

Molecular Epidemiology Analysis of TB in Five Regional States of Iran

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Background: The increasing prevalence of TB drug resistant strains in absence of recent transmission evidence, highlights the need for an improved control program, coupled with a need to improve detection rate and early diagnosis. IS6110-RFLP is a means of genotyping TB clinical samples. In this study IS6110-RFLP was used for specification and quick tracking of TB infection source, transmission and reactivation of infection, in Iran.

Materials and Methods: This study was carried out on 258 TB patients from Tehran, Mashhad, Isfahan, Shiraz and Ahwaz. DNA from positive cultures was extracted and digested by PVUII restriction enzyme. The digested sequences were separated based on the size on agar gel and then southern Blot was transferred on the membrane. IS6110 probe was marked by HRD and hybridized to the target parts along genome.

Results: Sixty-one strains (24%) showed similar patterns (Recent transmission) and 197 strains (76%) showed different IS6110 patterns (Reactivation). Average number of IS6110 copies was between 10-11 bands. Frequency of IS6110 similar pattern was 11.46 in Afghan immigrants and 10.68 in Iranians.

Conclusion: High diversity of IS6110, indicates that 76% of the patients have been infected through reactivation by different sources, while 24% have been infected due to recent transmission. Observing different antibiogram patterns in patients infected with the same strain indicated vast transmission of a single strain in the society. A susceptible strain can be changed into mono drug resistant and MDR strain in the transition period.

Key words: Tuberculosis, Epidemiology, Iran

INTRODUCTION

Tuberculosis (TB) is an infectious disease affecting millions worldwide and is caused by the *Mycobacterium tuberculosis* complex. In 2009, WHO estimated that there were about 9.4 million new cases, and 1.3 million deaths globally due to tuberculosis (1).

Frequency of drug resistant strains, in absence of evidence of recent transmission and in combination with many previously treated cases highlight the need for an improved control program, coupled with a need to

improve detection rate and early diagnosis (2). MDR-TB prevalence is higher among previously treated patients than among new cases (2). Velayati et al. in a recent study suggests that although the majority of MDR-TB bacilli are killed by treatment, a small number may undergo phenotypic changes to tolerate the cidal effect of the antimycobacterial agents (3).

Therefore, reaching a medical diagnosis and coming up with a therapeutic strategy are necessary for the identification of pathogenesis factors (4).

Molecular epidemiology is the applied science to study environmental risk factors, molecular identification, biochemical levels, etiological factors and prevention methods of TB in the society. IS6110-RFLP is used to specify the infection source, reactivation of infection, or infection transmission from one individual to another (5,6). It specifies the crisis line between clinical diseases and bacterial genetic map, providing medical instructions. IS6110 belongs to IS3 category of the family of insertion sequences (IS). This insertion sequence was reported to be specific for *Mycobacterium tuberculosis* complex and hence is extensively exploited for laboratory detection of tuberculosis and epidemiological investigations based on polymerase chain reaction (7).

Mycobacterium tuberculosis genome has several repeating polymorphic elements which are studied for separating isolates. Fingerprinting of various isolates shows that TB is due to the last transfer from a person to another (transmission) or reactivity of an existing infection (Reactivation) (8,9). Population is an indicator of the period during which the equal isolates have changed.

Since the transposition of repeating elements is a time-dependant process, the value of polymorphism between these elements is studied as an epidemiological transition factor.

Thus, study of *Mycobacterium tuberculosis* structural analysis in a society or a population provides us with some information about the history of evolution and distribution of special clones in the population. In geographic regions, diversity in mycobacterium strains indicates diversity of infection origin in different people afflicted with the infection in different places. Tehran, Mashhad, Isfahan, Shiraz and Ahwaz are populated cities that receive many immigrants from neighboring countries each year. Thus, these five cities of Iran were chosen to investigate the diversity of mycobacterium strains by the current study. IS6110-RFLP method was used to specify the epidemiologic pattern of *Mycobacterium tuberculosis* strains.

MATERIALS AND METHODS

This research was carried out on 258 samples of *Mycobacterium tuberculosis* separated from old TB patients presenting to Mycobacteriology Research Center to receive their TB drugs. Patients were selected from Tehran, Mashhad, Isfahan, Shiraz and Ahwaz, 5 major cities of Iran. Data was collected by trained technicians using standard questionnaires. Some information regarding sex, age, place of birth, close contacts, previous history of TB, Tuberculin test and related medical data were collected and entered into related forms. Primary isolation and culture from mucus samples was performed in accordance to the instructions of Mycobacteriology Methods (10).

Biochemical testing methods including production of niacin, catalase activity, nitrate revival, pigment production and growth speed were used to identify isolates (11). Determination of drug susceptibility to Isoniazid, Rifampin and Ethambutol was performed using standard method on medium LJ with the concentration of 0.2, 4, 40 and 2 microgram in milliliter, respectively (12).

Genomic DNA of all positive cultures was extracted by the QIAGEN DNA extraction kit Cat No: 51304 (13). Digestion of DNA was performed by PvuII enzyme. The digested fragments were separated from each other based on their size on the 0.8% agar gel. The size of bands indicated DNA volume required for Southern Blotting. Southern Blotting was performed by 1% agar gel. RFLP-IS6110 was performed on all samples according to the standard protocol (14). The probe supplemented IS6110 parts was marked by HRD and was hybridized to the target parts along genome (14). A film of Roche Radiology with high sensitivity was placed on membrane. After 5 minutes the film was placed into the Auto Processor and the developed film was studied.

