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Use of Mycobacterial Interspersed Repetitive Unit-Variable-Number Tandem Repeat Typing to Study *Mycobacterium tuberculosis* Isolates from East Azarbaijan Province of Iran

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Abstract: The aim of present study was to determine the genotypes of isolates from East Azarbaijan province by this method. We performed (MIRU-VNTR) analysis of strains, isolated from 127 patients during a period of September 2002 to March 2003 in tuberculosis centers of the province. Among 127 isolates, we found 93 distinct MIRU-VNTR patterns, including in 21 clustered patterns and 72 unique patterns from isolated strains. The discriminatory power of MIRU-VNTR typing in present study was high (HGDI = 0.9932) for isolates. In clusters similar patterns of Nakhichevanees patients and Iranian patients was revealed in three clusters which showed Nakhichevanees patients referred to tuberculosis centers of province could be a source for transmission of tuberculosis. Tuberculosis in this province is relatively in good condition. The allelic diversity of our samples was lower than previous studies. These results indicate that MIRU-VNTR can be a useful and first line tool for studying genetic diversity of *M. tuberculosis* isolates in regional setting such as East Azarbaijan province of Iran.

Key words: *Mycobacterium tuberculosis*, transmission, MIRU-VNTR, Iran

INTRODUCTION

Tuberculosis is the leading cause of death in adults from a single infectious agent, killing about 3 million people every year. One-third of the human population is thought to be infected by the causative agent Mycobacterium tuberculosis (Cole *et al*., 1998; Burgos and Pym, 2002; Asgharzadeh *et al*., 2007a) and about 200 million additional people are at risk for developing the disease in the next 20 years if current trends continue (WHO, 1998; Grange and Zunmla, 1999). Therefore, efficient disease control can be achieved only by epidemiological surveillance systems to accurately monitor epidemic trends at a regional and global level.

Genotyping of *M. tuberculosis* has an important role in epidemiological studies (Asgharzadeh *et al*., 2007b). A large number of DNA fingerprinting methods for typing *M. tuberculosis* isolates have been developed in recent years, all have significant drawbacks and only a few have been adapted for widespread use. IS6110, spoligotyping and variable number tandem repeat-mycobacterial interspersed repetitive units are the most widely used methods (Kremer *et al*., 1999; Barow *et al*., 2001; Cowan *et al*., 2002).

Variable Number Tandem Repeat (VNTR) typing is an invaluable tool for genotyping and provides data in a simple and format based on the number of repetitive sequences in so-called polymorphic micro- or mini-satellite regions (Mazors *et al*., 2001). Despite some attempts to develop equivalent approaches for typing bacterial pathogens, only limited application for bacterial molecular epidemiology could be developed up to now (van Belkum *et al*., 1998; van Belkum, 1999). Mazors *et al*. (1998) introduced VNTR for *M. tuberculosis* which named Mycobacterial Interspersed Repetitive Unite-variable Number Tandem Repeat (MIRU-VNTR), each isolates is typed by the number of copies of repeated unites at 12 independent loci scattered throughout the genome (Supply *et al*., 1997). The repeated units are 52 to 77 nucleotides in length and the number of repeated unites can be determined by the size of the entire locus (Supply *et al*., 2000), previous

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studies demonstrated the importance of MIRU-VNTR method for tracking epidemiological key events, such as transmission or relapse and provide nonambiguous data which are highly portable between different laboratories (Mozars *et al*., 2001; Frothinghom *et al*., 1998; Supply *et al*., 1997).

The East Azarbaijan Province is located in the north west of Iran and in neighborhood of Nakhichevan state of Republic of Azerbaijan. The estimated population of the province is 3,500,000 (Asgharzadeh *et al*., 2006). The capital city of the province is Tabriz. The estimated rate of TB in Iran in 2004 was 27 in 100,000 (WHO, 2006) but in East Azarbaijan province the estimated rate was lower; it can be due to low case finding or low prevalence of TB in this province.

The objective of this study is to genotype *M. tuberculosis* isolates from 127 patients with tuberculosis in East Azarbaijan province and determine manner of transmission in this province.

