of mycobacterium tuberculosis was prepared from the samples using Qiagen kit according to the instructions of the manufacturing company. Isoniazid resistance was evaluated using specific primers for inhA and KatG genes. Rifampin resistance was evaluated using MAS-PCR method with three specific alleles of rpobB codons and codons 516, 526 and 531. **Results:** Mycobacterium tuberculosis resistance to Isoniazid was 7.3%, to Rifampin 5.5% and to both drugs 1.8%. In our study, there were no association between drug resistance and gender, age, prison history, smoking, drug use, underlying disease, occupation, and HIV. **Conclusion:** According our findings that include prevalence of 7.3% Isoniazide resistance, 5.5% Rifampin resistance and 1.8% to both drugs, evaluating all newly diagnosed patients for resistance to standard anti-tuberculosis treatment seems rational.

Keywords: Drug resistance, isoniazid, mycobacterium tuberculosis, rifampin

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Introduction

TB is a bacterial disease caused by Mycobacterium Tuberculosis and sometimes Mycobacterium bovis and Mycobacterium africanum. People infected with Mycobacterium TB may develop TB at any time. This disease can affect most of the body tissues and organs especially the lungs. the highest chance of getting the disease is immediately after being infected.

Drugs are the basis of TB treatment. Currently, the main anti-TB drugs used in the treatment and control of disease are isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin.[1] MDRTB (multi drug resistance) refers to the cases resistant to at least isoniazid and rifampin. XDR TB is a case of resistance to isoniazid, rifampin, fluoroquinolone, and at least one of the three second-line injectable drugs (amikacin, capreomycin, kanamycin).[2] According to WHO, the number of patients diagnosed in 2015 was 13,000 in Iran, and the incidence was 16 in 100,000 per person. This year, the number of HIV-positive TB patients in Iran reached 350, with an incidence of 0.44% per one hundred thousand people.[3] One of the serious crises in the control of the TB epidemic is the diagnosis and treatment of MDR TB patients.

In 2015, about 480,000 new cases were reported, that also 100,000 cases of rifampin resistance should be added to the present state.

Among these cases, only 125,000 (20%) received appropriated MDR-TB treatment. Rifampin is one of the most important first-line drugs for TB treatment. Many studies have shown that more than 95% of rifampin resistance is because of point mutations in the rpoB gene (the RNA polymerase beta subunit encoding gene).[4] The most frequent mutations related to rifampin resistance in mycobacterium tuberculosis are replacements at codons 531, 526, and 516 in the rpoB gene, respectively. Mutations in these three codons are the factor for 70 to 95% of resistance to rifampin.[4] Isoniazid is another effective drug in TB treatment. Isoniazid resistance happens due to mutations in several genes, the most important of which are inhA and katG.[5] Conventional tests for the susceptibility of mycobacterium tuberculosis need at least 3–4 weeks. Nowadays Molecular reliable methods have replaced conventional methods. Some of these procedures are quick and accurate.[6] In Iran, prevalence of anti-TB drugs resistance is limited. According to the WHO recommendation, prior to treatment for anti-TB drugs, the susceptibility test for drugs should be determined. Currently, according to the our country protocol, antibiotic susceptibility testing is not done for all patients prior to treatment and resistance is diagnosed when the clinical response to first-line therapies is not occurred and the patient's sputum smear is still positive after the 5 months treatment.[4] The goal of our study was to evaluation of the frequency of resistance to 2 drugs (Isoniazid and Rifampin) by molecular investigation and it`s risk factors in new cases of smear positive pulmonary tuberculosis.

Materials and Methods

This descriptive cross-sectional study was conducted on the patients to refer to Ahvaz Jundishapur University of Medical Sciences health care centers with new diagnosis of smear positive pulmonary TB.

The study was approval by Ahvaz Jundishapur University's ethical committee (ID: IR.AJUMS.REC.1396.734). Consent to participate was obtained from all participants in 19-12-2017.

Incidental Sputum samples of newly 55 diagnosed smear positive pulmonary TB patients were collected, and sent to Reference Laboratory in Ahvaz, immediately. The samples taken from the cities affiliated to Ahvaz Jundishapur University of Medical Sciences health care were kept at 8 to 10°C and were sent to Ahvaz Reference Laboratory maximally within 24 hours in a cold box. These samples were decontaminated by Petroff method (sputum samples with the same or twice volume were added to normal NAOH 4% concentration to eliminated other bacteria and normal flora after hour and then All external factors except for TB bacilli [due to lipid wal] are eliminated).

