MAJOR ARTICLE

Impact of HIV Infection on the Recurrence of Tuberculosis in South India

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(See the editorial commentary by Chaisson and Churchyard, on pages 653–655, and the article by Glynn et al., on pages 704–711.)

Background. There is limited information on the relative proportion of reactivation and reinfection at the time of recurrence among human immunodeficiency virus (HIV)–infected and HIV-uninfected patients who are successfully treated for tuberculosis infection in India.

Methods. HIV-infected and HIV-uninfected patients with sputum culture–positive pulmonary tuberculosis were treated with short-course regimens and followed up for 36 months at the Tuberculosis Research Centre, South India. Bacteriologic recurrences were documented, and typing of strains was performed using 3 different genotypic techniques: restriction fragment length polymorphism (RFLP) by IS6110, spoligotyping, and mycobacterial interspersed repeat unit (MIRU)–variable number tandem repeat (VNTR). DNA fingerprints of paired *Mycobacterium tuberculosis* isolates (baseline and recurrence) were compared.

Results. Among 44 HIV-infected and 30 HIV-uninfected patients with recurrent tuberculosis during the period July 1999 to October 2005, 25 and 23 paired isolates, respectively, were typed using all 3 methods. Recurrence was due to exogenous reinfection in 88% of HIV-infected and 9% of HIV-uninfected patients ($P < .05$). Among recurrent isolates, the HIV-infected patients showed more clustering, as well as a higher proportion of drug resistance, including multidrug resistance.

Conclusions. In India, a tuberculosis-endemic country, most recurrences after successful treatment of tuberculosis are due to exogenous reinfection in HIV-infected persons and endogenous reactivation in HIV-uninfected persons. Strategies for prevention and treatment of tuberculosis infection must take these findings into consideration.

The human immunodeficiency virus (HIV) pandemic has increased the burden of tuberculosis, especially in populations where HIV infection is common and where the prevalence of tuberculosis was already high [1, 2]. The worldwide estimate is that nearly 2 billion people are infected with *Mycobacterium tuberculosis*, 33 million are infected with HIV, and 12 million are dually infected [3]. According to the World Health Organization (WHO) 2008 global report on tuberculosis, 700,000 of the 9.2 million new tuberculosis cases reported in 2006 occurred among HIV-infected people [4]. HIV has an effect on the incidence, prevalence, clinical presentation, diagnostic yield, and treatment options for patients with tuberculosis. The developing world has the highest burden of both diseases [5].

India has 20% of the global burden of tuberculosis infections, with 1.8 million new cases each year, as well as a large pool (~2.5 million) of HIV-infected individuals [4, 5]. This sets the stage for a deadly synergy between the 2 infections. The risk of developing tuberculosis disease for HIV-infected patients in India is estimated to be 7 cases per 100 person-years at risk, compared with the 10% lifetime risk of an immunocompetent host [6]. Even though the initial bacteriologic response to antituberculosis therapy is similar in both HIV-infected and HIV-uninfected patients, recurrences of tuberculosis infection are more frequent

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among coinfected patients, especially in countries with high rates of tuberculosis infection [7]. Recurrences of tuberculosis could be due to either exogenous reinfection or endogenous reactivation, and the 2 can be differentiated by molecular typing. For decades, the issue of the role played by exogenous reinfection versus endogenous reactivation has been debated [8, 9]. We have earlier shown that endogenous reactivation predominates in patients with tuberculosis not infected with HIV [10]. However, there are no data with regard to HIV-coinfected patients with tuberculosis from India and limited data from other parts of the world [11, 12]. It is likely that the presence of HIV in communities could modify the pattern of tuberculosis transmission.

There are several genotyping tools, including restriction fragment length polymorphism (RFLP) typing, spoligotyping, and mycobacterial interspersed repeat unit (MIRU)–variable number tandem repeat (VNTR) typing that are used to differentiate tuberculosis strains. Each of these techniques has its own advantages and disadvantages. It is preferable to use more than 1 method to accurately assess the source of the tuberculosis strain that is infecting patients. In the present study, we compared the relative proportions of reinfection or reactivation among recurrence isolates of *M. tuberculosis* from HIV-infected and HIV-uninfected patients enrolled in clinical trials and followed up at the Tuberculosis Research Centre (TRC), Chennai, India. All patients were well characterized at baseline, treated under similar conditions with short-course regimens, and followed up regularly to document treatment outcomes. Our aims were to study the role of HIV infection in tuberculosis transmission in a tuberculosis-endemic setting and also the relative performance of the 3 genotypic methods in classifying strains.