MATERIALS AND METHODS

Patient population and bacterial isolates: The study population comprised 127 patients with TB confirmed by culture from September 2002 to March 2003 in TB centers of the province. Samples which were collected and submitted for culture in tuberculosis centers of province, clinical isolates were recovered from sputum (n = 99), bronchial fluids (n = 19), abscess aspirates (n = 2), urine (n = 2), cerebrospinal fluids (n = 2), pleural fluid (n = 1), endometrial biopsy (n = 1) and neck mass biopsy (n = 1). Fourteen strains were from Nakhichevanees patients which were compared with Iranian strains for detecting probable transmission of the infection. Information about age, sex, geographical origin and history of tuberculosis were recorded. The species identification of the isolates was based on Polymerase Chain Reaction (PCR) method and standard microbiological tests (Rieder *et al*., 1998).

DNA used for the PCR analysis was extracted from cultured Mycobacterium's. Two loops full of bacteria's were suspended in 400 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and placed at 80°C for 20 min to kill the bacteria. DNA was extracted by lysozyme, SDS, proteinase K and CTAB. Extracted DNA after sedimentation with isopropanol and washing with ethanol 70% was redissolved in 100 μL TE buffer (van Soolingen *et al*., 1994).

MIRU-VNTR typing: PCR was performed in 20 μL volume that contained 5 to 50 ng of DNA, 0.5 μm of specific primers (Table 1) in the presence of 1.5 mM $MgCl_2$, 100 μm of each dNTP, 50 mM KCl, 20 mM Tris_HCl, pH 8.4 and 1.25 U recombinant DNA polymerase (Cinnagen co. Iran). DNA was amplified by general PCR. All PCRs were initiated by a 7 min denaturizing step at 94°C and completed by a 7 min extension step at 72°C. The temperature cycles for different types of PCRs were as follow, 35 cycles of 45s at 94°C, annealing temperature for 45 sec and 72°C for 55 sec. Annealing temperatures were used as follow: 65, 63, 68, 65, 59, 67, 59, 65, 64, 63, 68 and 65 for MIRU loci 2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39 and 40, respectively.

Table 1: Oligonucleotides used in MIRU-VNTR genotyping

Primer sequence (5'-3')								Locus of MIRU
TGG	ACT	TGC	AGC	AAT	GGA	CCA	ACT	2
TAC	TCG	GAC	GCC	GGC	TCA	AAA	T	
GCG	CGA	GAG	CCC	GAA	CTG	C		4
GCG	CAG	CAG	AAA	CGC	CAG	C		
GTT	CTT	GAC	CAA	CTG	CAG	TCG	TCC	10
GCC	ACC	TTG	GTG	ATC	AGC	TAC	CT	
TCG	GTG	ATC	GGG	TCC	AGT	CCA	AGT A	16
CCC	GTC	GTG	CAG	CCC	TGG	TAC		
TCG	GAG	AGA	TGC	CCT	TCG	AGT	TAG	20
GGA	GAC	CGC	GAC	CAG	GTA	CTT	GTA	
CTG	TCG	ATG	GCC	GCA	ACA	AAA	CG	23
AGC	TCA	ACG	GGT	TCG	CCC	TTT	TGT C	
CGA	CCA	AGA	TGT	GCA	GGA	ATA	CAT	24
GGG	CGA	GTT	GAG	CTC	ACA	GAA		
TAG	GTC	TAC	CGT	CGA	AAT	CTG	TGA C	26
CAT	AGG	CGA	CCA	GGC	GAA	TAG		
TCG	AAA	GCC	TCT	GCG	TGC	CAG	TAA	27
GCG	ATG	TGA	GCG	TGC	CAC	TCA	A	
ACT	GAT	TGG	CTT	CAT	ACG	GCT	TTA	31
GTG	CCG	ACG	TGG	TCT	TGA	T		
CGG	AAA	CGT	CTA	CGC	CCC	ACA	CAT	39
CGC	ATC	GAC	AAA	CTG	GAG	CCA	AAC	
GGG	TTG	CTG	GAT	GAC	AAC	GTG	T	40
GGG	TGA	TCT	CGG	CGA	AAT	CAG	ATA	

Negative controls consisted of the PCR components on reaction mixtures lacking mycobacterium DNA. PCR products were electrophoreses in 1.5% agarose gel and after staining with 0.5 μg mL^{-1} ethidium bromide visualized under UV light. The size of fragments was determined in comparing with 100 bp DNA ladder plus size marker (Fermentas) (Supply *et al*., 2001)

Statistical analysis: The MIRU-VNTR allelic diversity (h) at each of the loci was calculated by the equation.

$$h = 1-\Sigma\chi_i^2$$

where, χ_i is the frequency of the *i*th allele at the locus (Sun *et al*., 2004).

