Original article

Multidrug-Resistant Tuberculosis in North-West of Iran and Republic of Azerbaijan: A Major Public Health Concern for Iranian People

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ARTICLE INFORMATION

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ABSTRACT

Background: Republic of Azerbaijan is considered as an area with high prevalence of multi-drug resistant tuberculosis. Uncontrolled travelling of Azerbaijanis people to Iran is the issue that needs to be considered as an important issue.

Methods: This study was conducted on 32 patients with tuberculosis from Baku–Nakhchivan and 48 patients from Iran during 2012 to 2014. Colonies of *Mycobacterium tuberculosis* were examined after isolating them from patients using proportional method on Lowenstein-Jensen media regarding resistance encounter with Rifampin, Isoniazid and Ethambutol.

Results: Among *M. tuberculosis* isolates belonging to 32 foreign patients; 69%, 72% and 56% of them were resistant to Rifampin, Isoniazid and Ethambutol, respectively (multidrug resistance tuberculosis: MDR-TB: 62.5%). From 48 isolates of Iranian patients; 8%, 4% and 4% were resistant to Rifampin, Isoniazid and Ethambutol, respectively (MDR-TB: 2.1%).

Conclusions: Resistant strains are common in people from Baku-Nakhchivan. To prevent the transmission of these strains to Iranians, strategies such as; establishing a medical campus in border lines of both countries for clinical examinations and conducting screening tests regarding tuberculosis infection in applicants for entering Iran must be taken in to account.

Introduction

Tuberculosis is still discussed as an important factor of mortality and morbidity in the world which easily transmitted through breathing [1,2]. Since 1998 to 2030, 225 million new tuberculosis cases and 79 million cases of death related with tuberculosis are expected [3]. This disease will get more critical and hazardous when multi-drug resistant tuberculosis (MDR-TB), which is resistant to at least Isoniazide (INH) and Rifampin (RMP), the two most powerful anti-TB drugs, is created in these patients suffering from it. It will be necessary for a patient to be hospitalized and to be treated by second line anti-TB drugs. This chemotherapy will have higher toxic effects on patients and impose very high medical expenses on the public health system [3,4]. Therefore, finding the timely new cases of tuberculosis and continuous monitoring of public health systems in their treatment with four-drug combination regimen: RMP, INH, Ethambutol (EMB), Pyrazinamide (PZA) for two months and two-drugs combination regimen: RMP and INH for four months is a strategy that can reduce the drug resistance [5].

According to the reports of WHO in 2012[6]; there are 27 countries in the world considered as high MDR-TB burden countries. Republics of Azerbaijan and Armenia which are Northern neighboring countries of Iran are among those 27 countries. In general efficient management of the disease can be done only in epidemiological surveillance systems to accurately monitor epidemic trends at regional and global levels[7].

The aim of this study was to evaluate the prevalence of MDR-TB strains in Azerbaijani natives (Baku-Nakhchivan) regardless of the new cases or previously treated MDR-TB cases, in circumstances that they travel easily to the northern regions of Iran without effective health control, and at the end to compare it with north west Iranians.

Methods

This study was carried out on 32 patients from the Republic of Azerbaijan and 48 patients from North Western of Iran with pulmonary tuberculosis (smear positive) during

2012 to 2014. In a pilot study among 20 patients in target group selected randomly, the prevalence of resistance to mono drug (at least) was estimated 30%. By $Z=1.96$, $p=0.3$, $q=0.7$, $d=0.05$, 68 persons as sample size were estimated. Finally the numbers of 80 persons were considered for this study with possible missing cases. All of these patients had been referred to Tuberculosis and Lung Disease Research Center in Tabriz for diagnosis and treatment.

The main inclusion criterion was positive sputum smear and culture together with TB and exclusion criterion was smear positive and culture negative. We performed a cross-sectional study of the data in randomized form. All *P* values were two tailed; $P<0.05$ was considered to be statistically significant. The Chi-square test was used to compare the frequency of variables. All statistical analyses were conducted using SPSS version 14(Chicago, IL, USA).