METHODS

Study population and setting. The study was conducted at the TRC's clinics in Chennai and Madurai, South India, from July 1999 to October 2005. The Institutional Ethics committee had approved the clinical trials, and all patients provided written informed consent. Patients were evaluated for the presence of HIV and tuberculosis, as well as their suitability for clinical trials. A standardized questionnaire was administered by a physician, clinical examination was performed, and a chest radiograph was taken. HIV infection was diagnosed on the basis of 3 tests: samples found positive using the TRIDOT rapid test (J Mitra) were next tested with CombAids (Span Diagnostics, India), followed by an enzyme-linked immunosorbent assay (Lab Systems, India). Tuberculosis was suspected on the basis of clinical and radiographic features, and diagnosis was confirmed by mycobacterial culture.

Patients were recruited for clinical trials if they fulfilled the clinical and laboratory eligibility criteria. HIV-infected patients included in this analysis were from a pilot study of 6-month short-course treatment and a randomized clinical trial comparing 6 and 9 months of treatment (NCT 00376012). Patients received a standard short-course antituberculosis regimen consisting of ethambutol (1200 mg), isoniazid (600 mg), rifampin (450 or 600 mg for patients weighing <60 or >60 kg, respectively), and pyrazinamide (1500 mg) thrice-weekly for the initial 2 months (intensive phase, $2EHRZ_3$), followed by rifampin and isoniazid thrice-weekly for the next 4 or 7 months (continuation phase, $4/7RH_3$) [13]. All doses in the intensive phase and 1 dose per week in the continuation phase were directly observed. Surprise pill counts at home, urine tested for acetylisoniazid, and return of empty pill covers were done to check adherence. Patients included in the efficacy analysis had adherence >80%, and most of the bacteriologic recurrences came from this group. Patients did not have access to antiretroviral treatment during the course of this study.

Of the 25 HIV-negative patients with recurrent disease in the study, 12 had been treated with the same short-course regimen ($2EHRZ_3/4RH_3$) at the same dosages, whereas 11 were treated with regimens containing ofloxacin (3 months of daily isoniazid [300 mg], rifampin [450 or 600 mg, depending on the patient's weight], pyrazinamide [1500 mg], and ofloxacin [400 mg], followed by 1 or 2 months of thrice-weekly isoniazid and rifampin), as they were enrolled in a trial assessing the efficacy of fluoroquinolones [14]. Patients were reviewed clinically and with sputum examination every month up to 24 months and every 3 months thereafter, until 36 months from treatment initiation.

Bacteriologic methods. Three sputum samples were collected at baseline and every month during treatment, and 2 sputum samples were collected every month during the follow-up period. Sputum smears were examined for acid-fast bacillus (AFB) by fluorescence microscopy before being processed by the modified Petroff method and cultured on Lowenstein-Jensen medium for 8 weeks. Species identification was performed using standard biochemical tests and drug susceptibility tests by standard methods at the TRC, a WHO supranational reference laboratory for mycobacteria [15].

Molecular typing methods. Genomic DNA was extracted from *M. tuberculosis* cultures by standard cetyltrimethyl ammonium bromide (CTAB)–NaCl extraction method [16]. IS6110 DNA fingerprinting was done according to internationally accepted guidelines [17]. Spoligotyping was performed using standard methods [18]. In brief, the spacers between the direct repeats in the target region were amplified by using a primer set, primer Dra 5′-CC AAG AGG GGA CGG AAA C-3′ and biotinylated primer Drb 5′-GGT TTT GGG TCT GAC GAC-3′. The polymerase chain reaction (PCR) products were then hybridized to a Biodyne C membrane (Isogen Bioscience). This membrane contains immobilized synthetic oligomeric spacer sequences derived from the direct-repeat region of *M.*

tuberculosis $H_{37}Rv$ and *Mycobacterium bovis* BCG. Hybridized DNA was detected using an enhanced chemiluminescence kit (Amersham International), with exposure to X-ray film producing a pattern or profile reminiscent of a bar code.

