Original Article

Frequency of Beijing family of Mycobacterium tuberculosis in Mashhad, Northeast of Iran

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Received: 12 September, 2016, Accepted: 21 December, 2016

Abstract

Background: Beijing family of Mycobacterium tuberculosis (*M. tuberculosis*) is widespread in Asia and has been involved in outbreaks of multidrug-resistant tuberculosis in various parts of the world. The aim of the current study was to evaluate the frequency of Beijing family of *M. tuberculosis* in patients with tuberculosis in Mashhad, Iran.

Materials and Methods: Totally, 72 specimens of *M. tuberculosis* were collected from pulmonary samples of patients at Ghaem hospital in Mashhad (Iran) between April 2011 and May 2012. The authors used IS6110-based polymerase chain reaction (IS6110-based PCR) method to identify Beijing family of *M. tuberculosis*. Based on PCR results, strains belonging to Beijing and non-Beijing families were detected. Also, among members of Beijing family, ancient and modern subfamilies were distinguished.

Results: Beijing genotype was observed in five (6.9%) of 72 culture positive samples. In the present study, no cases of modern subfamilies were detected. *M. tuberculosis* had a higher frequency in men (61.1%) compared to that of women (38.9%). Our data demonstrated that IS6110-based PCR can be used to distinguish Beijing family from non-Beijing family, with high specificity and sensitivity.

Conclusion: The improvement of convenient and quick methods to detect and control Beijing family of *M. tuberculosis* in clinical samples is an interesting subject in areas where *M. tuberculosis* is prevalent. This method has the advantages of being quick, cost-effective, and requires comparatively less clinical laboratory equipment.

Keywords: Mycobacterium tuberculosis; Beijing lineage; Polymerase chain reaction

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Please cite this article as: Mirbagheri S Z, Meshkat Z, Naderinasab M, Ghadamsoltani T, Rostami S, Momen Heravi M, AskariV R. Frequency of Beijing family of Mycobacterium tuberculosis in Mashhad, Northeast of Iran. Arch Med Lab Sci. 2016;2(3):102-7.

Introduction

Tuberculosis (TB) is caused by different strains of Mycobacterium tuberculosis (M. tuberculosis) (1, 2). It is regarded as a major health problem worldwide with a significant rate of morbidity and mortality (3-6). TB is a contagious airborne infection which is primarily transmitted from person to person (7). The genus of Mycobacterium exhibits extensive genetic diversity (8).

Beijing genotypes are increasingly reported in different parts of the world. It is hypothesized that

Beijing family may have certain features, including an increased virulence, and a higher transmissibility. Furthermore, Beijing family seems to show multidrug resistance (9-13). Beijing family can be divided into ancient (atypical) and the modern (typical) subfamilies (14-17). In a study, it was found that the modern subfamily has a higher adaptability to human hosts and is more infectious compared to the ancient subfamily. The population genetic analysis have shown that among M. tuberculosis strains, the infection caused by modern Beijing subfamily is higher than the ancient (15). These strains are increasingly reported from different parts of the world (9).

Transmission of tuberculosis is influenced by the gender. The results indicated a higher prevalence of tuberculosis among men than in women (6, 18).

Several genotyping methods, such as IS6110-RFLP and spoligotyping, were provided to distinguish the Beijing family. However, there are several issues associated them such as being time consuming, not being cost effective, and the extensive technical requirements.

Therefore, there is an urgent need to develop experimental methods, such as PCR-based methods, which are quicker (19). Biological markers have been greatly developed for typing with recent methods (2). They can be used for epidemiological studies of infectious diseases.

One of the most widely targets used in molecular detection of M. tuberculosis complex (MBT) is sequence IS6110. This target can also be used to distinguish Beijing family from Non Beijing families (20).

The authors used the IS6110- based -PCR assay to evaluate the prevalence of Beijing strains. The quality of this straightforward and quick method was studied for epidemiological studies on Beijing family.

The aim of the current study was to determine the frequency of Beijing family of Mycobacterium tuberculosis in culture-positive specimens in Mashhad, Northeast of Iran.

Methods

Patient characteristics and Bacterial isolates. This cross-sectional study was performed from April 2011 to May 2012. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences. In total, 72 specimens of M. tuberculosis were collected from pulmonary samples of patients at Ghaem hospital in Mashhad (Iran).