DNA of the studied samples was collected using Qiagen kit according to the instructions of the manufacturing company. After DNA collected, specific IS6110 primers [Table 1] were used to identify mycobacterium species. M. TBH37Rv was used as a positive control and water as a negative control. Specific primers for inhA and KatG genes were used to examined isoniazid resistance. Moreover, specific rpobB codons (codons 516, 526, and 531) were used to evaluate rifampin resistance by MAS-PCR [Figure 1]. Non-bonding or nonspecific bonding to another site was considered as resistance. Table 1 shows the sequence of primers used in this study.

For newly diagnosed smear positive pulmonary TB patient, demographic information questionnaire, including occupational status, underlying diseases, cigarette and drug abuse, history of prison, prior treatment with anti-TB drugs, HIV infections and immunosuppressive drugs history were completed and their information to determine the risk factors of drug resistance were used.

Table 1: The sequences of primers used to identify rifampin and isoniazid resistant strains			
Reference	Size	5' 3'	Gene
27	190 bp	ATC CTG CGA GCG TAG GCG TCG G	IS6110-Tb1
		CAG GAC CAC GAT CGC TGA TCC	IS6110-Tb2
		GG	
28	292 bp	GCA GAT GGG GCT GAT CTA CG	KatGF
	270 bp	ATA CGA CCT CGA TGC CGC	KatGR
		GCG CGG TCA GTT CCA CA	inhAP-15
		CAC CCC CGA CAA CCT ATC G	inhAPF2
28	218 bp	CAG CTG AGC CAA TTC ATG GA	rpoB516
	185 bp	CTG TCG GGG TTG ACC CA	rpoB526
	170 bp	CAC AAG CGC CGA CTG TC	rpoB531
		TTG ACC CGC GCG TAC AC	RIRm

After collecting the statistical data, SPSS 17 was used for data analysis and statistical tests.

$$n = \left[\frac{Z^2_{1-\frac{\alpha}{2}} \; p(1-p)}{d^2} \right] \qquad \alpha = 0.05 \qquad Z_{1-\frac{\alpha}{2}} = 1.96$$

$$p = 0.29 \qquad d = 0.12$$

Firstly, descriptive data including mean and standard deviation for quantitative data and frequency and percentage for qualitative data was used. Chi-square test to determine the association between the qualitative variables was used.

Results

The study examined 55 patients with newly diagnosed smear-positive pulmonary TB. Among these, 46 (83.6%) were male and 9 (16.4%) were female. Their mean age was 38.78 ± 14.8 years. The oldest was 82 years and the youngest was 20 years [Table 2].

All of the female patients in this study were housewives. Also, 17 (30.9%) patients were unemployed, 17 (30.9%) were

Table 2: Demographic information of the studied population	
Variable	Number (present/amount)
Male	46 people (83.6%)
Female	9 people (16.4%)
Mean age	38.78±14.8
The oldest	82 years
The youngest	20 years

The most frequent age group was 20-40 years (67.27%) and 41-60 years (21.82%)

Figure 1: Result of MAS-PCR for Isoniazid and Rifampon Resistance

self-employed and 7 (12.7%) were workers. People who sell product were considered self-employed.

Isoniazid resistance in 4 patients (7.3%) and rifampin resistance in 3 patients (5.5%) was reported, also among these 1 patient (1.8%) was resistant to both drugs.

Among 4 patients with Isoniazid resistance, 1 was female and 3 were males. Also, 3 patients were in 20–40 years age group and one in 60–41 years age group. There was not statistically significant between Isoniazid resistance with gender and age (p.v >0.05). All three Rifampin resistant patients were males and those were 20- to 40-year-old age group, but no significant association was seen between gender, age, and Rifampin resistance (p.v > 0.05). The patient with resistance to both drugs was males and 24 years old. There was not significant association between gender, age and both drugs resistance. Two patients had a history of prison and 2 did not. Also, among 3 Rifampin resistance patients, 2 had a history of prison but there was no significant association between prison history and Rifampin resistance (p.v = 1). The patient with resistance to both drugs did not have a prison history. This was not statistically significant between prison history and drugs resistance (p.v = 0.491). Also, statistically significant association between smoking, drug abuse and resistance to isonizaid, rifampin, and both drugs was not observed (pv > 0.05). Also, we not found association between HIV infection, patients had a history of immunosuppressive drugs and drug resistance [Tables 3 and 4].

