Comparing Transmission of Mycobacterium tuberculosis in East Azarbaijan and West Azarbaijan Provinces of Iran by Using IS6110-RFLP Method

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**Abstract:** To investigate the genetic variation among Mycobacterium tuberculosis isolates from East and West Azarbaijan provinces of Iran and to evaluate the manner of recent transmission of tuberculosis (TB), we performed IS6110-based restriction fragment length polymorphism analysis of isolates. Restriction fragment length polymorphism (RFLP) typing performed on 165 culture-positive specimens from East and West Azarbaijan. Using IS6110 as a probe, Mycobacterium tuberculosis strains assigned to clusters based on identical DNA fingerprints. Rates of patients have clustered were 27.68% in East and 30.19% in West Azarbaijan. There was not statistically significant differences in clustering of patients in two provinces (p = 0.4533) but infection with Mycobacterium tuberculosis in males and females in two provinces were different (p = 0.0048). In East Azarbaijan there was not difference in transmission of tuberculosis between males and females, also in males and females belonged to clusters we couldn’t find statistical difference (p = 0.1833). The rate of active transmission of TB in West Azarbaijan was slightly more than East Azarbaijan. It can be due to different factors such as poor economic and less developed condition in West Azarbaijan.

**Key words:** Mycobacterium tuberculosis, IS6110, RFLP, Azarbaijan, transmission

**INTRODUCTION**

*Mycobacterium tuberculosis* is one of the most harmful human pathogens worldwide, causing about 8 million new tuberculosis cases and 2-3 million deaths yearly (De Boer et al., 2002). Tuberculosis (TB) remains a major health problem worldwide, but it is more prevalent in underdeveloped and developing countries, in which over 95% of cases occur (Vuković et al., 2003). It has estimated that between 19 and 43% of the world’s population were infected with *Mycobacterium tuberculosis*, but a few (5-10%) will develop active TB (American Thoracic Society, 2000).

DNA fingerprinting of *Mycobacterium tuberculosis* is a valuable tool to study tuberculosis (TB) epidemiology (Asgharzadeh et al., 2007), most strains of *M. tuberculosis* has insertion sequence of IS6110 (Cohn and Brien, 1998) that is the member of IS3 family (Fang et al., 1999) and amount of copies between strains are different from 0 to 22 (Kurepina et al., 1998). The presence of IS6110 in *M. tuberculosis* permits identifying of individual strains by DNA fingerprinting with RFLP analysis. IS6110-RFLP is most important and useful method for typing of *M. tuberculosis* (Van Embden et al., 1993). Typing of *Mycobacterium tuberculosis* strains is important for source tracing (Kiers et al., 1997) and revealing the transmission of tuberculosis in societies (Duriez et al., 2003; Maguire et al., 2002; Diaz et al., 2001; Pena et al., 2003; Barnes et al., 1996). It has generally assumed that the level of clustering among *M. tuberculosis* isolates from a certain region is associated with the level of recent transmission. On the contrary, non-clustered cases have considered indicating TB resulting from reactivation of latent infection (Vuković et al., 2003). Patients with TB whose isolates couldn’t be grouped into clusters, i.e., those with unique DNA fingerprints, are assumed to have disease that results from the reactivation of latent infection acquired in the past (Murray, 2002). The East and West Azarbaijan provinces are located in the Northwest of Iran, in neighbor-hood with each other (Fig. 1). Tabriz is the capital of East Azarbaijan province and Orumieh is the
capital of West Azerbaijan. The estimated rate of TB in Iran in 2002 was 29 in 100,000 and notification rate is 17 in 100,000 (WHO, 2004). However, TB incidence is not homogeneous in different parts of this country. In East Azerbaijan province the estimated rate of TB in 2002 was low; this can be due both to low case finding or low prevalence of TB in this part of the country. The aims of this study were to determine the genetic diversity of *M. tuberculosis* population in East and West Azerbaijan province and to detect the manner of transmission of the disease in these regions and comparing them.