The Hunter-Gaston Discriminatory Index (HGDI) described by Hunter and Gaston (Hunter and Gaston., 2003) was used as a numerical index for MIRU-VNTR discriminatory power. HGDI was calculated by using the following formula:

$$HGDI = 1-\left[\frac{1}{N(N-1)}\sum_{j=1}^{s} n_j(n_j-1)\right]$$

Where:

N = The total number of strains in the typing scheme.
s = The total number of different MIRU-VNTR patterns.
n_j = The number of strains belonging to the jth pattern.

Dendrogram was created by MSVP software.

RESULTS

The age of the patients ranged from 12 to 90 years which young patients (≤45 years old) represented 43% of study population. The male to female ratio was 1.3:1. Using MIRU-VNTR typing; 93 distinct were identified, including 21 cluster patterns and 72 unique patterns (Table 2). Fifty five isolates (43.3%) included in clusters. And the discriminatory power of MIRU-VNTR typing was high (HGDI = 0.9932) for our isolates. The minimum estimate for the proportion of tuberculosis that was due to transmission in East Azarbaijan Province is 26.8% (55-21)/127).

In 21 clusters (Fig. 1), the largest cluster comprised six patients, four of these patients were female and two were male, which this transmission can be happen by contact or by frequenting in the same place. The rest were residents from different parts of the city and no epidemiological linking was detected; however all were suffering from poor living condition. The second cluster was made up of four patients from Tabriz and Maraghe, a city about 200 km away from Tabriz and no connection between the patients were detected. Seven clusters were composed three patients and twelve clusters composed two patients.

Three cluster has been defined that shared MIRU-VNTR pattern between Iranian and Nakhichevanees patients. The first cluster composed one Nakhichevanees patients and two Iranian patients, the second cluster composed one Nakhichevanees and one Iranian patient and equally the last cluster composed one Nakhichevanees and one Iranian patient. One Cluster composed three Nakhichevanees patients with the same pattern, which were one female and two male.

The allelic diversities of the 12 MIRU-VNTR locus based on this study isolates. Locus 26 and 40 were highly discriminative (≥0.6), locus 16, 23, 24 and 31 were moderately discriminative (≥0.3) and locus 2, 4, 10, 20, 27, 39 were poorly discriminate (<0.3) (Table 3).

Table 2: MIRU-VNTR patterns of strains from East Azarbaijan Province of Iran

	MIRU-VNTR patterns at locus											
Strain No.	Loc 2	Loc 4	Loc 10	Loc 16	Loc 20	Loc 23	Loc 24	Loc 26	loc27	Loc 31	Loc 39	Loc 40
IP-48*	2	2	4	2	2	5	1	2	3	3	2	2
IP-49	2	2	3	2	2	4	2	3	3	3	1	2
IP-42	2	2	3	1	2	5	1	3	3	3	2	5
IP-35	2	2	3	3	2	5	2	4	3	3	1	3
IP-50	2	2	3	4	2	3	2	5	3	3	3	2
IP-20	2	2	2	3	2	5	1	5	3	3	2	3
IP-51	2	2	3	3	2	6	2	4	4	3	2	2
NP-1*	2	2	7	2	2	5	2	1	3	2	2	3
IP-53	2	2	3	2	2	6	2	4	3	3	1	2
IP-54	1	2	3	3	2	7	1	1	4	2	2	5
IP-22	2	3	4	2	2	5	2	4	3	3	2	3
IP-55	2	2	3	2	2	6	1	4	3	3	2	4
IP-24	2	2	2	4	2	5	1	5	3	4	2	3
IP-21	2	2	2	3	2	5	2	5	3	3	2	3
IP-25	2	2	2	4	2	5	1	5	3	4	2	3
IP-37	1	2	4	3	2	5	2	5	3	3	2	4
IP-56	2	2	3	4	2	5	2	2	3	3	2	4