MIRU-VNTR genotyping was performed by using multiplex PCR, X-Rhomadine-labeled MapMarker 1000, standard size (BioVentures), and ABI 3100 and ABI 3730-XL sequencers (Applied Biosystems). Sizing of the PCR fragments and assignment of the various VNTR alleles were performed by using customized GeneScan and Genotyper (version 2.0) or Genemapper software packages (PE Applied Biosystems). All results were entered into Microsoft Excel (version 2003) in a digital format [19]. Patterns obtained by the different methods were analyzed using Bionumerics software (version 5.0; Applied Maths). Separate databases were created for both HIV-infected and HIV-uninfected patients with tuberculosis, and RFLP images were scanned and loaded as the primary genotyping method. Spoligotyping and MIRU data were loaded into these databases as a character type in numerical code. These data were linked to the respective sample number so that for a single sample all 3 typing data were available for comparison. If the fingerprint patterns were identical (number and position of bands, spots, and peaks) by all 3 methods or differed by 1 band, spot, or peak, the recurrence was classified as reactivation, whereas if the patterns differed by 2 or more bands, spots, or peaks by any of the methods, the recurrence was classified as reinfection. Clustering analysis was done by similarity matrix using Jaccard coefficient, and dendrograms were drawn by unweighted pair group method using arithmetic averages (UPGMA) using Bionumerics software (Applied Maths).

Statistical Analysis. We used χ^2 test with Yates continuity correction or Fisher exact test as appropriate to compare proportions. A P value of $<.05$ was regarded as significant.

RESULTS

In total, 387 HIV-infected patients with culture-confirmed pulmonary tuberculosis were treated, and 306 (79%) were declared cured (all sputum specimen results were negative during the last 2 months of treatment). The mean age of the patients was 34 years; mean weight was 44 kg, 77% were men, and the median CD4 count was 160 cells/μL. Of the 44 (14.4%) documented recurrences, results from all 3 genotyping methods for both time points were available for 25 patients. We compared the demographic characteristics of patients whose isolates were not typed ($n = 14$) with those patients whose isolates were typed ($n = 15$). Patients were similar in terms of age (mean, 34 and 33 years, respectively), sex distribution, weight (mean, 42.6 and 44 kg, respectively), socioeconomic status, and median viral load (257,844 and 209,227 copies/mL, respectively). Mean CD4 cell count was 220 cells/μL in patients whose isolates were typed versus 165 cells/μL in those whose isolates were not typed.

Figure 1 is a schematic representation of patients enrolled in HIV clinical studies and followed up, for whom baseline *M. tuberculosis* isolates were available.

During the same period, a large number of HIV-uninfected patients with pulmonary tuberculosis were treated at the TRC. Genotypic results of paired isolates using all 3 methods were available for 23 patients; their mean age was 37 years, mean weight was 42.1 kg, and 79% were men. Tables 1 and 2 show the genotyping results and drug susceptibility pattern of all patients using the 3 different methods. We found that in 22 (88%) of 25 HIV-infected patients, the recurrence was due to a new strain of tuberculosis, whereas in 21 (91%) of 23 HIV-uninfected patients recurrence was due to the same strain ($P < .05$). To rule out cross-contamination as a reason for apparent exogenous reinfection, we examined serial isolates from several patients at and after recurrence and looked for consistency of results. The number of serial isolates obtained from patients ranged from 2 to 8, and all of them were concordant with their respective primary isolates. Among recurrences, 10 HIV-infected and 5 HIV-uninfected patients had isolates resistant to 1 or more drugs; 6 HIV-infected and 2 HIV-uninfected patients had isolates that were multidrug resistant. Figure 2 shows the timing of recurrence, by type and HIV status. Although most reactivation of disease tended to occur within 12 months of completion of treatment, at least half of all reinfections occurred after 12 months.

