Short Communication

Drug resistance pattern and associated risk factors of tuberculosis patients in the central province of Iran

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Abstract

Background: One of the fundamental issues of infectious disease treatment is drug resistance. The aim of the present study was to investigate the first-line anti-tuberculosis drug resistance rates and determine the risk factors related to multidrug resistant mycobacterium tuberculosis.

Methods: From March 2011 to September 2012, mycobacterial strains were collected from one hundred fifteen diagnosed smear positive patients in the central province of Iran and tested for drug susceptible against ethambutol, rifampicin, isoniazid and streptomycin and the risk factors influencing the development of drug resistance were determined.

Results: The mean age of patients was 52.23±19.75 years. The rate of multi-drug resistant tuberculosis (MDR-TB) was 7.8%. Our study revealed that there were significant associations between prior treatment, age < 45 years, positive smear result at the end of the second month and positive smear result at the end of the third month. However, there was no association found between gender, inhabitant, nationality, close contact with TB patient, HIV infection and size of mantoux test.

Conclusion: The results show that about 8% of TB cases in Arak are MDR TB. The age under 45 years, previous TB treatment and positive smear at the end of the second and third months of treatment were the main factors in the development of MDR-TB.

Keywords: Drug resistance, Mycobacterium tuberculosis, Risk factor.

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Tuberculosis (TB) is one of the important leading causes of death in humans and it remains a serious public health obstacle in the developing countries (1). Early detection and correct treatment of MDR strains of mycobacteria are the most effective measures for the management of multidrug-resistant (MDR) TB (2). Drug resistance is the capability of organisms to stay viable or to multiply within the presence of the concentration of the drug that may ordinarily destroy or inhibit cell growth (3). With the introduction of the first anti-tuberculosis in the world in 1943, drug resistance began to rise and became a major problem and threat for TB control programs in many countries (4). Multi-drug resistant TB is one of the major types of resistance that microorganisms are resistant to at least two drugs of isoniazid and rifampin. Drug-resistant TB is a result of poor management of treatment or transmission from patients with drug-resistant TB (5). The treatment of such cases is expensive and lengthy with higher morbidity and mortality (6). Currently, there are many challenges in the treatment of resistant TB notably for the first detection and effective treatment. Studies indicated that drug resistance is increasing and the native information of the drug resistance pattern of clinical isolates is important mostly used to take suitable treatment for preventing treatment failures and reducing the quantity of secondary cases of multi-drug resistant TB (7).

The purpose of this study was to evaluate the drug resistance patterns of MTB and determine the risk factors for multi-drug resistant TB so as to supply policy-makers with recommendations for proper management of these patients.

Methods

From March 2011 to Sepember 2012, all patients with suspected tuberculosis sputum samples were collected. The Ziehl-Neelsen staining technique was used to identify mycobacteria. Then the positive smears were submitted for culture and drug sensitivity test (DST). Sputum smear exam was performed in the health center laboratory, whereas culture was done at the provincial research laboratory. After getting informed consent, a designed questionnaire for demographic information, the history of previous TB treatment, result of sputum smear, tuberculin skin test and HIV status was completed for every patient. The sputum samples were decontaminated with sodium hydroxide (NaOH) then centrifuged and refined on Lowenstein-Jensen (LJ) culture media. The LJ culture media was incubated at 37°C and determined on days three and seven to identify contaminations or quick growth of atypical mycobacteria. The identity of MTB was recognized by the p-nitrobenzoic acid (PNB) and thiophene carboxylic acid hydrazine (TCH) resistance. Species other than MTB were excluded from the present analysis. For DST, the LJ medium was impregnated with ethambutol (EMB), rifampicin (RIF), isoniazid (INH) and streptomycin (SM), in conforming with the proportional technique as suggested by World Health Organization and Clinical and Laboratory Standards Institute(CLSI) (8, 9).

The concentrations were 2 µg/ml for EMB, 40 µg/ml for RIF, 4 µg/ml for SM, and 0.2 µg/ml for INH. If the microorganism grew on the medium, the particular drug was ≥1% compared to the control culture, the strain was detected as resistant and sensitive if growth rate was < 1% compared to control culture. For external quality control, microscopic smear exam and sputum culture were conducted by the Provincial Research Laboratory and for the internal quality control, a standard laboratory strain (H37Rv) was enclosed for every culture. This project has been approved by Institutional Review Board and Ethics Committee of Arak University of Medical Sciences. The prevalence of mono and multi- drug resistance among the new and antecedently treated cases was determined. The results were analyzed by SPSS statistics Version 19.0. Categorical information (MDR-TB and non-MDR-TB) was compared by chi-square and Fisher's exact tests. Multiple logistic regression analysis was performed to identify the risk factors for MDR-TB and the strength of association was measured by odds ratio (OR) with 95% confidence interval (95% CI) and a two-tailed p-value of <0.05 was considered significant.

