ORIGINAL ARTICLE

TRANSMISSION PATTERN OF TUBERCULOSIS USING RFLP-BASED IS6110


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Abstract

Background-The aim of this study was to determine IS6110 banding pattern of Mycobacterium tuberculosis (MTB) isolates for evaluation of tuberculosis (TB) transmission. These isolates were obtained from intermediate laboratories of six major provinces of Iran; East Azarbaijan, West Azarbaijan, Khorasan, Kerman, Kermanshah and Fars.

Methods-Restriction fragment length polymorphism (RFLP) was performed on 100 suitable isolates, which have been obtained from some laboratories thought Iran. Fingerprinting was done using the oligonucleotide 6110 a’ (5΄-GTGAGGGCATCGAGGTGGC) and 6110 b’ (5΄-GCGTAGGCGTCGGTGACAAA) primers.

Results-We observed two types of banding patterns among the typed strains: sixty-two percent of MTB strains had a high copy number of IS6110, whereas 33% had a low copy number. In addition, five MTB strains (5%) without any IS6110 banding pattern were detected. The analysis of banding pattern in MTB isolates revealed heterogeneous DNA fingerprinting. The computer-assisted dendogram system demonstrated 8% to 51% similarity among typed strains. According to the available data, similarity between 90% and 100% is considered as homogeneous DNA fingerprinting.

Conclusion-Since two banding patterns (low and high) have been detected, it could be suggested that two or more lineage for TB strains might exist in Iran, which requires further analysis. This study also suggests that in these cases, tuberculosis is characterized by the absence of obvious focuses of transmission.

Keywords • IS6110 • RFLP • Mycobacterium tuberculosis

Introduction

The World Health Organization estimates that perhaps as much as one-third of the world’s population or approximately 1.9 billion persons are or have been infected with Mycobacterium tuberculosis (MTB). The key to controlling the spread of tuberculosis include proper case finding, rapid diagnosis of tuberculosis and prompt initiation of effective chemotherapy. Besides, we need to learn more about epidemiological spread of diseases inside and across the borders of the country, which in turn may leads us to new interventional strategies.

Advances in molecular biology have provided powerful epidemiological tools for typing and detecting MTB deoxyribonucleic acid (DNA). The use of repetitive elements for fingerprinting in MTB isolates was initially described by Eisenach, et al. One of the insertion elements isolated by Eisenach was IS6110, which is a mobile genetic element present exclusively in the genome of members of the MTB complex and is present in multiple copies in most members of this complex. These properties have made IS6110 highly successful as a target for detecting MTB isolates.
DNA for diagnosis of TB and fingerprinting the strains of epidemiological interest.\textsuperscript{14,15} With availability of large number of MTB isolate from different parts of Iran in this center, we decided to perform restriction fragment length polymorphism (RFLP) based on IS6110 typing to determined transmission patterns of tuberculosis in different provinces of the country. This was a preliminary screening for future evaluation of TB transmission in Iran.

Materials and Methods

Mycobacterial strains

RFLP was performed on 100 suitable isolates which were received between September 1998 and February 1999 from selected laboratories of East and West Azarbaijan, Khorasan, Kerman, Kermanshah and Fars. Out of the 100 MTB isolates, 32 were from East Azarbaijan, 22 from West Azarbaijan, 25 from Khorasan, 7 from Kermanshah, 8 from Kerman and 6 from Fars provinces. They were composed of 37 drug-resistant and 63 drug-sensitive strains. From these 100 culture-positive slants, the colonies were transferred to 7H9 broth medium and were cultured grown for 2-3 weeks at 37\textdegree C. Thereafter, they were killed by heating in 80\textdegree C water bath for 30 minutes prior to being used for DNA extraction.

Isolation of genomic DNA and restriction endonuclease digestion

The isolation of genomic DNA was carried out by the method described by Whipple, et al. The cells were suspended in 400 \mu L of TE buffer (0.01 M Tris- HCl, 0.001 M EDTA, pH 8.0) and treated with 8000 U of lipase (Sigma) for 2 hours at 37\textdegree C. Then 4 mg/mL of lysozyme (Sigma) was added and incubated for 1.5-2 h at 37\textdegree C. Addition of 2mg/mL of proteinase K and 1% of SDS into the sample mixture was followed and treated for 16 h at 56\textdegree C. After adding 0.4 volume of cold 5 M potassium acetate solution the sample was gently mixed by inversion and placed on ice for 10 minutes before centrifugation. DNA was extracted from the supernatant by adding an equal volume of saturated phenol-chloroform and isoamylalcohol.

