

Beijing strains of *Mycobacterium tuberculosis* in smear-positive tuberculosis patients in North-West and West of Iran

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Abstract

Background: Tuberculosis (TB) is the leading cause of morbidity and mortality among chronic infectious diseases. The goal of this cross-sectional study (2012–2014) was to examine the prevalence of *Mycobacterium* TB (MTB) Beijing strains in regions near the Iranian border and to identify any epidemiological links.

Materials and Methods: To this end, MTB isolates were harvested, from 64 HIV-negative, pulmonary smear-positive TB patients from the Iranian border provinces of East Azerbaijan (North-West), Kurdistan (West), and Kermanshah (West) (2012–2014). Isolates were subjected to restriction fragment length polymorphism (RFLP) analysis, using the insertion sequence IS6110 as a probe (IS6110 RFLP), and drug susceptibility testing by the proportion method. We gathered demographic and clinical data using a questionnaire and reviewing patient records. Results were analyzed with Gel Compare II 6.6 and SPSS-18.

Results: The mean age of the patients was 54.4 years and 46.9% were male. The prevalence of Beijing strains among the isolates was 9.4% (17.6% in the Western provinces and 0% in East Azerbaijan). There was a statistically significant relationship between the Beijing strains and drug resistance and also between these strains, and the recurrence of TB in patients that had previously received treatment ($P = 0.02$ and $P = 0.04$, respectively).

Conclusions: Finally, the prevalence of Beijing strains in Western Iran was greater than expected. Our results therefore indicate that regional and cross-border tracing may be necessary to control spread of this organism.

Key Words: Beijing strains, drug resistance, *Mycobacterium tuberculosis*

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INTRODUCTION

Mycobacterium tuberculosis (MTB) is the causative agent of TB. Although an ancient disease, TB is still the leading cause of mortality among chronic infectious

diseases.^[1] This is of particular importance in countries, such as Iran, which have a high prevalence of TB. Despite these efforts, however, approximately 14,000 new cases are reported annually in Iran,

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and the mean prevalence of TB was estimated at 21 cases per 100,000 population by the World Health Organization (WHO) in 2013.^[2] While the emergence of multidrug-resistant (MDR) forms of the disease are cited as a threat to these efforts aimed at controlling the spread of TB, it is not clear how drug resistance affects disease dynamics.^[3]

According to the WHO, drug resistant MTB strains were identified in 5% of the new cases and in 48% of patients with a history of TB treatment in Iran (2014). Although Iran is ranked globally as having an intermediate prevalence of TB and MDR-TB,^[2] it shares common borders with countries that have a high prevalence of both. The Azerbaijan Republic has a prevalence of 124 cases/100,000 population and primary MDR of 39%, and Iraq has a prevalence of 73 cases/100,000 population and primary MDR of 18%.^[2] It is therefore essential to track and control TB transmission across these borders. Previous research indicated a link between the incidences of drug resistance in a given region and the prevalence of certain MTB strains. Particularly, the MTB Beijing strains were reported to be responsible for widespread resistance to antibiotics commonly used to treat TB.^[4,5] The prevalence of the Beijing strains in Azerbaijani Republic prisoners and Iranian population was reported to be 71%^[4] and 10%, respectively,^[5] but information on Iraq was not accessible.

Prevalence of the Beijing strains in Northwest and West Iran has not been determined, and it is not clear from previous research whether the presence of the special strain increases the likelihood of drug resistance. As such, the identification of strain types and the status of drug resistance is key factors for treating and controlling the spread of TB in these regions. The IS6110 restriction fragment length polymorphism genotyping technique is a standard method that has been utilized to analyze MTB strains in multiple epidemiological studies.^[6]

We therefore employed this technique to determine the prevalence of MTB Beijing strains among TB patients from two different populations of Turkish and Kurdish subjects in three provinces on the Iranian border.