Isolates identification

In the first step, the three morning sputum samples of all these patients were received, and then digested and decontaminated with Petroff method. Then, the direct smear was prepared from each of the specimen sediment and was stained with Ziehl-Neelsen method. These sediments were also cultured on Lowenstein-Jensen (LJ) media (Hi-Media, Bombay, India) and incubated at 37°C for 6-8 weeks. All isolates were identified using differential tests such as niacin production, nitrate reduction, growth rate (> or < 7 days) and pigment production [8].

Drug susceptibility test (DST)

Sensitivity of isolated *Mycobacteria* to first line anti-TB drugs, (Sigma Chemical Co., St. Louis, MO), with a specific concentration as shown in Table 1, were conducted on 80 foreign and Iranian isolates by proportional method[8].The criterion used for drug resistance was growth of 1% or more of the bacterial population on media containing the critical concentration of each drug [9].

Table1: Preparation of antibiotics stock solutions and serial dilution to reach the target concentration on LJ medium. (The 100% assay of the drugs is assumed)

Antibiotics	Result	Solvent/Diluents	Concentration
0.2 gr	+	10 ml [a]	$C_1 = 0.02$ gr/ml
1.0 ml C_1	+	9 ml DW	$C_2 = 0.002$ gr/ml
1.0 ml C_2	+	9 ml DW	$C_3 = 200$ µgr/ml
1.0 ml C_3	+	99 ml LJ	$C_{target\ for\ EMB} = 2.0$ µgr/ml
0.1 ml C_3	+	99.90 ml LJ	$C_{target\ for\ INH} = 0.2$ µgr/ml
2.0 ml C_2	+	98 ml LJ	$C_{target\ for\ RMP} = 40.0$ µgr/ml

C: Concentration, **LJ:** Lowenstein-Jensen, **DW:** Distilled water
[a] DW for EMB or INH and HCl 0.1 N for RMP

Results

Among *M. tuberculosis* isolates belonging to 32 foreign patients (of Republic of Azerbaijan), 22 patients were found to be resistant to RMP and 23 patients were resistant to INH, (MDR-TB: 62.5%). Of 48 isolates belonging to Iranian patients, 4 isolates were resistant to RMP and 2 isolates were resistant to INH, (MDR-TB: 2.1%) (Table 2).

A comparison between the frequencies of drug resistant isolates from two patient groups showed that RMP, INH and EMB resistant species in foreign patients are significantly 9, 18 and 14 times higher than Iranian patients, respectively.

Table 2: First line anti-tuberculosis drugs resistance in *M. tuberculosis* strains between two groups

Drug susceptibility test	Iran, n (%)		Azerbaijan, n (%)		*P* value
	Sensitive	**Resistant**	**Sensitive**	**Resistant**	
Rifampin (RMP)	44 (91.7)	4 (8.3)	10 (31.3)	22 (68.7)	0.005
Isoniazid (INH)	46 (95.8)	2 (4.2)	9 (28.1)	23 (71.9)	0.005
Ethambutol (EMB)	46 (95.8)	2 (4.2)	14 (43.7)	18 (56.3)	0.005
MDR-TB (INH, RMP)	-	1 (2.1)	-	20 (62.5)	0.001
MDR-TB (3 drugs)	-	0 (0.0)	-	14 (43.7)	0.001

Discussion

So far, multi drug-resistant TB have been surveyed in two studies in North West of (East Azerbaijan province) Iran. In a study conducted by Heidarnejad &Nagili[10], in the period from 1999 to 2000, only one MDR-TB was reported among 155 TB patients. In another study conducted from 2007 to 2008, Maleki & Moadab have reported 3 cases of MDR-TB from 103 TB patients[11].