Discussion

Nowadays, drug resistance is a serious problem against TB control program in most of the countries. This resistance is constantly increasing and given the limited number of effective drugs, their resistance is considered as a threat in TB control program.

In our study, examined 55 patients with newly diagnosed smear-positive pulmonary TB. resistance of mycobacterium TB to isoniazid was 7.3%, to rifampin 5.5% and to both drugs 1.8%. In Germano manuel *et al.*,[7] isoniazid resistance was 5.8% and rifampin resistance 3.4%. In Akaninyene Otu *et al.*[8] resistance to isoniazid was 2%, to rifampin alone 0% and resistance to both 3%. In a study by Nyagosya Range *et al.*,[9] isoniazid resistance was 3.6%, rifampin resistance 0.8%, and resistance to both 3.6%. In the study by Asaad *et al.*,[10] isoniazid resistance was 33.8% and rifampin resistance 23.5%. In a study by Abhishek Agarwal *et al.*[11] among men with smear-positive pulmonary TB, isoniazid resistance was 13.2% and rifampin resistance 4.2%. In Dasarathi Das *et al.*,[12] resistance to isoniazid was reported to be 2.56% and rifampin resistance 0%. In Kelemework Adane *et al.*,[13] resistance to rifampin was 2.59% in new patients and 6.74% total, resistance to rifampin was 3.89% and 5.62% total, and resistance to both drugs was 1.29% and 2.24% total. In Hadizadeh *et al.*,[14] isoniazid resistance was 11.3% and rifampin resistance was 10.7%. In a study by Pourhajibagher M *et al.*[15] among patients with suspected
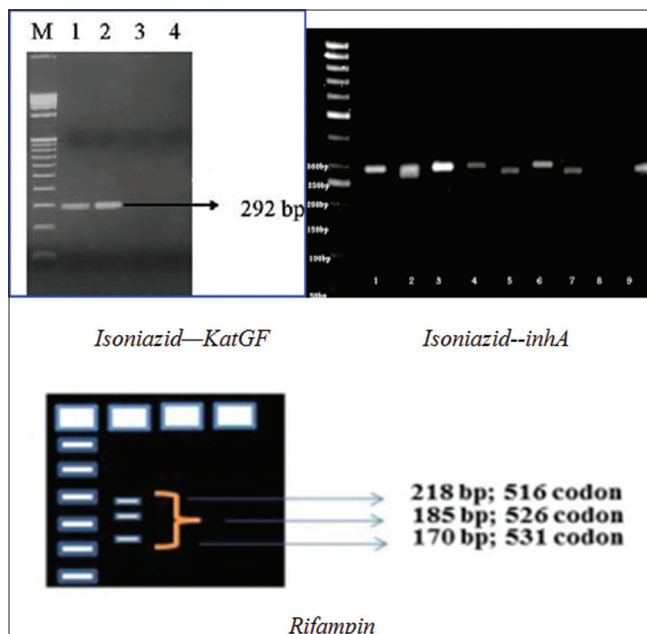
Table 3: Distribution of drug resistance according to the variables							
	Gender		Underlying diseases	Imprisonment	Smoking	Drug abuse	HIV infection	History of immunosuppressant drugs
	Male	Female						
Resistance to isoniazid	3	1	0	2	3	1	0	0
Resistance to rifampin	3	0	0	2	3	2	0	0

Table 4: The patients' status in terms of the variables						
	Underlying diseases	Smoking	Drug abuse	Imprisonment	HIV infection	Taking immunosuppressant drugs
Has	4	26	16	28	4	0
Does not have	51	29	39	27	51	55

TB, isoniazid resistance was 5.08% and 6.77%, respectively, and rifampin resistance was reported 1.7%. In Farazi A *et al.*,[16] isoniazid resistance in new cases was 2.9% and 2.6% in total, and rifampin resistance in new cases was 1% and total 1.7%. In Khosravi AD *et al.*,[17] resistance to rifampin and isoniazid was reported to be 15% and 13.7%, respectively. In Tavanaee Sani A *et al.*,[18] resistance to isoniazid was 7%, resistance to rifampin 7% and resistance to both 4%.