Synthesis of oligonucleotides

Oligonucleotide primers were synthesized by means of a DNA synthesizer (Milligen / Biosearch, Istituto Di Microbiologia, Universita Cattolica Del Sacro Cuore, Largo Francesco vito, Roma, Italy). The oligonucleotide 6110a (5'-GTGAGGCGCAT-CGAGGTGGC) and 6110 b (5'-CGTAGGCCT-CGGTGACAAA) were based on the IS6110 sequence. With these primers the 245bp fragment of IS6110 was produced by Polymerase chain reaction (PCR) and used as a probe in southern blot analysis.

Southern blot hybridization

Mycobacterial genomic DNA was digested with \textit{PvuI} and electrophoretically separated on 0.8% agarose gel (Figure1). After denaturation, DNA was transferred to the nitrocellulose membrane by vacuum blotting for 1 h and hybridized with digoxigenin (dig)-labelled probe. DNA was prepared by the method described by the manufacturer. Hybridization and washing were done at 68\textdegree C.

PCR procedure

Polymerase chain reaction (PCR) was performed by the procedure described previously by Maniatis, et al.\textsuperscript{18} The samples were amplified using the gene AMP kit (Perkin Elmer Cetus, Norwalk, CT, USA) through 35-40 cycles in a programmable thermal cycler (Perkin Elmer Cetus) with a 2-step denaturation for 1.5 minutes at 92\textdegree C, annealing and extension for 2 minutes.
at 70°C. The amplification products, together with 123bp lader fragment, were electrophoresed on 2% agarose gel and visualized the amplicons by ethidium bromide fluorescence.

Labelling of DNA probes
The IS6110-specific DNA probe of 245 bp was amplified by PCR and electrophoresed on agarose gel. Each DNA probe was electroeluted and precipitated with LiCL, dried and resuspended with TE, and labelled with dig-dUTP using a dig DNA labelling kit (Boeringer/ Mannheim, Germany). The detection of dig-labelled DNA was carried out according to the manufacturer’s recommendations.

Computer-assisted analysis of RFLP patterns
The fingerprint patterns of the isolates were pair-wise compared both by the computer-assisted analysis Gel ComparR and by visual observation. The distance between the two profiles was calculated according to the Dice index; the algorithm used to produce the dendrogram from these distances was the unweighed pair group method of analysis (UPGMA).

Results
The results demonstrated that two types of banding patterns for IS6110 were present in these strains; the MTB strains (33%) had low copies of IS6110 (4-6 bands) in their chromosomal DNA and MTB strains (62%) and had high copies of IS6110 (6-14 bands). In addition, five MTB strains (5%) were without any IS6110 banding pattern. The pattern of DNA fingerprinting of 54 chosen culture-positive slants from Azarbaijan (East and West) showed that the strains had multiple IS6110-containing restriction fragments and each strain showed a unique pattern (Figure 2). The computer-assisted dendrogram system also demonstrated no noticeable similarity in banding pattern among the strains. The similarity among them was a minimum of 10% and a maximum of 50% (Figure 3).

The DNA fingerprinting of 25 culture-positive slants from Khorasan province showed a few banding patterns with less than 30% similarity among them (Figure 4). However, we found a set of identical strains, which had no epidemiological linking according to available data.

Similarly, 21 chosen culture-positive slants, received in NRITLD from intermediate laboratories of Fars, Kerman and Kermanshah provinces were used for DNA fingerprinting. The computer-assisted dendogram showed a minimum of 8% and a maximum of 51% similarity with IS6110 having multiple banding patterns (Figure 4). However, in Fars province a set of identical strains (Figure 5) was detected. Reviewing their information, our data revealed that they were epidemiologically-linked strains.

Figure 2. The DNA fingerprinting pattern of IS6110 in MTB strains collected from Azarbaijan (East and West).