Table 2: Continued

	MIRU-VNTR patterns at locus											
Strain No.	Loc 2	Loc 4	Loc 10	Loc 16	Loc 20	Loc 23	Loc 24	Loc 26	loc27	Loc 31	Loc 39	Loc 40
IP-57	2	2	4	2	2	5	2	4	3	5	1	3
IP-58	2	2	3	4	2	5	2	6	3	3	2	3
IP-59	2	3	4	3	2	5	2	4	3	3	2	2
IP-43	2	3	4	3	2	5	2	3	3	2	2	3
IP-29	2	2	3	3	1	5	2	5	3	3	2	3
NP-8	2	2	4	3	2	5	2	6	3	5	2	3
IP-30	2	2	3	3	1	5	2	5	3	3	2	3
IP-60	2	2	5	4	2	5	2	3	2	2	1	3
NP-9	1	2	5	3	2	5	2	5	3	2	2	5
NP-12	2	2	6	2	2	5	1	1	3	3	2	2
NP-5	2	2	5	2	2	5	2	4	3	3	2	4
IP-8	2	2	4	3	2	3	2	5	3	3	2	2
IP-9	2	2	4	3	2	3	2	5	3	3	2	2
IP-5	2	2	4	3	1	3	2	4	3	3	2	2
IP-62	2	2	4	1	2	4	2	5	3	2	2	4
IP-63	2	2	4	3	2	4	1	5	3	2	2	5
IP-31	2	2	3	3	1	5	2	5	3	3	2	3
NP-2	2	3	4	2	2	5	2	4	3	3	2	3
IP-15	2	2	4	3	1	3	2	3	3	3	2	2
IP-20	2	2	2	3	2	5	2	5	3	3	2	3
IP-64	2	4	3	3	2	5	2	5	3	3	2	4
IP-52	2	2	4	3	2	3	2	5	3	3	2	2
IP-3	2	2	4	3	1	3	2	4	3	3	2	2
IP-13	2	2	5	3	2	5	1	4	3	3	2	3
IP-65	2	2	5	3	2	6	1	5	3	2	2	4
IP-6	2	2	4	3	2	3	2	5	3	3	2	2
NP-10	2	2	6	2	1	5	2	2	3	2	2	3
IP-7	2	2	4	3	2	3	2	5	3	3	2	2
IP-19	2	2	5	3	2	5	1	4	3	3	2	3
IP-64	2	2	3	3	2	5	2	5	2	3	2	1
IP-18	2	2	2	3	2	5	1	5	2	2	2	3
IP-65	2	2	4	3	2	2	2	5	3	3	2	2
IP-66	2	2	2	3	2	5	2	6	3	3	2	3
IP-28	2	2	3	3	2	5	1	2	3	3	2	4
IP-67	2	2	2	2	2	1	2	1	3	3	2	3
IP-1	2	3	3	2	1	4	1	5	3	2	2	5
IP-68	2	1	2	3	2	5	2	3	2	3	2	3
IP-2	2	3	3	2	1	4	1	5	3	2	2	5
IP-69	2	2	5	4	2	5	2	4	3	3	2	3
IP-70	2	2	3	3	2	5	1	1	3	2	2	2
IP-36	2	2	3	3	2	5	2	4	3	3	1	3
IP-71	2	2	4	3	2	2	2	3	3	3	2	2
IP-72	1	2	5	3	2	6	1	4	3	2	2	8
IP-73	2	2	3	2	2	5	1	2	3	3	3	3
IP-74	2	2	3	3	2	5	2	3	3	3	1	3
NP-11	1	2	5	4	2	5	2	4	3	2	2	4
IP-75	2	2	4	3	1	5	2	6	2	3	1	3
IP-76	2	2	5	2	1	5	3	3	2	3	2	3
IP-77	2	2	6	2	2	5	2	3	3	3	1	2
IP-78	2	2	5	3	1	8	2	4	2	3	2	3
IP-38	2	2	3	3	2	3	1	2	3	3	2	2
IP-79	2	2	4	3	2	5	4	2	3	2	2	2
IP-40	2	2	2	4	2	5	2	4	3	3	2	3
IP-46	2	2	4	1	2	5	1	3	3	3	2	4
IP-80	2	2	4	3	2	3	2	3	3	3	2	2
IP-81	2	2	5	3	2	5	2	2	3	3	2	3
IP-82	2	3	4	3	2	3	2	2	3	3	2	2
NP-12	2	2	5	1	2	3	1	2	2	3	2	3
IP-84	2	3	5	2	2	5	2	1	3	3	2	2
IP-16	2	2	4	3	1	3	2	3	3	3	2	2
IP-85	2	2	2	3	2	5	2	1	2	2	2	3
IP-86	2	2	5	4	2	2	2	2	3	3	2	3
IP-87	2	2	2	3	2	5	2	2	3	3	2	1
IP-88	2	2	3	2	2	5	2	2	3	3	2	5
IP-33	2	2	2	3	2	5	1	2	3	3	2	3
IP-89	2	1	5	2	2	5	2	2	2	3	2	2
IP-90	2	2	4	2	2	5	1	2	3	3	2	2