MATERIALS AND METHODS

Taking patients

In this cross-sectional study, from 2012 to 2014, 64 patients with positive pulmonary smears from East Azerbaijan ($n = 30$), Kurdistan ($n = 17$), and Kermanshah ($n = 17$) provinces located in the Northwest and West Iran were investigated. This study was approved by the Medical Ethics Committees

of Tabriz medical university; moreover, patients were asked and provided for written informed consent before interviews.

Drug susceptibility testing

Drug susceptibility testing was carried out in Löwenstein–Jensen (LJ) medium according to the standard proportion method recommended by the WHO and the International Union Against TB and Lung Disease and by the following materials: isoniazid (INH): 0.2 mg/l, rifampicin (RMP): 40 mg/l, ethambutol (EMB): 2 mg/l, and streptomycin (STM): 4 mg/l.^[6]

Clinical inclusion criteria

All patients were HIV negative and received directly observed treatment, short-course (DOTS). Sputum-positive pulmonary TB was defined as TB in patients with at least 2 initial sputum smear examinations positive for acid-fast *Bacilli* (AFB), one sputum smear test positive for AFB and radiographic abnormalities consistent with active pulmonary TB, or one sputum smear specimen positive for AFB and culture positive for MTB.^[7] All MTB complexes were identified by culturing on LJ medium. The samples containing non-TB *Mycobacterium* were excluded from this study.

Extraction of genomic DNA

DNA was extracted from clinical isolates grown on LJ medium as described.^[7]

IS6110-restriction fragment length polymorphism

For fingerprinting, PVU II-digested total DNA was electrophorized and separated using 1% agarose gel in Tris-acetate buffer. After performing Southern blot using the Kepler method and nylon membrane cross-linking, DNA hybridization was accomplished (using ECL system; Amersham, Little Font, Buckingham ECL System, Shire, United Kingdom) by a 245-bp internal polymerase chain reaction fragment probe that was amplified using two pairs of primers: INS-1 (5' CGT-GAG-GGC-ATC-GAG-GTG-GC) and INS-2 (5' GCG-TAG-GCG-TCG-GTG-ACA-AA).^[7]

The thermocycler (PeQ lab, primus 96) condition followed were initial denaturation at 95°C for 5' 35 cycles of 95°C for 30', 62°C for 30' and the final extension at 72°C for 5'.

The PVU II-digested total DNA of the H37RV reference strain was used as the external size standard in each Southern blot experiment.

Computer analysis

Fingerprinting patterns of IS6110 were analyzed using Gel Compare II software (WINDOWS 7, VERSION 6.6,

APPLIED MATH, KOTRIJK, BELGIUM). The strains we examined that exhibited >80% similarity to the 19 Beijing reference strains obtained from the research of Kremer *et al.* were considered to be Beijing strains.^[4] To this end, autoradiograms were scanned at an optical resolution of 190 dpi, the positions of the IS6110 fragments of the evaluated samples and the reference Beijing strains were then normalized with an internal marker, and their accuracy was verified using the IS6110 banding pattern of the H37RV strain. Dendrograms were designed by hierarchical unweighted pair clustering group method analysis algorithm (UPGMA) and analyzed by Statistical Package for the Social Sciences (SPSS-18) software (IBM SPSS, Chicago, Illinois, USA) was used to perform Chi-square tests for comparing variables in the Beijing and non-Beijing strains.

RESULTS

A total of 64 MTB isolates were evaluated. The mean age and age range of the patients were 54.4 and 15–83 years, respectively. Thirty of the samples (46.9%) were obtained from East Azerbaijan, whereas 17 each (26.6%) were obtained from Kermanshah and Kurdistan. All patients were HIV-negative (ELISA test) and were monitored according to the DOTS (directly observed treatment, short-course) protocol during treatment. The prevalence of drug resistance among the isolates was 14.1% in nine cases. Specifically, three isolates (4.7%) were resistant to streptomycin (STM), two each (3.1%) were resistant to RMP, one (1.5%) was resistant to isoniazid, and one was (1.5%) resistant to INH, STM, and EMB, and the two MDR strains exhibited resistance to IHN, RMP, and EMB, and to IHN, RMP, and STM.