The samples were processed and decontaminated by modified Petroff's method for culture of M. tuberculosis. Isolates of M. tuberculosis were cultured on Lowenstein-Jensen medium and were incubated at 37ºC for at least three weeks (21). M. tuberculosis isolates were identified according to its specific growth rate and, colony morphology on LJ medium (22). The stocks of samples were prepared and stored at -20 ºC.

DNA extraction and PCR. DNA of bacterial isolates was extracted by simple boiling method (23). Sequence of the oligonucleotide primers Ris1 and Ris2 (Metabion, Swedish) corresponding to the 3′ and 5′ termini of IS6110, (Table 1) were used to amplify IS6110. PCR mixture contained 0.8X of 10X buffer, 1.6 mM of 50 mM MgCl2, 0.16 mM of dNTPs, 0.04 u/25µl Taq DNA polymerase (CinnaGen, Iran), 1 µM of each primers and 1 µl of template DNA in a total 25 µl reaction volume.

For IS6110 amplification, PCR was performed as follows: denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 45 s, and extension at 72ºC for 45 s; with a final extension step of 72 °C for 5 min.

Based on the PCR products after gel electrophoresis, Beijing strains of M. tuberculosis were detected as strains with two (290bp, 470bp) or three (260bp, 290, 470bp) bands. The single band of 470 b, was regarded as non-Beijing (Figure 1).

The amplified fragments were analyzed by electrophoresis on a 12% polyacrylamide gel, stained with ethidium bromide, and visualized under UV light.

Results

Among the 72 isolates, all samples were positive for M. tuberculosis according to culture on Lowenstein Jensen medium. Of 72 isolates with MTB, 44 (61.1%) belonged to men and 28 (38.9%) belonged to women. All patients were Iranian.

Five samples (6.9%) showed two bands of 4.70kb and 2.90kb, identical to that of ancient

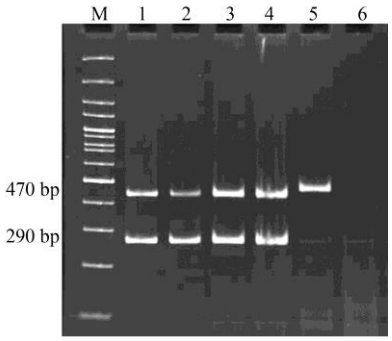

Figure 1. PCR products of IS6110. M: 100 bp DNA size marker; Lane 1: positive control; Lane 6: negative control; Lane numbers 2, 3 and 4, Beijing strains (ancient subfamily); Lane 5 non-Beijing genotype.

Table 1: Sequences of primers used in design and development of IS6110 -based-PCR assay

Name	Sequence	Size Of Product
Ris1 (forward primer) (36)	5′-GGCTGAGGTCTCAGATCAG -3′	Modern subfamily 260bp, 290, 470bp ancient subfamily 290bp, 470bp
Ris2 (reverse primer) (36)	5′-ACCCCATCCTTTCCAAGAAC -3′	

subfamily of Beijing, and were distinctive from other clinical samples (Fig. 1). Totally, among the 72 isolates, five isolates (6.9%) belonged to the Beijing family while 67 were non-Beijing (Table 2). Four patients with Beijing genotype were woman (80%) and one was man (20%). The ,modern subfamily and ancient subfamily of the Beijing family are summarized in Table 3. No ,modern subfamily was detected in samples.

Discussion

Tuberculosis is an ancient disease being a threat to the health of millions of people. In 1993, WHO reported it as a global threat for the world's health. However, it still remains a health issue in the world (9). According to a recent report from WHO, one third of the world's population (more than two billion people) are infected with Tuberculosis. Annually, it is responsible for a global mortality rate of about two million people. If this trend continues as the same rate, more than 40 million people are

Table 2: Patient characteristics for M. tuberculosis isolates

Characteristic	No. (%) Of Patients (N=72)
Sex	
Male	44 (61.1)
Female	28 (38.9)
Genotype	
Beijing	5 (6.9)
Non-Beijing	67 (93.1)

Table 3: Patient characteristics for Beijing family

Characteristic	No. (%) of patients (n=5)
Sex	
Male	1 (20)
Female	4 (80)
ancient sublfamily	5 (100)
Modern subfamily	0 (0)

estimated to die due to infection with Tuberculosis, in the next 25 years, worldwide. Beijing subfamily of Mycobacterial tuberculosis was first discovered in 1995 (24). This genotype has specific characteristics including multi-drug resistance and a higher rate of transmission and pathogenicity (9, 10, 13). Beijing family is classified into ancient (atypical) and modern (typical) groups (14, 15, 17).