In Iran, compared to neighboring countries, the incidence of tuberculosis is considerably low (21 cases per 100,000 populations). However, because of the long border with high TB-burden countries such as Afghanistan, Pakistan, Azerbaijan and Armenia, prevention of the transmission of MDR-TB is a priority for Iran national TB control programs[12]. Directly observed treatment short- course (DOTS) strategy, fixed dose combinations of anti-TB drugs (FDCs) and the continuation of the treatment regimen for at least 6 months, are very critical factors about tuberculosis cases. The development of new TB cases or previously treated TB cases will have different therapeutic regimens and outcomes [5].

This cross- sectional study was performed to survey the general status of the MDR-TB in the Republic of Azerbaijan (Baku- Nakhchivan) travelers and residents of the north west of Iran. In the communities that the DOTS strategy has been followed seriously, the treatment failure rate has been significantly reduced, in comparison with communities which have had a routine self-administration of treatment at home. As a result of DOTS strategy, the rate of drug resistance caused by incomplete and inadequate treatment [13], significantly reduced. According to the WHO report [6], the poor performance of DOTS and also incomplete and inadequate treatment in TB patients in Russia has been a major cause of treatment failure and increase in the amount of drug resistance. India and China [14,15], the two countries which host more than one-third of the world's TB cases, could significantly reduce drug resistance during recent years by the implementation of the DOTS strategy which corrected drug administration and completed the adequate treatment. In 2011, the latest information on previously treated TB cases with MDR in countries such as Belarus, Estonia, Kazakhstan, Kyrgyzstan, Moldova, Tajikistan, Uzbekistan and Russia that was reported by the WHO indicated that, MDR-TB was more than 50% of the total cases of previously treated TB in these countries. WHO estimated that the percentage of new TB cases with MDR-TB and previously treated TB cases with MDR-TB have been 22% and 55%, respectively at Republic of Azerbaijan in 2007 [16,17]. This burden of disease in the Republic of Azerbaijan has situated it among the countries in the top of the list of countries with high MDR-TB burden. This information represents the failure of the treatment in the TB-patients of this country, which may be caused by lack of proper implementation of the DOTS strategy. Besides, these

data can point out that Eastern European and Central Asian countries are hot regions for MDR-TB, with nearly one-third of new cases and two-thirds of previously treated tuberculosis cases affected by MDR-TB in some settings [16].

Since 2007, only 13 countries in which the rate of extensively drug-resistant TB (XDR-TB) cases, defined as MDR-TB plus resistance to a Fluoroquinolone and at least one of three injectable second-line drugs (Amikacin, Kanamycin or Capreomycin) have had more than 10% of the total MDR-TB cases in the world, and Republic of Azerbaijan is one of these countries [16,18].

In 2011, WHO was able to report the quantity and quality of TB diagnosis laboratories in 23 countries from 27 high MDR-TB burden countries. Unfortunately, Azerbaijan was one of the four countries from which the WHO has not provided the report [16].

Incomplete and inadequate treatment, monotherapy or low quality drug consumption and treatment for less than 6 month period can be some of the reasons that can lead to the development of drug resistance.

In this study, our estimates of MDR-TB patients from the Republic of Azerbaijan population were close to WHO estimates in 2007 regardless of the previous treated TB cases or new TB cases. This subject can be caused by the indiscriminate entry of the patients into the northern cities of Iran to detect and management of their diseases. This subject may be related to weakness in the diagnosis and treatment of tuberculosis in their country. This study suffered from some limitation of course, since patients of Republic of Azerbaijan stayed in Iran for a short period of time (just for medical reasons) and they were not also available after returning to their country. Therefore, the data about them were very limited.

Conclusions

MDR-TB strains are common in Baku-Nakhchivan. To prevent from transmission of these strains to Iranians, preventative strategies such as establishing a medical campus in border lines of both countries to carry out clinical examination and conducting screening tests such as PPD test and sputum direct smear examination regarding tuberculosis infection for travelers to Iran must be done.