RESULTS

A total of 258 under-study patients were separated based on their city of origin; 152 patients were from Tehran, 36 from Mashhad, 32 from Shiraz, 16 from Ahwaz and 22 from Isfahan.

Our findings showed that, 107 (42%) were females and 151 (58%) were males. Among 59 Afghan immigrants, 29 (49%) were females and 30 (51%) were males. No meaningful correlation was found between incidence of tuberculosis in males and females and incidence of tuberculosis was slightly higher in men.

The average age of patients was 44.67 yrs. (range 2- 86 yrs, both being Iranian). Overall, 134 (52%) were sensitive to all 5 drugs; 52 (20%) were resistant to one drug and 72 (28%) were resistant to all 5 drugs (MDR). The hybrid bands in each strain specified number of IS6110 copies. Number of IS6110 copies varied from 0 to 20. Average number of IS6110 copies was 10.82 bands. Using the frequency extent of IS6110 bands, epidemiologic spread pattern of *Mycobacterium tuberculosis* strains was specified.

Frequency of IS6110 similar pattern was 11.46 in Afghan immigrants and 10.68 in Iranians. In the present study, it was revealed that the most common strain of mycobacterium tuberculosis had 6 to 12 IS6110 patterns. In this study, the strains had 0 to 20 copies and the average number of copies of IS 6110 was 10.82 bands. Also, it was revealed that among 72 multi- drug resistant (MDR) cases, 19 patients were placed in 13 identical groups who had 9-20 copies of IS6110, respectively; which means that noticeable changes were seen in number of IS 6110 bands and most MDR cases had between 10-15 copies.

Investigating digested patterns showed that, 197 strains (76%) displayed a unique pattern; while, 61 strains (24%) showed 30 other patterns which were classified in 30 groups. The similar pattern was considered as the identical pattern (Recent transmission) and different patterns were considered as reactivation. As a result, 61 patients (24%) had been infected with tuberculosis through recent infection (cluster) and 197 patients (76%) were afflicted due to reactivation. Number of patients in both groups varied from 2 to 3. Sixty-one other patients were divided into two numerical groups; 29 out of 30 groups (97%) consisted of 2 patients. The remaining one group (3%) included 3 patients. This study showed that 48% of patients afflicted with tuberculosis were multi- drug resistant and non-MDR

resistant. Among 72 multi-drug resistant (MDR) cases, 53 strains showed identical unique pattern and 19 strains showed 13 different groups. The last group played a critical role in transmission of the recent infection. Thirteen patients (21%) with a recent infection were Afghan immigrants; 47 patients (79%) were Iranian. In fact, 13 patients (21%) who were afflicted with tuberculosis due to recent infection (cluster) were younger than 30 years old and 48 patients (79%) were over 30 yrs. While, 143 Non-cluster patients (72%) were over 30 years and 54 patients (28%) were younger than 30 years of age. The average age of the subjects was 42.16 years old.

DISCUSSION

In this study, genetic fingerprinting method was used to specify the epidemiologic pattern of *Mycobacterium tuberculosis* strains. RFLP analysis is the gold standard for *M. tuberculosis* genotyping; but this specification is limited to complex strains of *Mycobacterium tuberculosis* and is special in each strain.

Today, drug-resistant TB is considered as a global health problem (15). This study showed that incidence of multi-drug resistant strains (MDR) had increased compared to the previous studies (16). In a similar study carried out on TB patients in Huston, it was shown that people with identical genetic pattern commuted and collaborated in certain places (17). In another study, no correlation was found between people with the same social status in different groups (18). In a study conducted in various parts of the world it was shown that transmission rate of the resistant strains was 30% to 40% lower than that of susceptible strains (19). Also, in a research carried out in South Africa, it was reported that transmission of resistant strains was 70% to 80% lower than that of susceptible strains (20). In this study no special drug- resistant pattern of IS6110 was detected. This indicates that mycobacterium tuberculosis genome does not change in patients' body after its mutation.

It is assumed that the strain was susceptible at first and through transmission from one host to another

(unsuccessful treatment cases), the strain changed to MDR type. In fact, positioning and number of IS6110 along the genome of *Mycobacterium tuberculosis* showed no correlation with drug-susceptibility.

No relevant IS6110 pattern was obtained in different drug susceptible isolates. In some mono-drug resistant *Mycobacterium tuberculosis* strains, the number and condition of IS6110 were similar to those of susceptible and MDR strains. In a different study, two mono-drug resistant strains (one to Isoniazid and the other one to Rifampicin) were compared with sensitive multi-drug resistant strains. In our research, different IS6110 pattern was not seen (21).

Previous studies showed that more than 90% of active cases of tuberculosis in Iran resulted from reactivation of an infection contracted years before, and that recently transmitted diseases had a minor role (22). The high rate of initial resistance in MDR-TB cases and the high rate of MDR-TB in a young age group are indicators of recent transmission (15).

Our study showed that diversity of the observed strains was more than the identical strains; 76% of 258 studied cases showed different IS6110 patterns. Number and position of IS6110 band along the genome of 61 cases were similar to each other (24%) which were departed in 30 different groups.

Some recent studies have shown that polymorphism degree (diversity) of DNA depends on the geographic region of the separated strains (17, 18).

In a research in France, 83% of strains were disseminated through recent transmission and 17% were obtained of various strains. In another research, 90% strain diversity and only 10% incidence were seen (23). High percentage of genetic diversity in strains indicates diversity of mycobacterium strains in different geographical locations. It can be concluded that these strains may have entered by the immigrants. Rushing people with low socio-economic condition from different places to big cities such as 5 under study cities is regarded as a reason for reactivation of infection.

Conflict of interests

The authors declare that they have no conflict of interests.

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