Results

Among the one hundred- twenty smear positive TB patients entered into the study, five cases were excluded (two cases infected with nontuberculosis mycobacteria and cultures of three cases were contaminated). One hundred fifteen cases determined as MTB were finally analyzed. Most of them (91.3%) were Iranian, the mean age was 52.23±19.75 years and fifty six (48.7%) patients were men and fifty nine (51.3%) were women. Among them, 103 (89.6%) were new cases and twelve (10.4%) were those previously treated. Sixteen (13.9%) patients had close contact with tuberculosis patients, three (2.7%) were HIV+ and 65 (56.6%) cases were urban.

The smear of 18 (15.6%) patients were paucibacillary, 37 (32.2%) were 1+, 31 (27%) were 2+ and 29 (25.2%) were 3+ in microscopic examination. The DST results showed that seventy eight (67.8%) strains were sensitive to all first-line antibiotics tested in our study and thirty seven (32.2%) were resistant to at least one drug. The proportions of mono-drug resistance were 7% for EMB, 2.6% for INH, 1.7% for RIF and 2.6% for SM (table 1). Among all isolates, 9 (7.8%) strains were MDR-TB and 2 (1.7%) strains were resistant to all four first-line drugs. There was the association of initial smear grading of 2+ and 3+ with drug resistant tuberculosis (p=0.0284, OR=2.56) however, its association with MDR-TB was not important (p=0.6867, OR=0.52) (table 2). Multivariate analysis of risk factors associated with MDR-TB showed that in previously treated cases, were considerably more than the new cases (p=0.0174). The frequency of MDR-TB was higher in age under forty-five years old (p=0.0428) and in patient with positive smear at the end of second (p=0.0390) or third (p=0.0339) months of treatment.

Table 1. First line anti-tuberculosis drugs resistance in M. tuberculosis strains (new vs previously treated cases)

	Isoniazid		Rifampicin		Ethambutol		Streptomycin	
	previously treated	new	previously treated	new	previously treated	new	previously treated	new
mono drug resistance	0	3 (2.9%)	1 (8.3%)	1 (1%)	1 (8.3%)	7 (6.8%)	1 (8.3%)	2 (1.9%)
two drug resistance	2 (16.7%)	6 (5.8%)	1 (8.3%)	2 (1.9%)	1 (8.3%)	6 (5.8%)	1 (8.3%)	6 (5.8%)
three drug resistance	3 (25%)	2 (1.9%)	2 (16.7%)	2 (1.9%)	1 (8.3%)	2 (1.9%)	0	5 (4.9%)
four drug resistance	2 (16.7%)	0	2 (16.7%)	0	2 (16.7%)	0	2 (16.7%)	0
Total	7 (58.3%)	11 (10.7%)	6 (50%)	5 (4.9%)	5 (41.7%)	15 (14.6%)	4 (33.3%)	13 (12.6%)

