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Short Communication

Differentiation of modern and ancestral *Mycobacterium tuberculosis* in Northwest region of Iran by screening for the presence of TbD1

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***Mycobacterium tuberculosis* is responsible for considerable human morbidity and mortality worldwide. Based on the presence or absence of *M. tuberculosis* specific deletion (TbD1), *M. tuberculosis* isolates are divided into ancestral and modern strains. The aim of this study was to differentiate ancestral and modern *M. tuberculosis* in northwest of Iran. 165 *M. tuberculosis* strains were isolated from West and East Azarbaijan provinces of Iran. Ancient and modern *M. tuberculosis* isolates were distinguished by primer specific polymerase chain reaction (PCR). This study showed that 49 (29.7%) of the isolates were modern *M. tuberculosis* and 116 (70.3%) were ancestral *M. tuberculosis*. The prevalence of modern *M. tuberculosis* in West Azarbaijan was relatively higher than that of East Azarbaijan. Considering the increasing rate of modern *M. tuberculosis* in the studied region and in world, which has resulted in multi-drug resistance and low preventive effect of bacillus of calmette and guerin (BCG) vaccine, the fast diagnosis, prevention, treatment and more controlling programs of the infection, is important in this region.**

Key words: TbD1, modern tuberculosis, ancestral tuberculosis, PCR.

INTRODUCTION

Mycobacterium tuberculosis is one of the most successful bacterial pathogens in the history of mankind. Despite the many studies on the pathogenesis of this organism, it is remains as a major health problem worldwide (Asgharzadeh and Kafil, 2007) and it is more prevalent in underdeveloped and developing countries, in which over 95% of cases occur (Vukovic et al., 2003). Eight million new cases of tuberculosis are reported to occur every year, with up to three million deaths per year (Zhang et al., 2000). This worldwide problem is increasing due to several factors, including multi drug-resistant strains and co-infection with human immunodeficiency virus (Gleissberg, 1999). Based on the presence or absence of *M. tuberculosis* specific deletion (TbD1), *M. tuberculosis* strains are respectively, divided into ancestral and modern strains (Sun et al., 2004). Modern strains include Beijing/W, Harlem, Africa X and Delhi and ancestral strains include East- Africa and India (Sun et al., 2004). Beijing genotype is one of the members of modern *M. tuberculosis* that have been dominant since the mid-1950s and have remained so in the 1990 in the countries outside East Asia (Qian et al., 1999). It is likely that these ancestral strains predominantly originated from endemic foci, whereas modern *M. tuberculosis* strains that have lost TbD1 may represent epidemic *M. tuberculosis* strains (Brosch et al., 2004). It has even been hypothesized that vaccination with *Mycobacterium bovis* bacillus of calmette and guerin (BCG) may protect well Beijing/w strains, suggesting that they are escape variants (López et al., 2003; Van Soolingen et al., 1995; Hermans et al., 1995). Thus, identification and treatment of modern TB and specially Beijing genotype that show resistance to multi-drug and vaccination is very important (Rafiei, 2007).

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This study was aimed for the differentiation of ancestral *M. tuberculosis* strains and modern strains in the region based on the implementation of molecular methods. *M. tuberculosis* was isolated from patients in East and West Azarbaijan in Iran and was analyzed by PCR method.

MATERIALS AND METHODS

All isolates of *M. tuberculosis* were collected from patients who were referred to the central TB laboratory of West and East Azarbaijan Tuberculosis and Lung Disease Research centers. A total of 165 *M. tuberculosis* isolates were used for PCR (polymerase chain reaction) analysis (53 from west Azarbaijan and 112 from East Azarbaijan). The isolates were identified as *M. tuberculosis* by standard biochemical test, including production of niacin, catalyses activity, nitrate reduction, pigment production and growth rate. DNA used for the PCR analysis was extracted from cultured mycobacterium. Two loops full of bacteria was suspended in 400 µl of TE buffer (10 mM Tris -Cl, 1 mM EDTA, pH = 8.0) and incubated at 80°C for 20 min to kill the bacteria. DNA was extracted by modified lysozyme, SDS, proteinase K and CTAB (Asgharzadeh et al., 2008). PCR was performed in 50 l volumes that contained 20-200 ng DNA, 0.5 µM specific primers (TBF1: CTA CCT CAT CTT CCG GTC CA, TBR1: CAT AGA TCC CGG ACA TGG TG) in the presence of 1.5 mM $MgCl_2$, 200 M of each dNTP and 2U DNA polymerase (Cinnagen, Iran). DNA was amplified by general PCR. An initial denaturation of 7 min at 94°C was followed by 35 cycles of denaturation at 94°C 30s, annealing at 54°C for 40 s, extension at 72°C for 4 min and a final extension at 72°C for 10 min. The negative control was consisted of the PCR components in the reaction mixtures without the mycobacterium DNA. PCR products were analyzed in agarose gels after staining with 0.5 µg ml^{-1} ethidium bromide and visualized under UV light. The amplicons were 2638 and 485 bp long for the ancient *M. tuberculosis* strains in which the TbD1 region was present and for the modern *M. tuberculosis* strains in which the TbD1 region was absent, respectively (Sun et al., 2004).