IS6110 RFLP data revealed that both groups of patients had a high proportion (45% and 47%, respectively) of IS6110 single copy *M. tuberculosis* isolates. There was no significant difference in the distribution of IS6110 types between the 2 groups. Eleven percent of HIV-infected and 20% HIV-uninfected patients with tuberculosis were infected with 2–5-copy strains, although no-

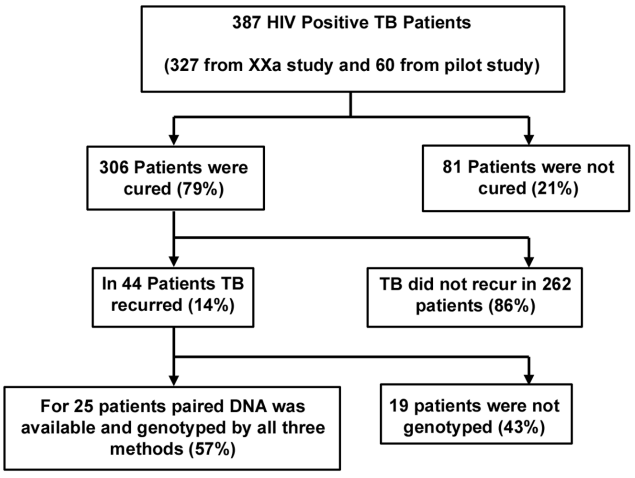

Figure 1. Profile of patients enrolled in human immunodeficiency virus (HIV) clinical trials and followed up, for whom baseline *Mycobacterium tuberculosis* isolates were available.

Table 1. Genotyping of Paired Tuberculosis Isolates from Human Immunodeficiency Virus (HIV)–Positive Patients

Patient	Sex	Age	RFLP	Month	Exogenous or Endogenous	Spoligotyping	Spoligo	ST	MIRU	SHRE
1	M	27	2J	0	Exo	474377777413771	EAI5	340	8 2 3 4 3 5 2 4 6 1 4 1 3 10 6	SSSS
			6AB	7		477777777413071	EAI3_IND	11	5 2 4 4 3 3 2 4 6 1 4 1 3 6 6	SSSS
2	M	33	1B	0	Exo	777777777003731	EAI5		5 2 3 2 3 4 2 2 6 0 0 0 5 12 4	SSSS
			3F	20		477777777413071	EAI3_IND	11	5 2 4 4 3 4 2 4 6 1 4 1 3 6 2	SSSS
3	M	43	1A	0	Exo	477777777413071	EAI3_IND	11	2 4 5 4 4 3 3 2 6 1 2 5 4 0 1	SSSS
			1A	8		477777777413771	EAI3	126	2 4 5 4 4 3 6 0 0 1 2 5 4 0 1	SSSS
4	M	30	11R	0	Exo	577777774000771	ORPHAN		2 5 3 5 3 3 2 3 3 4 3 3 7 3 5	SSSS
			1A	41		474377777413771	EAI5	340	0 2 3 4 3 5 2 3 6 1 4 1 3 7 5	SSSS
5	M	27	11S	0	Exo	000000000003771	Beijing	1	2 6 3 3 1 4 3 3 4 4 3 4 7 4 9	SSSS
			11AO	18		777777777700771	U	124	2 5 3 5 3 3 2 3 3 4 3 3 7 3 5	SSSS
6	M	63	1A	0	Exo	401777777413071	EAI3_IND	473	5 2 4 4 3 4 2 4 6 1 10 1 3 6 4	SSSS
			3A	20		473777777413771	EAI5	340	5 2 2 4 3 5 2 4 6 1 4 1 3 6 2	SSSS
7	F	25	1A	0	Exo	477777777413071	EAI3_IND	11	5 2 4 4 3 4 2 4 6 1 4 1 3 6 2	SRSS
			1A	14		477777777413071	EAI3_IND	11	5 2 4 4 3 5 2 4 6 1 2 1 3 6 5	SSSS
8	M	30	1A	0	Exo	477777777413071	EAI3_IND	11	5 2 4 4 3 5 2 4 6 1 2 1 3 7 6	SSSS
			3AI	35		777777777413700	EAI5	138	5 2 0 6 2 3 2 2 5 2 5 1 7 7 5	SSSS
9	F	30	1A	0	Endo	477777777413071	EAI3_IND	11	7 1 5 4 3 4 2 4 6 1 4 1 3 7 7	SSSS
			1A	12		477777777413071	EAI3_IND	11	7 2 5 4 3 4 2 4 6 1 4 1 3 7 7	SSSS
10	F	27	3G	0	Exo	477777777413071	EAI3_IND	11	5 2 4 4 3 4 2 4 6 1 4 1 2 6 4	SSSS
			1A	24		477777777413071	EAI3_IND	11	2 1 2 5 1 3 1 2 3 4 3 1 2 3 8	SSSS
11	M	37	14J	0	Exo	703777740003771	CAS1_DELHI	26	2 7 3 6 4 5 4 2 4 2 2 1 2 4 8	SSSS
			3X	9		403777760003771	ORPHAN		8 2 3 4 3 5 2 3 6 1 4 1 3 7 5	SSSS
12	M	25	1A	0	Endo	477777777413771	EAI 5	126	5 2 2 4 3 5 2 4 6 1 4 1 3 6 3	SSSS
			1A	7		477777777413771	EAI 5	126	5 2 2 4 3 5 2 4 6 1 4 1 3 6 2	SSSS
13	M	40	6AB	0	Exo	475773777413071	ORPHAN		4 2 4 4 3 4 2 3 6 1 4 1 2 7 5	SSSS
			6AB	17		477777777413071	EAI3_IND	11	4 2 4 4 3 4 2 3 6 1 4 1 2 7 5	SSSS
14	M	29	1E	0	Exo	777777737760731	ORPHAN		2 1 2 5 1 3 3 4 3 4 3 3 2 4 0	SSSS
			15K	37		000000000003771	Beijing	1	2 0 0 3 3 5 4 4 4 4 5 2 6 6 8	RRRS

