Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 4:198-202

Comparative efficacy of MIRU-VNTR and IS 6110-RFLP for differentiation of *Mycobacterium tuberculosis* isolates

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Abstract

The aim of this study was To characterize Mycobacterium tuberculosis isolates by MIRU-VNTR typing and IS 6110 – RFLP and to compare the efficiency of MIRU-VNTR typing and IS 6110 – RFLP in discriminating Mycobacterium tuberculosis isolates. In Mycobacterium genome a large number of insertional and repetitive elements have been identified. In the genome of Mycobacterium tuberculosis insertation sequence IS 6110 is present.. In Mycobacterium tuberculosis, 41 mini satellites like structure have been identified. They are specific class of variable number of tandem repeats at different loci varies between strain. MIRU-VNTR, is a widely accepted technique for strain typing of Mycobacterium tuberculosis. Analysis of fingerprinting of M.tuberculosis strains showed the presence of both low (5%) and multiple (95%) IS6110 copy number strains. After genotyping the same isolates by MIRU-VNTR typing it is showed that MIRU-VNTR typing is more sensitive than IS 6110-RFLP. As MIRU-VNTR typing is less time taking, less DNA are required and Bands obtained are clear so easy to interpretate.

Key Words: MIRU-VNTR, IS 6110, *Mycobacterium tuberculosis*

INTRODUCTION

The famous German microbiologist Robert Koch isolated and described the causative agent of tuberculosis, *Mycobacterium tuberculosis* in 1882. At one time tuberculosis was the single most important infectious disease of humans and amounted for one seventh of all deaths worldwide. Even today tuberculosis still accounts for almost 3 million deaths per year, more than 5% of all deaths and up to one third of the world's populations have been infected with *M.tuberculosis.*

IS 6110 RFLP [1] is current gold standard method for *Mycobacterium tuberculosis* typing and is extensively used for epidemiological and population based studies [2].

There are several PCR based methods used in genotyping are RAPD [3,4] AFLP [5,6], DR based method [7], VNTR typing [8], MIRU-VNTR typing [9], Spoligotyping [10].

Mycobacterium interspersed repetitive units (MIRUs) and VNTR sequences are scattered throughout the *Mycobacterium tuberculosis* genome. 12 out of 41 MIRU loci present in the *Mycobacterium tuberculosis* H_{37} Rv genome correspond to human mini satellite like VNTR region among non-related isolates of different geographical origin [11]. A PCR based typing method by using these 12 loci provides a resolution comparable to that of *IS 6110-RFLP*.

The complementarily of information about genetic relationship inferred from MIRU-VNTR and *IS 6110-RFLP* typing would be highly significant for epidemiological study of tuberculosis.This study has been undertaken to

genotype *Mycobacterium tuberculosis* isolates of Fatehabad area of Agra district based on MIRU-VNTR typing and *IS 6110* – RFLP.

MATERIALS AND METHODS

Mycobacterium tuberculosis isolates were taken from the Repository maintained by NJIL & OMD Agra, Isolation of DNA was done by a method adopted by van Soolingen [12]. Probe was prepared by PCR amplification of 245 bp IS6110 fragment using INS1 (5' CGT GAG GGC ATC GAG GTG GC-3') and INS2 (5'GCG TAG GCG TCG GTG ACA AA-3') from standard *M.tuberculosis* (H37Rv). Probe was labeled with a non-radioactive substance digoxigenin (DIG) by the random primed DNA labeling technique using DIG DNA labeling and detection kit.

Restriction of the DNA samples was done using the restriction enzyme PvuII. PvuII cut *M.tuberculosis* genome at a specific site with insertion sequence 5'-CAG/CTG-3'3'-GTC/GAC-5'.

For MIRU-VNTR typing 20 isolates was amplified for12 MIRU loci (2,4,10,16,20,23,24,26 27,31,39,40) will be amplified individually with primers specific for sequences flanking the units . The reaction mixture will be prepared by using the Hot Star Taq DNA Polymerase kit. The PCR product was analyzed by agarose gel(2%) electrophorosis . The size of amplicons was estimated by compararision with ladder and number of repeats was calculated with the help of gel documentation system.

RESULTS

In India very less work has been done so far targeting MIRU-VNTR loci. All the study that was done earlier show that there were no clustering present among the MIRU loci . Present study show that there were clustering case present among two isolates that means the patients to whom these two isolates belongs were infected with same strains of *Mycobacterium tuberculosis* or there is cross contamination present among these two isolates .All the MIRU-VNTR genotyping repeats are analyzed by Hunter Gaston Index that show the most discriminatory locus among the 12 MIRU loci.

Table 13. MIRU-VNTR genotypes of *M.tuberculosis* isolates

S.no	Isolates	MIRU 2	MIRU 4	MIRU 10	MIRU 16	MIRU 20	MIRU 23	MIRU 24	MIRU 26	MIRU 27	MIRU 31	MIRU 39	MIRU 40
1	T-1	2	5	4	4	1	7	2	2	3	4	1	3
2	T-2	2	2	5	5	2	4	1	4	3	4	2	3
3	T-3	2	2	6	6	2	4	1	6	3	4	2	3
4	T-4	2	2	7	7	1	4	1	6	3	4	3	3
5	T-5	2	2	3	3	2	4	1	6	3	4	3	3
6	T-6	2	6	3	3	2	5	2	1	3	5	3	4
7	T-7	2	2	1	1	2	4	1	4	3	2	2	3
8	**T-8**	**2**	**2**	**5**	**5**	**2**	**4**	**1**	**6**	**3**	**4**	**2**	**3**
9	**T-9**	**2**	**2**	**5**	**5**	**2**	**4**	**1**	**6**	**3**	**4**	**2**	**3**
10	T-10	2	2	1	1	2	4	1	4	3	1	1	3
11	T-11	2	2	5	5	2	1	1	5	3	5	3	2
12	T-12	2	2	5	5	2	4	1	3	3	5	3	3
13	T-13	1	2	3	3	2	5	2	5	3	2	2	3
14	T-14	2	5	6	6	2	5	1	2	3	6	2	2
15	T-15	2	1	6	6	2	5	1	4	2	5	4	2
16	T-16	2	2	5	5	2	5	1	8	2	5	4	2
17	T-17	2	2	4	4	2	5	1	5	2	5	2	2
18	T-18	2	2	3	3	2	4	1	7	2	5	2	2
19	T-19	2	2	3	3	2	4	1	7	2	5	2	2
20	T-20	2	2	4	4	2	4	1	6	2	5	3	2
21	$H_{37}R_V$												
22	Negative												