Among the isolates tested, 6 (9.5%) were of the Beijing genotype [Figure 1]. Four patients from Kurdistan and two from Kermanshah provinces comprised these six; thus, the prevalence of Beijing strains in patients from Western provinces was 17.6% (six out of 34 patients), whereas no Beijing strains were detected in the patients from East Azerbaijan. This relationship was statistically significant ($\chi^2 = 5.8$, $P = 0.003$). There was also a statistically significant link between the Beijing strains and drug resistance ($\chi^2 = 9.5$, $P = 0.02$). Two of the six Beijing strains exhibited resistance to STM, whereas another was MDR (resistant to IHN, RMP, and STM). Furthermore, as four of the six patients infected with Beijing strains had previously received treatment for TB, there was a statistically significant link between these strains and the recurrence of TB ($\chi^2 = 15.4$, $P = 0.04$). The mean ages of patients in the Beijing and non-Beijing

clusters were 65.7 (standard deviation [SD] = 16.9) and 56.3 (SD = 21.9), respectively, which was not statistically different ($t = 0.72$, $P > 0.05$). Likewise, no significant association was found between gender and strain type ($\chi^2 = 0.67$, $P > 0.05$).

DISCUSSION

The goal of this study was to determine whether the Beijing strains are prevalent in Iran. As such, 64 isolates were harvested from TB patients in the Iranian provinces of East Azerbaijan (North-Western border), Kermanshah, and Kurdistan (Western border). The prevalence of the Beijing strains in these patients was 9.4%. Its prevalence in some Western and Central Asian locales was 0% in Syria and Lebanon,^[8] Turkey 0.5%,^[9] Palestine 0.64%,^[10] Azerbaijani prisons 70.8%,^[4] Armenia 46%,^[11] Kazakhstan 70.4%,^[12] and Saudi Arabia with a rate of 5.8%.^[13] The prevalence of the Beijing strains in different parts of Iran is estimated at 10% in Shiraz,^[5] 5.5% in Markazi Province,^[14] and 7.1% in Khorasan.^[15] Contrary to our expectations, Beijing strains were not isolated from patients in East Azerbaijan. Given the high incidence of this strain in the Azerbaijan Republic^[4] and the high level of border traffic between Azerbaijan and Iran, we predicted a higher prevalence of the Beijing strains in this population. Meanwhile, 66.7% of the Beijing isolates were harvested from patients native to Kurdistan, and 33.3% were harvested from patients from Kermanshah. These results indicate the risk for spread of the Beijing strains was higher in the Western regions and that, therefore, there could possibly be a high risk of transmission at the Iran-Iraq border.

The link between the Beijing strains and an increase in antibiotic resistance is currently controversial. In some parts of the world, for example, in Russia, Estonia, and Azerbaijan, a very strong statistical relationship between the Beijing lineage and drug resistance was reported.^[16-18]

In contrast, several other studies failed to identify a link between these factors.^[19-20] Interestingly, Buu *et al.* found that all MDR Beijing strains tested were resistant to streptomycin, indicating that streptomycin resistance may be a marker for MDR in these strains.^[21] In addition, we detected a link between patients that had previously received TB treatment and the presence of Beijing strains, which was consistent with previous findings.^[22,23] A limitation of this study was that due to cost restrictions, and therefore limits to the size of the sample, we were unable to conduct an extensive and strong analysis.