Table 2: Continued

	MIRU-VNTR patterns at locus											
Strain No.	Loc 2	Loc 4	Loc 10	Loc 16	Loc 20	Loc 23	Loc 24	Loc 26	loc27	Loc 31	Loc 39	Loc 40
IP-12	2	2	5	3	1	5	1	4	3	3	2	3
IP-34	2	2	2	3	2	5	1	2	3	3	2	3
IP-91	2	2	3	2	2	6	2	4	3	3	2	4
IP-92	2	2	2	4	2	5	2	2	3	3	2	3
NP-4	2	2	5	2	2	5	2	4	3	3	2	4
IP-93	2	2	3	3	2	6	2	4	2	3	1	3
IP-27	2	2	3	3	2	5	1	2	3	3	2	4
IP-4	2	2	4	3	1	3	2	4	3	3	2	2
IP-26	2	2	3	3	2	5	1	2	3	3	2	4
IP-94	2	3	3	3	5	5	2	2	3	4	2	3
IP-95	2	2	5	3	2	6	2	3	3	3	2	3
IP-96	2	2	4	3	2	1	1	2	3	3	2	3
IP-97	2	2	3	2	2	5	2	3	3	3	2	3
IP-39	2	2	3	3	2	3	1	2	3	3	2	2
IP-98	2	2	3	2	2	5	2	5	3	3	2	4
IP-32	2	2	2	3	2	5	1	2	3	3	2	3
NP-3	2	2	5	2	2	5	2	4	3	3	2	4
IP-22	2	3	4	2	2	5	2	4	3	3	2	3
IP-99	2	2	3	3	2	6	2	5	3	3	2	2
IP-100	2	3	2	3	2	5	2	5	3	1	2	3
IP-10	2	2	4	3	2	3	2	5	3	3	2	2
IP-41	2	2	2	4	2	5	2	4	3	3	2	3
IP-101	2	3	3	3	2	5	2	6	3	3	2	1
IP-102	2	2	4	2	2	6	2	6	3	2	2	2
IP-103	2	2	5	1	2	5	2	4	3	3	2	3
IP-104	2	3	2	3	2	5	2	5	2	2	2	3
NP-6	1	2	4	3	2	5	2	5	3	3	2	4
IP-105	2	2	3	1	2	5	2	5	2	3	2	5
IP-106	2	2	3	2	2	5	2	3	3	3	2	1
IP-45	2	2	4	1	2	5	1	3	3	3	2	4
IP-44	2	3	4	3	2	5	2	3	3	2	2	3
NP-13	2	3	4	4	2	5	2	4	3	2	2	5
IP-109	2	3	3	3	2	3	2	5	3	3	2	3
IP-110	2	3	2	3	2	5	2	2	3	3	2	4
IP-11	2	2	5	3	2	5	1	4	3	3	2	3
NP-14	2	2	3	1	2	5	1	3	3	3	2	5
IP-111	2	2	4	1	2	5	2	1	3	3	2	3
IP-112	2	2	2	1	2	5	2	3	3	2	2	4
IP-17	2	2	2	3	2	5	1	5	2	2	2	3
IP-113	2	2	3	3	2	3	2	3	2	3	2	2
IP-114	2	3	4	1	2	5	2	4	3	2	2	4
IP-115	2	2	3	3	2	5	2	3	3	3	2	3
IP-116	2	2	3	3	2	5	2	2	3	3	2	3