**MATERIALS AND METHODS**

**Bacterial strains:** All isolates of *M. tuberculosis* were collected from patients who referred to central TB laboratories of Orumieh and Tabriz Tuberculosis and Lung Disease Research Center since March 2004 to March 2005. Finally, the RFLP patterns of 165 *M. tuberculosis* isolate which 53 were isolated from West Azerbaijan and 112 from East Azerbaijan were determined. The isolates have identified as *M. tuberculosis* by Ziehl-Neelsen staining and standard biochemical tests, including production of niacin, pigment production and growth rate on Lowerstein-Jensen Medium.

**IS6110- RFLP:** Extraction of DNA from *M. tuberculosis* strains were performed by the standardized protocol described by Van Snellenberg et al. (1994). The IS6110 DNA probe was prepared by in vitro amplification of a 245-bp fragment, using the polymerase chain reaction (PCR). The oligonucleotides INS-1 (S'-CGT GAG GGC ATC GAG GTG GC) and INS-2 (S'-CGG TAG GCG TCG GTG ACA AA) [TIB-MOLEBIOL, Germany] were used to amplify a 245 bp fragment from purified chromosomal *M. bovis* BCG DNA by PCR. This fragment was purified and labeled by Digoxigenin. DNA fingerprinting performed as described by Van Embden et al. (Van Snellenberg et al., 1994; Van Embden et al., 1993). The extracted mycobacterium DNA digested with PvuII enzyme (Cinnagen, Iran) and restriction fragments were separated by 0.8% agarose gel electrophoresis at 20 V for 18 h. Then, the fragments transferred, from the gels to positively charged nylon membranes. Hybridization performed by using a 245 bp probe of insertion sequence IS6110 and detected by colorimetric system. A mixture of PvuII-digested supercoiled DNA ladder (Sigma, USA) and HaeIII-digested φX174 DNA (Fermentas, Lithuania) used as internal marker. PvuII-digested genomic DNA of *M. tuberculosis* reference strain Mtu4323 used in each southern blot experiment as an external size marker. RFLP patterns of the isolates compared by visual examination. A cluster defined as a group of two or more isolates from different patients whose RFLP fingerprints were identical with respect to both the number and molecular size of all bands (Asgharzadeh et al., 2006).

**Statistical analysis:** All patients included classified into two groups, clustered and non-clustered. Categorical data compared by chi-square test (or Fishers exact test). p-values below 0.05 has considered significant.

**RESULTS**

From March 2004 to March 2005 totally 171 pulmonary and extra pulmonary TB patients were subjected. Four isolates of *M. tuberculosis* with insufficient DNA were excluded. Two *M. tuberculosis* was isolated from West Azerbaijan assumed to be cross-contaminated. Out of 165 isolates, 112 (67.88%) were originated from East Azerbaijan and 53 (32.12%) from West Azerbaijan. Eighty nine (53.94%) *M. tuberculosis* were isolated from male patients and 76 (46.06%) belong to female patients. The age of patients ranged from 2.5 to 88 years.

Clustering rate was determined on 154 isolates. This contained five or more copies of IS6110. Among these isolates, 123 different patterns were observed. As shown in Fig. 2 and 3, RFLP typing revealed a variable number of hybridizing bands that ranged from 0-17 and majority of strains (93.3%) had at least five copies. The average copy number of IS6110 per strain was 7.3 that only 11 (6.7%) strain had less than five IS6110 copies which four strains were from East Azerbaijan and seven strains were from
Fig. 2: No. of IS6110 copies in *M. tuberculosis* strains isolated from East Azarbaijan

Fig. 3: No. of IS6110 copies in *M. tuberculosis* strains isolated from West Azarbaijan

Fig. 4: Twenty two IS6110-RFLP patterns of *Mycobacterium tuberculosis* strains isolated from patients; Mt: RFLP Pattern of *Mycobacterium tuberculosis* reference strain Mt14323