Figure 3. Computer-assisted dendrogram system demonstrated that the similarity of strain was from 8% to 51%.
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Discussion

The determination of banding patterns using RFLP based on IS6110 seems to be a useful tool for the evaluation of TB transmission. It is based on the assumption that different individuals who are infected with identical genotypes of M. tuberculosis strains are epidemiologically linked, whereas those with different genotypes are unrelated.9,8,13 Thereby, the present study aimed to investigate the banding pattern of MTB isolates collected from patients with pulmonary tuberculosis in different geographical regions of Iran using RFLP-based IS6110 for determination of TB transmission.

Our results demonstrate that the similarity of banding among MTB strains was between 8% to 51% (Figure 3). These fingerprinting patterns reflect the differences in copy number and sites of insertion in the chromosome due to duplication and/or movement of the element in the collected MTB strains. From the epidemiologic point of view, the available data on patterns of IS6110 by computer scanning states that similarities of less than 90% of MTB strains would reflect reactivation and not transmission of the disease in the community.8,14,15

The low rate of banding similarity of IS6110 MTB strains in an Iranian population was similar to the observation of researchers in other countries14-20, especially countries with a population with various geographical origins and where MTB strains from different regions are found. In France21, Vachee and his co-worker in 1999 demonstrated that out of 158 cases of bacteriologically confirmed tuberculosis, 126 patients were infected with genetically different isolates according to IS6110 banding patterns. Thereby, he concluded that in northern France, tuberculosis was characterized by the absence of obvious transmission focuses. Our observation and the results of European studies10,16,21 are in contrast with the epidemiological investigation in the USA.22,23 The studies performed in New York22 and San Francisco in 1990-1992 and 1991-1992 showed an 85% and 100% similarity of banding pattern respectively which could reflect the high transmission rate of tuberculosis. They observed up to 30-35 persons living together who had been infected by the same strains.

In the present investigation, 2 sets of identical strains were detected. One set from Fars province, which had epidemiological link, and the other set from Khorasan province with no epidemiological link. Reviewing their registration files, one can see that the identical strains of Fars province were from patients staying in the same localities. One of the patients worked in a shop in this region, but the other actually was a resident there. This single finding can reconfirm that IS6110 could be a very useful tool in tracing the transmission rate among epidemiologically related tuberculosis patients. In a similar observation, Curtis, et al24 detected eight cases with identical IS6110 DNA fingerprinting, all of which were infected by patients with extensive cavitary disease and who had stayed at home for almost 8 months before seeking medical care and being diagnosed. Furthermore, Kiers and

Figure 4. The MTB strain collected from Khorasan province had few a IS6110 banding in comparison to other states.

Figure 5. Two identical strains of MTB in Fars province could were detected and found to be epidemiologically linked.
his co-worker\textsuperscript{25} suggested the use of RFLP typing in international tracing of infection sources. He demonstrated that the source of MTB in infected children with tuberculous meningitis in the Netherlands was in the United Kingdom.

The major consideration for determining the usefulness of strain-typing methods is its specificity.\textsuperscript{11} The specificity of the fingerprinting pattern is dependent upon the number of bands obtained.\textsuperscript{10-12} Although IS6110 was originally described as a repetitive element present in 1-20 copies, many isolates of MTB are now known to possess one or few \textsuperscript{1-5} IS6110. Accordingly, a study performed by two main National Centers for Tuberculosis in Vietnam\textsuperscript{26} showed that 1.8\% of their MTB strains had no IS6110 in their genomes. In addition, the percentage of strains with only one IS6110 was high, and consistent with reports of the MTB strains in India.\textsuperscript{27} Similarly, our DNA fingerprinting revealed that 33\% of MTB strains had a low copy number of IS6110 (1-6 band).

Although, the number of MTB strains in this study was not sufficient to judge any specific grouping or clustering in Iran, similar reports by Fonkong and his colleagues\textsuperscript{28} show that low and high copy strains of MTB have a separate lineage which might be due to differences in their transmission. Two or more lineages for TB strains exist in Iran, which have to be analyzed. We suggest further studies to be performed in this field.

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**References**


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