*: IPs are Iranian patients number and NPs are Nakhichevanees patients number

Table 3: Allelic diversity of each MIRU-VNTR locus

	No. of strains with MIRU-VNTR locus											
No. of alleles	2	4	10	16	20	23	24	26	27	31	39	40
1	7	2		19	15	2	36	8			11	4
2	120	107	23	28	111	3	91	23	16	25	113	32
3		17	40	76		17		16	109	97	3	60
4			37	14		6		32	2	3		20
5			23			87		36		2		10
6			3			10		7				
7			1			1						
8						1						1
Allelic diversity	0.4	0.29	0.16	0.58	0.22	0.5	0.4	0.75	0.24	0.37	0.2	0.68

Allelic diversity (h) was defined as $h = 1 - \Sigma\chi_i^2$, where χ_i is the frequency of the *i*th allele at the locus

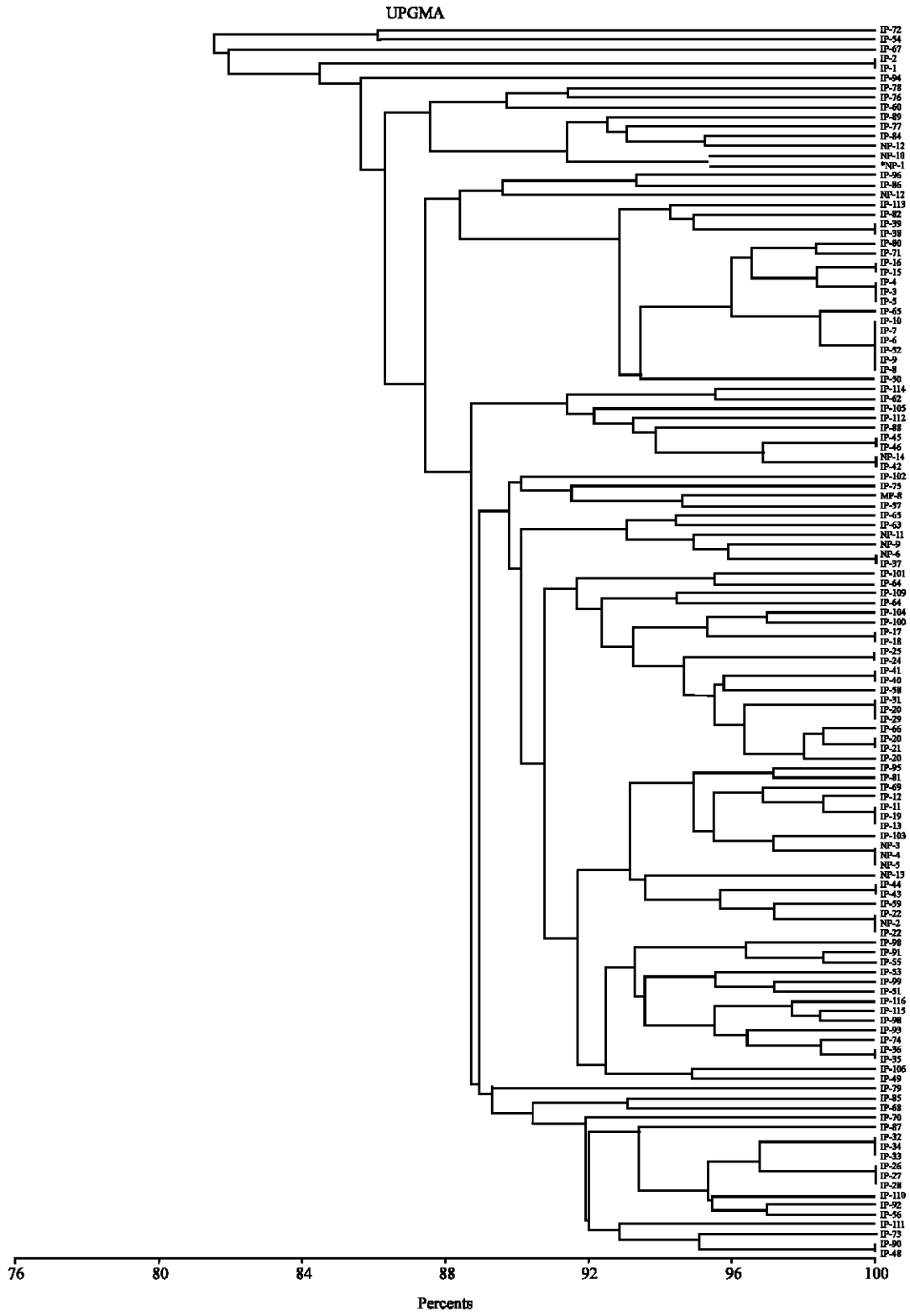

Fig. 1: Dendrogram of isolates in this study. It revealed twenty one clusters and similarity of our isolates