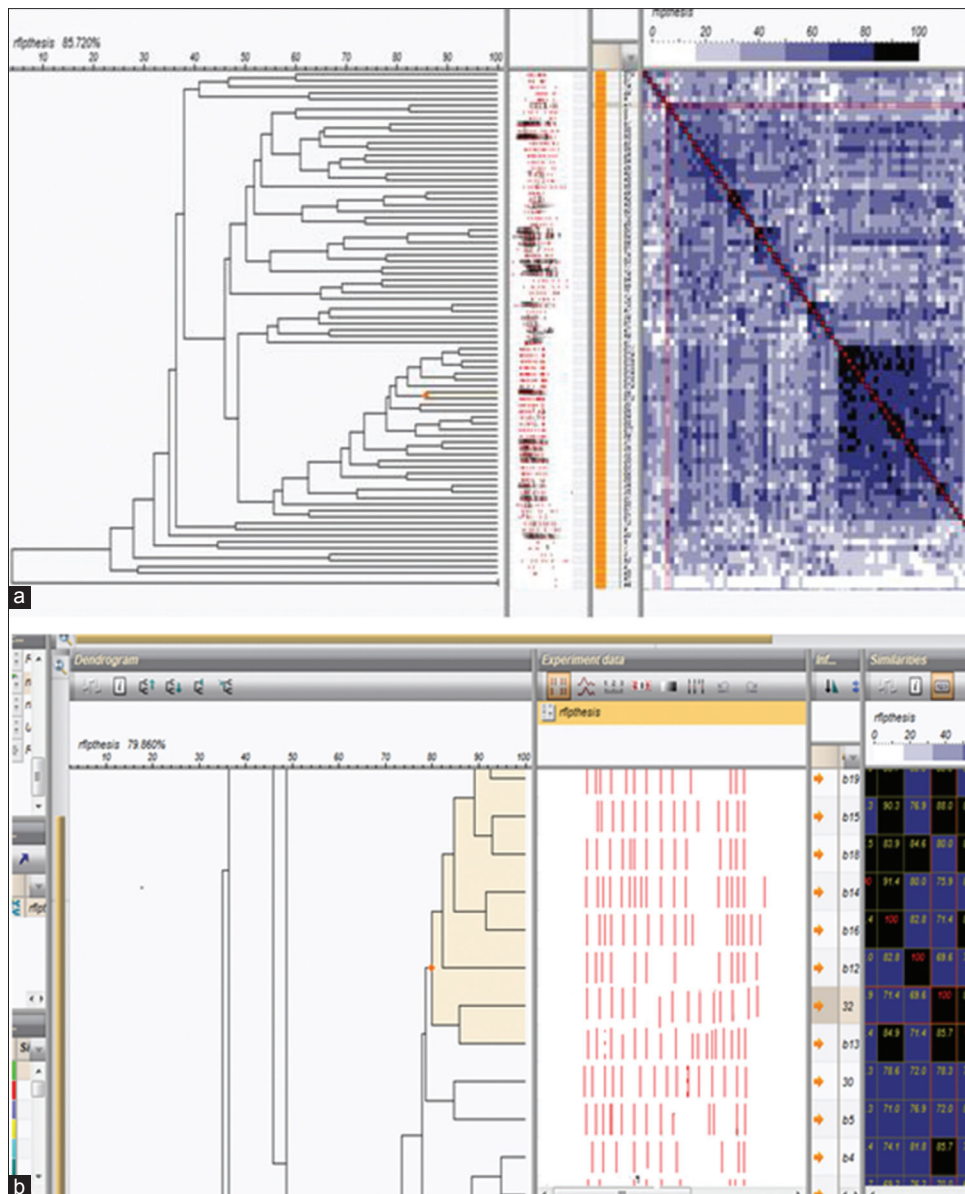


Figure 1: (a) Dendrogram reflects the similarity of IS6110 restriction fragment length polymorphism patterns with the PVU II restriction fragments depicted from left to right molecular weight. Nineteen references of Beijing strains are marked by black squares and six detected Beijing strains (of 64 *Mycobacterium tuberculosis* strains) marked by gray squares (on left). Over 80% similarity between reference Beijing and present study strains are presented as Beijing strains. (b) Part of Dendrogram, closer view

CONCLUSIONS

The prevalence of the Beijing strains in Western Iran was higher than expected. Our results indicate that regional and cross-border tracing research may be necessary to monitor and prevent future spread of MDR-MTB strains near the Iran-Iraq border.

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Conflicts of interest

There are no conflicts of interest.

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