West Azarbaijan. The clustered isolates comprised 30.52% (47 strains) of the total strains and they formed 16 clustered consisting of two to ten cases each. One hundred and seven strains (69.48%) had a unique RFLP patterns (Fig. 4). Transmission rate of West Azarbaijan was 23.91% [(16-5)/46] whereas it was 18.52% [(31-11)/108]
Table 1: Prevalence of tuberculosis and patients clustered and non-clustered in West and East Azarbaijan

<table>
<thead>
<tr>
<th>Patients in clusters</th>
<th>Tabriz (East A.)</th>
<th>Orumieh (West A.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>52 (46/93)</td>
<td>57 (69/81)</td>
<td>0.0048</td>
</tr>
<tr>
<td>Female</td>
<td>60 (53/57)</td>
<td>16 (13/19)</td>
<td></td>
</tr>
<tr>
<td>Clustered</td>
<td>31 (27/88)</td>
<td>16 (36/19)</td>
<td>0.4533</td>
</tr>
<tr>
<td>Non-clustered</td>
<td>77 (68/75)</td>
<td>30 (56/69)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis show percentage

for East Azarbaijan. In East Azarbaijan females slightly more infected with tuberculosis but in West Azarbaijan males more infected with tuberculosis (p = 0.0048) (Table 1).

**DISCUSSION**

A crucial aspect of any TB control program is the ability to determine the manner of transmission and where transmission is occurring in order to prevent further spread of infection and prevent active disease by identifying newly infected patients and providing them with prevention therapy (Asgharzadeh et al., 2007). In our analysis over a 1 year period, 165 *M. tuberculosis* isolates from 112 East Azarbaijan isolates and 53 West Azarbaijan isolates characterized by RFLP. Of the 165 isolates in this study, 11 (6.7%) strains showed fewer than five copies of IS6110, which 3.6% of East Azarbaijan isolates and 13.2% of West Azarbaijan isolates showed fewer than five copies of IS6110. Farina et al. (2004) have reported that 5.4% of strains from Tehran had low banding patterns also Doroudchi et al. (2000) have reported that 3.2% of strains from Fars province had low copies of IS6110, these show more prevalence of low copy number of IS6110 in West Azarbaijan. Zero-copy strains previously described with slightly higher frequencies noted in patients from Vietnam, China, Thailand and India (Rasolofo-Razanamparany et al., 2001). Moreover, it agrees with hypothesis of Van Soolingen et al. (1999) that certain genotypes of *M. tuberculosis* are associated with particular geographic region. The percentage of clustered strains in East Azarbaijan was 27.68% and in West Azarbaijan was 30.19%, which were lower than observed on Tehran (43%) (Farina et al., 2004), Paris (36%) (Gutierrez et al., 1998), New York (37%) (Alland et al., 1994) and Botswana (42%) (Lockman et al., 2001). There was some difference in clustering rate between East and West Azarbaijan. The proportion of clustered patients in West Azarbaijan was higher (30.19%) than in the East Azarbaijan (27.68%), suggesting a more active transmission of tuberculosis in West Azarbaijan. This may reflect the poor economic and less developed condition in West Azarbaijan. Difference between females and males patients in East and West Azarbaijan was significant (p = 0.0048) (Table 1). In addition, in West Azarbaijan tuberculosis was more frequent in males. It was similar with other parts of world (Alland et al., 1994; Gutierrez et al., 1998). However, in East Azarbaijan tuberculosis have a same prevalence in males and females. It can be due to different factors such as social structure and different culture of this province, also in East Azarbaijan females more infected by tuberculosis than males in West Azarbaijan (Table 1). This might be due to deprivation of the female populations in East Azarbaijan, as reflected by lower education, more poverty and malnutrition.

Finally it can be concluded that IS6110 DNA fingerprinting helped to study the manner of transmission in regions and high average copy number of IS6110 per isolate in East and West Azarbaijan of Iran confirm the usefulness of this method for epidemiological studies in this regions.

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**REFERENCES**