Table 2. Univariate analysis of association of risk factors with MDR-TB

Variable		Non-MDR-TB No (%)	MDR-TB No (%)	Odd ratio (95% CI)	P-Value
Age	Age < 45 yr	12 (32.4%)	8 (21.6%)	10.66 (1.17-97.18)	0.0227
	Age ≥ 45 yr	16 (43.2%)	1 (2.7%)		
Gender	Male	14 (37.8%)	3 (8.1%)	0.5 (0.10-2.41)	0.4624
	Female	14 (37.8%)	6 (16.2%)		
Type of patient	New Case	25 (67.6%)	4 (10.8%)	10.42 (1.76-61.67)	0.0115
	Previous TB treatment	3 (8.1%)	5 (13.5%)		
Nationality	Iranian	25 (67.6%)	7 (18.9%)	0.42 (0.06-3.03)	0.5773
	Immigrant (Afghans)	3 (8.1%)	2 (5.4%)		
Inhabitant	Urban	17 (46%)	8 (21.6%)	0.19 (0.02-1.77)	0.2204
	Rural	11 (29.7%)	1 (2.7%)		
Close Contact with TB patient	Yes	2 (5.4%)	2 (5.4%)	0.27 (0.03-2.27)	0.2436
	No	26 (70.3%)	7 (18.9%)		
HIV infection	Yes	1 (2.7%)	1 (2.7%)	0.3 (0.02-5.29)	0.4324
	No	27 (73%)	8 (21.6%)		
Sizes of mantoux test	<15mm	11 (29.7%)	5 (13.5%)	0.52 (0.11-2.36)	0.4580
	≥15mm	17 (46%)	4 (10.8%)		
Degree of sputum smear	≤1+	10 (27%)	2 (5.4%)	0.51 (0.09-2.96)	0.6867
	≥2+	18 (48.7%)	7 (18.9%)		
Smear result at the end of the second month	Positive	4 (10.8%)	5 (13.5%)	7.50 (1.39-40.56)	0.0231
	Negative	24 (64.9%)	4 (10.8%)		
Smear result at the end of the third month	Positive	2 (5.4%)	4 (10.8%)	10.4 (1.48-73.00)	0.0220
	Negative	26 (70.3%)	5 (13.5%)		

Table 3. Significant risk factors associated with MDR-TB reported in current and previous studies in Iran

Reference	Time	Design	Factor
Current study	Mar 2011 - Sep 2012	Prospective laboratory based study	age under 45 years, previous TB treatment , positive smear at the end of second and third months of TB treatment
Merza et al.	Dec 2000 - Jun 2005	Retrospective laboratory notes based study	Age < 45, Male sex, Previous treatment, Immigrant (Afghan) Poor living conditions, Un-employment
Shamaei et al.	Mar 2000 - Feb 2003	Retrospective hospital notes based study	Age > 65, Immigrant (Afghan) Previous treatment
Mirsaeidi et al.	Jun 2003 to Sep 2004	Anterograde patient interview study	Previous treatment
Baghaei et al.	2002 - 2005	Retrospective hospital based study	Previous treatment, Immigrant (Afghan), Positive sputum smear

Discussion

Currently, the emergence of MDR and XDR (extensively drug-resistant) tuberculosis in the world is becoming a major health problem (4, 10). World Health Organization has enforced a worldwide stewardship program, whereas, findings showed regional variations in trends of drug-resistance (11). MDR-TB patients were seemingly younger than sixty five years (12, 13). In this study, we found that the frequency of MDR-TB was higher in patients younger than forty five year old, which was the same as the findings from thirteen countries of Europe (14).

A systematic review analysis in Europe implied that the danger of MDR-TB for patients younger than forty five years was more than among the older patients and the risk of MDR was up to ten times higher in antecedently treated cases than in untreated ones (12). In several studies, it was found that the history of anti-tuberculosis treatment had been related to MDR-TB (15). Our study is consistent to the previous reports that showed the importance of previous treatment history as a risk factor of drug resistance (table 3) (16-20). Identification of patterns of transmission is considerable in TB management because acquired resistance and primary resistance need different management methods (21). The DST results showed that 32.2% in which almost one third of all TB cases were resistant to at least one drug and the proportion of MDR-TB among all 115 TB cases were 7.8% which means that in every thirteen cases of TB might have MDR-TB. In this study, univariate and multivariate analysis showed four relevant risk factors of MDR-TB. Age under 45 years, previous TB treatment, positive smear result at the end of the second and third months. Preventing the development of drug resistant, TB should continue to be the top priority for all countries, however, managing the MDR-TB cases that emerge is a part of the Stop TB strategy and should be a component of all TB programs and developing rapid detection and management of drug resistant cases is of great urgency.

In this study, we found the association between conversion at the end of intensive phase of treatment and the risk of MDR-TB. Drug susceptibility test should be strengthened and the patients infected with sensitive strains will get DOTS, those with mono-drug resistance will get intensive DOTS to prevent multidrug resistance, and the patients with MDR-TB will get the treatment for rescue of life (22). The limitation of this study is the potential recall bias while responding to a history of prior anti-TB treatment and it cannot empirically be determined whether MDR-TB cases in this study had had primary drug resistance or were acquired through inadequate and/or incomplete prior TB treatment. In conclusion, the results show that about 8% of TB cases in Arak are MDR TB. The age under 45 years, previous TB treatment and positive smear at the end of the second and third months of treatment were the main factors in the development of MDR-TB.

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