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Use of DNA Fingerprinting in Identifying the Source Case of Tuberculosis in East Azarbaijan Province of Iran

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In recent years inspite of medical advancement, tuberculosis remains as a worldwide health problem. Identifying the source of transmission of infection is necessary for decreasing of tuberculosis (TB). Determining the variety of TB strains by DNA fingerprinting help to this work. The aim of this study was to determine tuberculosis transmission dynamics in East Azarbaijan province by focusing on prevention of its transmission. In an attempt to identify new epidemic and transmission, Restriction Fragment Length Polymorphism (RFLP) typing was performed on 119 culture-positive specimens in East Azarbaijan. Using IS6110 as a probe, *Mycobacterium tuberculosis* strains were assigned to clusters based on identical DNA fingerprints. Twelve clusters were found among total of 38 strains. The clusters included 26 patients that infected by 12 another's. Ninthy-three distinct IS6110 patterns were revealed. Eighty-one of these patterns were unique and 12 were shared by 2 to 8 strains. The minimum estimate for transmission in East Azarbaijan province was 22%. RFLP typing is a useful instrument to more knowledge about transmission and the occurrence of micro-epidemics and international source tracing, as we found a Nakhichevanees patient referred to tuberculosis center of province could be a source for transmission of tuberculosis.

Key words: *Mycobacterium tuberculosis*, RFLP, IS6110, transmission, DNA fingerprinting

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INTRODUCTION

Despite medical advancement, tuberculosis (TB) remains as a health problem and tubercle bacillus continues to claim more lives than any other single infectious agent. In the recent years have seen increased incidence of tuberculosis in both developing and industrialized countries (Cole *et al.*, 1998). Between a third and a half of the world's population is infected with *Mycobacterium tuberculosis* (Burgos and Pym, 2002). Each year, there are about one million deaths due to TB (Soini and Musser, 2001).

Most strains of *M. tuberculosis* have 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 (Kuepina *et al.*, 1998). The presence of IS6110 in *M. tuberculosis* permits identifying of individual strains by DNA fingerprinting with Restriction Fragment Length Polymorphism (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 (Alland *et al.*, 1994; Durmaz *et al.*, 2003; Gutierrez *et al.*, 1998; Maguire *et al.*, 2002; Diaz *et al.*, 2001; Pena *et al.*, 2003; Barnes *et al.*, 1996).

The province of East Azarbaijan is located in North West of Iran and is neighbors with Nakhichevan state of Republic of Azerbaijan. The estimate rate of tuberculosis (TB) for Iran in year 2002 was 29 in 100,000 but in East Azarbaijan the estimate rate was lower. This can be due to low case finding or low prevalence of TB in this province but TB in Republic of Azerbaijan is prevalent with multidrug- resistance (Pfyffer *et al.*, 2001).

In this study the aim was to investigate the transmission of tuberculosis in East Azarbaijan province of Iran by RFLP- hybridization method to decrease transmission of TB in that province.

MATERIALS AND METHODS

Patient population and bacterial isolates: The study population comprised all patients from whom at least one sample was positive for *M. tuberculosis* by culture which collected in four TB centers of the province from September 2002 to March 2003. A total of 125 isolates of *M. tuberculosis* were collected from different patients of whom fourteen patients were from Nakhichevan state of Azerbaijan and 111 patients were from East Azarbaijan of Iran. The species identification of the isolates was based on PCR method and standard microbiological tests (Rieder *et al.*, 1998).

RFLP analysis: RELP analysis was performed as described by Van Soolingen *et al.* (1994). Extracted mycobacterium DNA was digested with PvuII, subjected to electrophoresis and hybridized with a 245 bp PCR-amplified probe directed against the right arm of IS6110. After hybridization, the insertion sequences were visualized with a colorimetric system, the DIG DNA labeling and detecting kit (Roche-Germany) by following the manufacturer's instruction. A mixture of PvuII-digested supercoiled DNA ladder (Sigma) and HaeIII-digested Φ 174 DNA (Fermentas) was used as an internal marker. PvuII - digested genomic DNA of *M. tuberculosis* reference strain Mtl 4323 was used as external marker in each gel. The IS6110 fingerprinting pattern was compared by visual examination (Van Soolingen *et al.*, 1994). A cluster of *M. tuberculosis* was defined as two or more isolates with exhibited the same number of copies (six or more) of the IS6110 fragment with identical molecular sizes. All strains were compared to detect probable transmission of infection between patients.

RESULTS

Six isolates from 125 patients could not be used for the RFLP because the amount of DNA for RFLP-hybridization was not enough. The RFLP patterns of 119 isolates were determined. The copy number of IS6110 in each of isolates varied from 0 to 18 (Fig. 1). Ninety three distinct IS6110-RFLP patterns were revealed. Eighty one of these patterns were unique and 12 were shared by 2 to 8 strains. Thirty eight strains (31.9%) belonged to one of 12 clusters that were found among the total of 119 strains. The largest cluster comprised 8 patients; other clusters included 5 and 4 patients each. Three clusters harbored 3 patients and six clusters comprised 2 patients each.