Table 1. ***(Continued.)***

Patient	Sex	Age	RFLP	Month	Exogenous or Endogenous	Spoligotyping	Spoligo	ST	MIRU	SHRE
15	M	40	12AF	0	Exo	703777740003011	ORPHAN		2 7 3 6 5 5 4 2 4 2 2 2 2 3 8	SSSS
			1A	8		777777777700771	U	124	0 2 4 4 3 4 2 4 4 1 3 1 3 4 6	SRSS
16	M	32	1A	0	Exo	477777777413071	EAI3_IND	11	2 4 5 4 4 3 4 2 4 1 2 4 4 7 1	SSSS
			1A	15		477777777413771	EAI 5	126	2 4 4 4 4 3 4 3 6 1 2 6 4 6 1	SRRR
17	M	45	9W	0	Exo	777777663560771	T1	573	3 4 2 2 4 3 3 2 1 1 5 2 2 0 2	RSSS
			10S	20		777777607760771	LAM9	42	5 4 2 1 4 2 3 3 1 1 5 3 2 4 2	SRRR
18	M	36	1A	0	Exo	401367777413071	ORPHAN		2 2 4 4 3 4 2 4 6 1 4 1 3 6 4	SSRS
			1A	17		401777777413071	EAI3_IND	473	5 2 4 4 3 4 2 4 6 1 4 1 3 6 4	SSRS
19	M	33	6T	0	Exo	477777777413071	EAI3_IND	11	2 4 5 4 4 3 6 0 0 1 2 4 4 0 1	SSSS
			6T	20		477777777413071	EAI3_IND	11	2 4 5 4 4 3 0 2 0 1 2 4 4 0 1	SRSS
20	M	32	9X	0	Exo	777777774000771	ORPHAN		2 5 3 5 3 3 2 3 3 4 3 3 3 3 5	SSSS
			1A	9		777777777413771	EAI5	236	2 2 3 3 3 3 2 3 3 1 3 1 5 6 8	SSRS
21	M	27	1A	0	Exo	477777777413031	EAI5	355	2 4 2 4 4 3 3 3 6 1 2 4 4 4 1	SSSS
			10S	7		777777607760771	LAM9	42	5 4 2 1 4 2 3 3 1 1 5 3 2 0 2	SRRR
22	M	27	1A	0	Exo	477777777413031	EAI5	355	2 4 2 4 4 3 6 3 6 1 2 4 4 0 1	SRSR
			10S	17		777777607760771	LAM9	42	5 4 2 1 4 2 3 2 1 1 5 3 2 0 2	SRRR
23	F	21	1A	0	Endo	477777777413071	EAI3_IND	11	5 2 3 4 3 4 2 4 6 1 4 1 3 6 6	SSSS
			1A	24		477777777413071	EAI3_IND	11	5 2 4 4 3 4 2 4 6 1 4 1 3 6 6	SSSS
24	M	27	3V	0	Exo	477777777413001	ORPHAN		2 4 5 4 4 3 6 2 0 1 2 4 5 0 1	SSSS
			7X	16		477777777413071	EAI3_IND	11	2 4 5 4 4 3 3 3 1 1 2 4 4 0 1	SRRS
25	F	38	14G	0	Exo	703777740000171	CAS1_DELHI	1789	2 7 3 6 5 5 2 2 4 2 3 2 2 5 6	SSSS
			10V	24		777777774020771	H1	47	2 5 4 5 3 3 2 3 3 4 3 3 3 3 7	SSSS