DISCUSSION

MIRU-VNTR is a PCR-based method for typing *M. tuberculosis* isolates that require amplification followed by size analysis of 12 independent loci; it can be highly effective in detecting the sources of infection and providing data for improving the control programs of tuberculosis. In this study we used MIRU-VNTR to investigate tuberculosis transmission in East Azarbaijan province of Iran. From one hundred and twenty seven isolates, fifty five isolates (43.3%) included in one of twenty one clusters, which show high transmission of tuberculosis in our region. But it was lower than Singapore with 55.3% (Sola *et al*., 2003) and Spain with 71% (de Viedgma *et al*., 2005) also the minimum estimate of transmission of tuberculosis in East Azarbaijan by MIRU-VNTR was 26.8% which was higher than San Francisco with 24% (Borgdorff *et al*., 2000) or London with 14.4% (Magurie *et al*., 2002) but it was lower than South Africa with 50% (Godfrey-Fausset *et al*., 2000). This rate of clustering among *M. tuberculosis* strains, suggesting that the majority of the tuberculosis cases in East Azarbaijan are due to reactivation.

It is known that in mixed populations the degree of DNA polymorphism is greater (van Embden *et al*., 1993). Although immigration from other parts of the country is almost in existent in East Azarbaijan. There is considerable number of patients from Nakhichevan (during our study, 11% of initial strains were isolated from nakhichevanees patients). In addition, the percentage of infection in Nakhichevan and Republic of Azerbaijan is high (WHO, 2006; Pfyffer *et al*., 2001). We often face with traveling from this region and in this study fourteen isolates that were affected by tuberculosis were from Nakhichevan. Six Nakhichevanees patients (42.85%) included in one of the four clusters, which one of the clusters composed three nakhichevanees patients that show internal transmission of tuberculosis between them but in next three clusters we had Nakhichevanees patients and Iranian patients with the same MIRU-VNTR pattern. Therefore, we can conclude these Nakhichevanees patients were the source of spread infection in East Azarbaijan by traveling and residing in this region, similar transmission introduced previously in San Francisco (Casperm *et al*., 1996), Denmark (Troels *et al*., 2001) and Spain (de Viedgma *et al*., 2005). In the other hand, multidrug resistance in Republic of Azerbaijan strains is high (Pffyfer *et al*., 2001). Treatment of tuberculosis in Iran is entirely free, so these patients refer to our province for treatment and it cause new transmission and more expenses for country. Furthermore it should be noted that the study was performed during a period of six month only and because of the characteristics of disease transmission and development a study using a large time frame should be performed to evaluate the real clustering rate.

In a study on tuberculosis transmission in Paris, 70% of the patients were male sex and male sex was a risk factor for clustering (Gutierrez *et al*., 1998). In agreement with mentioned study, in our study, however not significant, prevalence of tuberculosis was higher for male (58.27%) than female (41.73%). In our study, no difference was observed between the younger or older age categories; unemployment and generalized poor living condition could be the reason for this.

In our study allelic diversity of the 12 MIRU-VNTR was low, especially in loci 2, 4, 10, 20, 27 and 39. Low diversity of MIRU-VNTR loci apparently allow to the available data for a philogenetic analysis (Mokrousov *et al*., 2004). The allelic diversity of our samples was lower than previous studies (Sun *et al*., 2004; Sola *et al*., 2003).

The result of this study showed that sanitation of tuberculosis in East Azarbaijan is relatively in good condition and both of reactivation of latent infection and transmission of infection are effective in prevalence of tuberculosis in our province. In order to prevent transmission of tuberculosis from Nakhichevan, new tuberculosis centers should be establish near the border to prohibit traveling of Nakhichevanees patients and long staying of them.

In conclusion MIRU-VNTR as a simple, automation, a short turnaround time, high throughput, perfect and reproducible technique, can be use for study manner of transmission and epidemiological links in regional studies and patterns allow comparing results with other laboratories as a global investigation. MIRU-VNTR typing will be likely prevailing as a first line method for genotyping of *M. tuberculosis*.

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