RESULTS AND DISCUSSION

In this study, 165 culture-positive specimens from East and West Azarbaijan province of Iran were studied; 53 (32%) were from West Azarbaijan and 112 (68%) were from East Azarbaijan. Using PCR method, all *M. tuberculosis* strains were tested and the obtained data were analyzed. Based on the analysis, 49 isolates were found to be modern *M. tuberculosis* while 116 (70.3%) were ancestral *M. tuberculosis*. Among the 53 isolates from west Azarbaijan, 19 (35.85%) were modern *M. tuberculosis* and 34 (64.15%) were ancestral *M. tuberculosis* (Table 1) whereas among the 112 isolates from East Azarbaijan, 30 (26.79%) were modern *M. tuberculosis* and the reminding (73.21%) were ancestral *M. tuberculosis*. Statistical analysis by Fisher test showed that differences between groups were not significant.

In this study, we studied the prevalence of the modern and ancestral *M. tuberculosis* strains in East and West Azarbaijan provinces of Iran by specific primers for the subject. The study showed that prevalence of modern *M. tuberculosis* in West Azarbaijan was a little more than that of East Azarbaijan in the study period time (March 2004 and 2005). Higher prevalence of modern *M. tuberculosis* strains in West Azarbaijan was not a surprise, because it is located in a borderline of Iran and the rate of immigration from neighboring countries is high in the province. Published studies have shown that migration (Bifani et al., 1991) and close contact among the population can cause the spread and rapid dissemination of *M. tuberculosis* strains from one geographic area to another (Valway et al., 1998) and also the distribution of *M. tuberculosis* genotypes is reportedly associated with the geography, ethnicity and population migrations (Dou et al., 2008). Epidemiologic studies have revealed that different genotypes of *M. tuberculosis* may be prevalent in different geographic regions and that genotype distribution is closely associated with population migrations (Mokrousov et al., 2005). Beijing strains as the most prevalent member of modern TB have strong resistance to anti-tuberculosis drugs and can rapidly spread throughout the world (Toungoussova et al., 2002). Furthermore, it has been reported that Beijing genotype is predominant and a highly transmissible strains, which can be found in distinct geographic locations. This support the idea that, modern TB strains are more virulent than other strains of *M. tuberculosis* (Caminero et al., 2001).

Table 1. Result of the genetically differentiation of ancestral and modern isolates of *Mycobacterium tuberculosis*.

Region	Ancestral (%)	Modern (%)	Total (%)
West Azarbaijan	34 (64.15)	19 (35.85)	53(32)
East Azarbaijan	82 (73.21)	30 (26.79)	112 (68)
Total	116 (70.3)	49 (29.7)	165(100)

Therefore, their identification and discrimi-nation from ancestral *M. tuberculosis* strains, which have low resistance to anti-tuberculosis drugs, is very important. In addition, the study of their epidemiology is an important factor for planning health programs in the future. In recent years, the rapid dissemination of *M. tuberculosis* strains belonging to one particular genetic lineage especially Beijing genotype of modern tuberculosis strains has suggested that BCG-vaccinated individuals may be more prone to the infection by this strains (Anh et al., 2000). Increased transmission is another hypothesis that could explain the rapid intercontinental spread of these strains (Dou et al., 2008). The prevalence of tuberculosis is probably affected in this region because west Azarbaijan is located in the borderline of the country with countries such as Iraq, Turkey and Azarbaijan as neighbors. Thus, it is likely that various factor play a role in favoring the spread of the *M. tuberculosis* strains and that the most important ones may differ from one geographical area to another. Thus, it is important to undertake studies to identify which factors are the most significant to consider when setting up a *M. tuberculosis* controll programs and

for rapid diagnosis of the infection. Preventive health programs should be planned in the East and west Azarbaijan provinces of Iran, especially in west Azarbaijan to limit the spread of the modern TB strains. Due to the low preventive effect of BCG in controlling the spread of modern *M. tuberculosis*, new *M. tuberculosis* vaccines or DNA vaccines development is recommended. In conclusion, because of the growing prevalence of modern *M. tuberculosis* in the studied region and the low preventive effect of BCG vaccine in controlling modern *M. tuberculosis*, it is recommended that more tuberculosis controlling strategies and studies for the development of new vaccines be carried out.

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