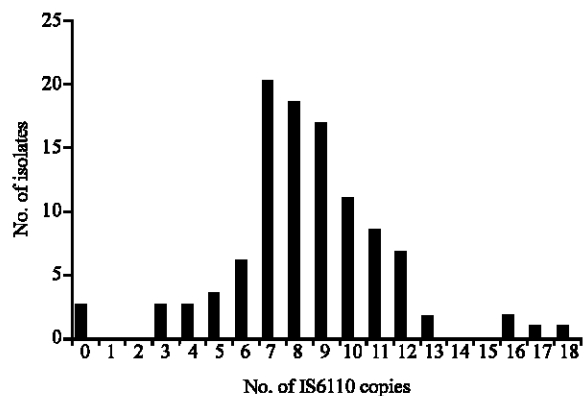


Fig. 1: Number of IS6110 copies in *M. tuberculosis* strains from 119 patients

One cluster with two Iranian patients has been defined that shared RFLP pattern with one strain of the Nakhichevanees patient that showed the probable transmission of infection from Nakhichevan. The rate of diversity of the patterns by RFLP analysis with IS6110 for the *M. tuberculosis* strains in this study was 78.2%, the minimum estimate for the proportion of tuberculosis that was due to transmission in East Azarbaijan province was 22% (38-12/119).

DISCUSSION

A crucial aspect of any TB control program is the ability to determine 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 preventing therapy. In our analysis over a 6 month period, 119 *M. tuberculosis* isolates from 119 TB patients were characterized by RFLP. These patients had been referred to TB centers from East Azarbaijan of Iran or Nakhichevan state of Azerbaijan country.

East Azarbaijan our province is neighbor with countries which have just become independent such as Republic of Azerbaijan and especially Nakhichevan state that percentage of infection and transmission in this region is high (Pfyffer *et al.*, 2001) and we often faces with traveling from this region. Fourteen patients who were affected by tuberculosis and referred to the TB centers of our provinces were from Nakhichevan. During the study, one of these patients was the source of infection of 2 Iranian patients, because they were in one cluster and being in the same cluster concluding the same source, so he was the source of spread infection in Tabriz by traveling and residing in this region. In similar transmission a traveling salesman introduced a New York strain into San Francisco (Casper *et al.*, 1996), or English patient transmitted TB to 37 patients in Netherland (the Netherlands 28; the UK 7; Surinam 1; Morocco 1) (Kiers *et al.*, 1997) and immigration of Somalians to Denmark increase the rate of TB during 1990 to 1999 in Denmark (Troels *et al.*, 2001). The importance of this case is that the treatment of TB in Iran is entirely free so foreign patients refer to our province for treatment and it cause new transmission and more expenses for us.

Our study confirmed that prolonged and causal contacts both can results in extensive transmission of tuberculosis. Between twelve clusters the largest one was 8 patients that all were from Tabriz. In this cluster, contact was limited, occurring in the setting of work, home or bus.

The minimum estimate of transmission of tuberculosis in East Azarbaijan province was 22% that was higher than Zurich with 11% (Pfyffer *et al.*, 1998) and London with 14.4% (Maguire *et al.*, 2002) But it was lower than South

Africa with 50% (Godfrey-faussett *et al.*, 2000), Tarrant County of Texas with 36% (Weis *et al.*, 2002), San Francisco with 24% (Borgdorff *et al.*, 2000) Lower rate of TB in East Azarbaijan may be due to a growing public awareness of TB or revival of the tuberculosis screening programme by the Tuberculosis and Lung Disease Center in Tabriz.

The result of this study show that sanitation of TB is relatively in good condition and both of reactivation of latent infection and transmission of infection are effective in prevalence of tuberculosis in East Azarbaijan. The outbreak described in this study have not been identified without DNA fingerprinting of *M. tuberculosis* isolates, which emphasizes the importance of DNA fingerprinting data for identifying unusual transmission patterns in unexpected setting.

In our opinion in order to prevent tuberculosis transmission from Nakhichevan, new tuberculosis center should be established near the border to prohibit traveling of Nakhichevanees patients and long staying of them in inns. We also suggest that recognition of photographs of patients with TB can be a useful tool for identifying links between patients.

Finally, it can be concluded that IS6110 DNA fingerprinting helps to find epidemiological links between tuberculosis cases and this technique also helps us to estimate the magnitude of recent transmission of tuberculosis in East Azaerbaijan province of Iran. In order to increase the knowledge of epidemiology of the disease, other studies in a larger period of time is recommended.

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