In the study, 55 patients with newly diagnosed smear-positive pulmonary TB. Isoniazid resistance was 7.3% which is almost similar to that observed by Pourhajibagher M *et al.* (6.77%),[15] Tavanaee Sani A *et al.* (7%),[18] Germano manuel *et al.* (5.8%).[7]

In the present study Rifampin resistance 5.5% which is nearly similar to that findings by Abhishek Agarwal *et al.* (4.2%),[11] and both drugs resistance is 1.8% which is slighty higher than reported by Kelemwork Adane *et al* (1.29%).[13] Isoniazid and rifampin resistance in other study seem are higher than us because of the higher patient group and cases with previous history of TB.[10,17,19]

Unlike our study, gender dominance was in favor of men (83.6%) In Farazi *et al.* (51.3%),[10] Pourhajibagher M *et al.* (65%)[9] gender dominance was in favor of women and other studies were similar to present study, Higher prevalence in men is probably due to more exposure to risk factor such as prison.

The highest age group in our study was 20–40 years (65.5%). therefore, our study was similar to the studies by Abhishek Agarwal *et al.*,[11] Germano Manuel *et al.*,[11] Nyagosya Range *et al.*,[9] Tavanaee Sani A *et al.*[18] Pourhajibagher M *et al.*[15] and this factor not evaluated in other study.

In our study mean age: 38.78 years was close to Nyagosya Range[9] (34.22 years), Akaninyene Out[8] (34. years), Kelemework Adane[13] (36.8 years), Park[19] (31.1 years), Abhishek[17] and Pourhaji *et al.*[15] but was different to Tavanaee[18] and Velayati,[20] this is due to the difference in age between the youngest and oldest.

Jobs patient in our study not similar to Akaninyene Out[8] and Kelemwork Adane[13] because of this difference was due to the examined population and other study this factor was not evaluated.

In our study among 4 Isoniazid resistance patients, 1 was female and 3 were males and three of these patients were in the 20–40 age group. All three Rifampicin resistance patients were males, and all were in 20–40 age group. The patient resistant to both drugs was male, and he was 24 years old. There was no significant association between age, gender, and drug resistance in our study that is close to Germano Manuel *et al.*[7] and Akaninyene Out *et al.*[8] but different to Farazi *et al.*[16] that is among 9 MDR patients 6 patients were females and 3 were males this difference is due to the MDR and new TB patients.

In our study, among 4 patients with isoniazid resistance, 2 had a history of prison, and 2 out of 3 patients with rifampin resistance, had a history of prison but there was not significant association between prison history and drug resistance. In Tavanaee Sani *et al.*[18] among 10 patients with drug resistance, 3 had a history of prison that is no significant association between prison history and drug resistance as our results and this factor not evaluated in other study.

In our study among 4 patients with Isoniazid resistance, and all 3 patients with Rifampin resistance had a history of smoking as well as the patient with resistance to both drugs had a history of smoking. There were no significant association between smoking and drug resistance is similar to Tavanaee Sani *et al.*,[18] Abhishek Agarwal *et al.*[17] and this factor was not evaluated in other studies.

In our study among 4 patients with Isoniazid resistance, 1 had drug abuse and among 3 patients with Rifampicin resistance, 2 had drug abuse. There was no significant association between drug abuse and drug resistance. similar Tavanaee Sani *et al.*[18] and this factor was not evaluated in other studies.

In present study, the patients with drug resistance were HIV negative. In Abhishek Agarwal *et al.*,[11] all subjects were HIV-negative but in studies by Nyagosya Range *et al.*[9] in Tanzania and in Kelemework Adane[13] in Ethiopia HIV in patients with drug resistance was 38.46% and 19.1%, respectively, there were no significant association between these two factors. The cause of the more prevalence of HIV among patients in both studies are the more prevalence of HIV in the African population and this factor was not evaluated in other studies.

Conclusion

According our findings that include prevalence of 7.3% Isoniazide resistance, 5.5% Rifampin resistance and 1.8% to both drugs, evaluating all newly diagnosed patients for resistance to standard anti-tuberculosis treatment seems rational. If according to the current national protocol, the drug resistance testing is delayed until treatment failure, treatment is delayed moreover MDR seems to be on a rise in the new cases of tuberculosis in our country.

Limitation of the study

The main limitation in this study is the small sample size also with our method of analysis (PCR), it is not possible to detect all gene`s resistance.

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Conflicts of interest

There are no conflicts of interest.

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