Acknowledgments

Conflict of interest statement

The authors declare no conflict of interest.

References

1. Rashedi J, Asgharzadeh M, Moaddab SR, Sahebi L, Khalili M, Mazani M, et al. Vitamin D receptor gene polymorphism and vitamin D plasma concentration: Correlation with susceptibility to tuberculosis. *Adv pharm bull*. 2014;4(Suppl 2):607-611.

2. Ducati RG, Ruffino-Netto A, Basso LA, Santos DS. The resumption of consumption: a review on tuberculosis. *Mem Inst Oswaldo Cruz*. 2006;101(7):697-714.

3. Shah NS, Wright A, Bai G-H, Barrera L, Boulahbal F, Martín-Casabona N, et al. Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis*. 2007;13(3):380-387.

4. Merza MA, Farnia P, Tabarsi P, Khazampour M, Masjedi MR, Velayati AA. Anti-tuberculosis drug resistance and associated risk factors in a tertiary level TB centre in Iran: a retrospective analysis. *J Infect Dev Ctries*. 2011;5(7):511-519.

5. World Health Organization. *Treatment of tuberculosis: guidelines*. 4th ed. Geneva: WHO; 2010.

6. World Health Organization. *Global tuberculosis control-surveillance, planning and financing: Russian Federation*. Geneva: WHO; 2005.

7. Asgharzadeh M, Kafil HS, Roudsary AA, Hanifi GR. Tuberculosis transmission in Northwest of Iran: using MIRU-VNTR, ETR-VNTR and IS6110-RFLP methods. *Infect Genet Evol*. 2011;11(1):124-131.

8. Rafi A, Moaddab SR. *Principles of mycobacteriology*. Tabriz; Sotudeh Publications: 2003.

9. World Health Organization. *Anti-tuberculosis drug resistance in the world*. 4th global report. Geneva: WHO; 2008.

10. Heidarnejad H, Nagili B. Primary resistance of mycobacterium tuberculosis to Isoniazid, streptomycin, Rifampin, and Ethambutol in pulmonary tuberculosis. *Arch Irn Med*. 2001;4(1):1-4.

11. Maleki R M, Moaddab SR. Drug susceptibility pattern of Mycobacterium tuberculosisstrains to first and second line drugs in Tabriz, Iran. *Iran J Med Microbiol*. 2009;3(1):18-24.

12. Nasiri MJ, Dabiri H, Darban-Sarokhalil D, Rezadehbashi M, Zamani S. Prevalence of drug-resistant tuberculosis in Iran: systematic review and meta-analysis. *Am J Infect Control*. 2014;42(11):1212-1218.

13. Sharma S, Mohan A. Multidrug-resistant tuberculosis. *Indian J Med Res*. 2004;120(4):354-376.

14. Dewan PK, Lal SS, Lonnroth K, Wares F, Uplekar M, Sahu S, et al. Improving tuberculosis control through public-private collaboration in India: literature review. *BMJ*. 2006;332(7541):574-578.

15. Xianyi C, Fengzeng Z, Hongjin D, Liya W, Lixia W, Xin D, et al. The DOTS strategy in China: results and lessons after 10 years. *Bull World Health Organ*. 2002;80(6):430-436.

16. World Health Organization (WHO). *Global tuberculosis report 2012*. WHO/HTM/TB/2012. Geneva: WHO; 2012.

17. Gelmanova I, Keshavjee S, Golubchikova V, Berezina V, Strelis A, Yanova G, et al. Barriers to successful tuberculosis treatment in Tomsk, Russian Federation: non-adherence, default and the acquisition of multidrug resistance. *Bull World Health Organ*. 2007;85(9):703-711.

18. Masjedi MR, Farnia P, Sorooch S, Pooramiri MV, Mansoori SD, Zarifi AZ, et al. Extensively drug-resistant tuberculosis: 2 years of surveillance in Iran. *Clin Infect Dis*. 2006;43(7):841-847.