IS 6110-RFLP Genotyping:
The same 20 isolates are selected as analyzed by MIRU-VNTR were also analyzed by *IS 6110-RFLP* genotyping. At one time 12 isolates are taken for this experiment, so two experiment was done for 20 isolates. After southern transfer pattern of *IS 6110-RFLP* was obtained . The number of *IS 6110* copies per isolate varies from 0-15 bands.
The result of *IS 6110-RFLP* of *Mycobacterium tuberculosis* isolates can be classified into two group-

A Group - with low copies of IS 6110 having 1-4 bands.
B Group - with high copies of IS 6110 having 5-15 bands.

Percentage of different isolates classified according to IS 6110 copy number.

S.No.	*IS 6110* Group	No.of Isolates	Percentage
1.	Group A(1-4)	1	5%
2.	Group B(5-17)	19	95%

Out of 20 isolates only one isolate belong to low copy number group rest of 19 isolates belong to high copy number group. So 5% isolate belong to Group A and 95% isolate belong to Group B.

As it was analysed by MIRU-VNTR that two isolates show the clustering , the same two isolates also show the clustering in *IS6110-RFLP* pattern also.

All the previous study that was done by *IS6110-RFLP* in North Indian *M.tuberculosis* isolates show the prevalence of High copy number pattern of *IS6110-RFLP* . This present study also supports the previous result that North Indian *M.Tuberculosis* isolates belong to low copy number *IS6110-RFLP* pattern and these isolates known as the Delhi type strains.

Table 2.Distribution of *IS 6110* copies in isolates

S.No.	Isolates	Copy Number
1.	T-1	7
2.	T-2	10
3.	T-3	6
4.	T-4	12
5.	T-5	10
6.	T-6	12
7.	T-7	5
8.	**T-8**	10
9.	**T-9**	9
10.	T-10	6
11.	T-11	11
12.	T-12	7
13.	T-13	12
14.	T-14	8
15.	T-15	9
16.	T-16	11
17.	T-17	1
18.	T-18	7
19.	T-19	12
20	T-20	10

DISCUSSION

Although the number of copies of *IS6110* can range 0-19, population based molecular epidemiological studies report that most strains contain 8-18 copies, a number sufficient to enable discrimination between the majorities of strains[13] . In the present study *IS6110* DNA fingerprinting and its comparison with MIRU-VNTR typing was done Out of 20 *M.tuberculosis* different hybridization patterns were obtained suggesting differences in copy number and genomic location of element. Results show that maximum number of isolates having multiple *IS6110* copies along with few copies in less number. Two subpopulations of *M.tuberculosis* were found,the first subpopulation is defined by strains that have multiple *IS6110* copies (more than 5), the second is defined by strains that have low copy numbers i.e. less than 5.The third subpopulation of zero copy number was not found in our studies, as reported in previous studies from north India. Our observations are in concordance with studies from North Indian isolates, which also revealed high percentage of multiple copies [14,15,16].

In India very less work have been done so far based on MIRU-VNTR genotyping so information is lacking in Indian setting .By the help of MIRU-VNTR typing 20 MIRU-VNTR profiles were obtained Among these isolates clustering was present among two strains (T-8 and T-9) Hunter Gaston Index was calculated for each of these 12

loci and it was found to be 0.100, 0.363, 0.811, 0.668, 0.189, 0.590, 0.268, 0.863, 0.442, 0.694, 0.694 and 0.563 for locus 2,4,10,16,20,23,24,26,27,31,39 and 40 respectevily .It showed that MIRU loci 26 was most discriminatory locus and MIRU locus 2 was least discriminatiry . In case where MIRU-VNTR profile were identical another genotyping method can be used to confirm the clustering , as in this study IS6110 RFLP was taken and they were found to be similar with this system also.This two step strategy would be expected to increase the accuracy of outbreak investigations and to considerably accelerate epidemiological studies of *Mycobacterium tuberculosis* in defined geographical setting of Northern India .It would be importent to generate the result for a diffrent group and also by analysis of a significant number of isolates.

Among the 20 isolates , two isolates showed the clustering .These two isolates show similar pattern by *IS 6110* RFLP also.

CONCLUSION

Analysis of fingerprinting of *M.tuberculosis* strains showed the presence of both low (5%) and multiple (95%) *IS6110* copy number strains. After genotyping the same isolates by MIRU-VNTR typing it is showed that MIRU-VNTR typing is more sensitive than *IS 6110-RFLP*. As MIRU-VNTR typing is less time taking , less DNA are required and Bands obtained are clear so easy to interpretate.

To investigate molecular epidemiology among the isolates from Agra rural, a well planned prospective study needs to be carried out on a large number of samples for drawing firm conclusion of usefulness of the typing method.

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