Genotyping methods currently in use to detect this strain, such as Spoligotyping and IS6110-RFLP are hard-to-perform, time-consuming and costly (25, 26). In the current study, a simpler procedure was used to study the frequency of this strain among patients at Ghaem hospital in Mashhad, Iran.

In the present study, IS6110-based PCR was used to detect Beijing strains among 72 isolates of Mycobacterium tuberculosis. Five (6.9%) belonged to Beijing family and 67 were non-Beijing. All Beijing cases were of ancient subfamily (none belonged to the modern subfamily).

The Beijing strains among all cases of tuberculosis have a worldwide distribution. In East Asia, North America, Oceania, and Europe, 50%, 16%, 13%, and 4% of the samples, respectively, are of the beijing strains (27). In a study based on DNA fingerprint database from Denmark, analysis of 97% of all culture-positive tuberculosis samples showed that 1.0% of Danish-born patients and 3.6% of

immigrants (from 85 countries) had Beijing genotypes(28).

In a study by Gui Lianli et al in Tuberculosis control center of Tianjin (China) in 2010, 91.6% of Mycobacterium tuberculosis isolates from patients (753 of 822) was shown to belong to Beijing family. This was a higher rate than that of other neighbor regions to this area (1). Olabisi Ojo et al. in Ireland in 2010, found a prevalence rate of 5.9% for Beijing subfamily using Spoligotyping method (29). Also, a study by Tianli Li et al in China in 2010, using MIRU-VNTR, found a frequency of 86.23% (188 of 218) for the cases of Beijing family among isolates of Mycobacterium tuberculosis (30).

These figures demonstrated that Beijing family of M. tuberculosis was not highly prevalent in Mashhad, northeast of Iran, and it was lower than the rates in other countries. Our study showed that a great proportion of isolates were of the ancient subfamily and no cases of modern subfamily were detected in M. tuberculosis isolates. A study in Japan using VNTR genotyping showed that the ancient family had the highest proportion of Beijing family (31). In Contrast, in northwest of Russia, 96% of the samples belonged to the modern subfamily (26). The authors studied the relationship between sex and the frequency of M. tuberculosis infection. The results indicated the association of being infected with ancient subfamily and being female. Also, a higher proportion of men showed TB disease compared to women. This was in conformity with the fact that in most countries, tuberculosis had a higher prevalence among men (6).

To gain a better understanding of the transmission and prevalence of Beijing family of M. tuberculosis in Mashhad, the authors attempted to determine the frequency of Beijing strains using IS6110-based PCR. Comparison with the Spoligotyping method (as the standard method), enabled us to assess the accuracy of this method. Previously, in a study from 2004 to 2005, the frequency of Beijing subfamily of M. tuberculosis was determined using both spoligotyping method and IS6110-based PCR. They were shown to have similar results. The authors found that the prevalence of Beijing genotype was 6.9% (5 isolates). In a previous study using spoligotyping method in Mashhad, the prevalence of Beijing genotype was reported to be 7.1% (32). Therefore, using both methods, almost the same results were obtained.

Several genotype-base methods have recently been developed to detect Beijing genotype. Simple PCR or real-time PCR-based are used to detect genomic regions of Beijing subfamily (5, 33, 34). In previous studies, different methods were used to detect Beijing subfamily by using IS6110-RFLP fingerprinting (25), or by rehybridizing membrane by Beijing-specific probes (20). However, these methods were proved to be time-consuming and hard-to-perform.

IS6110-based PCR which was used in this study is a simple technique, compared with spoligotyping, and is cheaper and easier to set up. Spoligotyping is not always convenient in experiment. Thus, when smaller sets of strains or single isolates are to be analyzed simultaneously, it becomes rather expensive (35). Therefore, a simpler method based on standard PCR and poly acrylamide gel electrophoresis (i.e., IS6110 based-PCR) for early detection of Beijing strains and its subfamily could be more useful.

Conclusion

IS6110-based PCR may also be a useful method to control the global TB epidemiology. It yields results within a day and is rapid to be used for a high throughput detection of Beijing strains from large isolates archives. This is an ideal technique for use in areas of the world where Beijing strains are prevalent. Furthermore, it is relatively inexpensive; and it is easily adaptable to a standard molecular biology laboratory even in less developed countries.

Conflicts of Interest

There is no conflict of interest among authors.

Acknowledgment

The current study was financially supported by the Deputy of Research, Mashhad University of Medical Sciences (Grant No. 900276). The authors are thankful to the Microbiology and Virology laboratory of Buali Research Institute.

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