NOTE. MIRU, mycobacterial interspersed repeat unit; RFLP, restriction fragment length polymorphism; SHRE, streptomycin, isoniazid, rifampin, and ethambutol; ST, spoligotype.

copy strains were rare (Figure 3). Figure 4 shows the spoligotyping data of isolates from HIV-infected and HIV-uninfected patients with tuberculosis at baseline and recurrence. *M. tuberculosis* isolates of EAI3 clade were more common among HIV-infected patients (49%) than HIV-uninfected patients with tuberculosis (38%) ($P < .05$), whereas the EAI5 clade was more common among HIV-uninfected patients (26% vs 18%; $P <$ .05). The Beijing genotype was present in 4% of HIV-infected patients and none of the HIV-uninfected patients.

There were 6 MIRU clusters of 2 isolates each, which were differentiated by spoligotyping or IS6110 RFLP (data not shown). Table 3 shows 4 spoligotyping and IS6110 clusters ranging from 2 to 10 isolates, which were differentiated by MIRU. Tables 4 and 5 show the clustering of strains among

Table 2. Genotyping of Paired Tuberculosis Isolates from HIV-Negative Patients with Tuberculosis

Patient	Sex	Age	RFLP	Month	Exogenous or Endogenous	Spoligotyping	Spoligo	ST	MIRU	SHRE
1	M	45	1A	0	Exo	477774377413771	ORPHAN		2 4 5 4 4 3 7 3 2 1 2 4 4 7 1	SRSS
			1A	24		477743774413071	ORPHAN		2 4 5 4 4 3 7 3 6 1 2 4 4 7 1	RRSR
2	F	23	1A	0	Endo	777777777413731	EAI1_SOM	48	2 2 5 3 4 3 9 6 9 2 2 5 5 4 1	RRSS
			1A	24		777777777413731	EAI1_SOM	48	2 2 5 3 4 3 9 6 9 2 2 5 5 4 1	RRRS
3	M	65	2G	0	Exo	477777777413771	EAI5	126	2 4 5 2 4 3 6 3 6 1 2 5 4 4 1	SSSS
			4E	24		700176777760671	ORPHAN		4 3 2 4 4 3 3 3 3 4 5 3 3 8 1	SRSS
4	M	51	2H	0	Endo	777777777760600	T1	243	4 4 2 4 4 3 2 3 3 4 5 3 3 8 3	SSSS
			2H	18		777776777760600	ORPHAN		4 4 2 4 4 3 2 3 3 4 5 3 3 8 3	SSSS
5	M	56	1A	0	Endo	467777777413071	ORPHAN		2 5 5 0 4 3 6 3 6 1 2 5 4 4 1	SSSS
			1A	18		477777777413071	EAI3_IND	11	2 5 5 0 4 3 6 3 6 1 2 5 4 4 1	SSSS
6	M	51	1A	0	Endo	467777757413071	ORPHAN		2 4 7 4 4 3 6 3 6 1 2 4 4 4 1	SSSS
			1A	18		467777757413071	ORPHAN		2 4 7 4 4 3 6 3 6 1 2 4 4 4 1	SSSS
7	M	50	1A	0	Endo	476377777413771	ORPHAN		2 3 5 3 4 3 7 3 6 1 2 5 4 5 1	SSSS
			1A	12		474377777413771	ORPHAN		2 3 5 3 4 3 7 3 6 1 2 5 4 5 1	SSSS
8	M	40	2C	0	Endo	477777777413071	EAI3_IND	11	2 4 2 4 4 3 7 3 6 1 2 4 4 3 1	SSSS
			2C	12		477777777413071	EAI3_IND	11	2 4 2 4 4 3 7 3 6 1 2 4 4 3 1	SSSS
9	M	53	1A	0	Endo	477777777413031	EAI5	355	2 4 5 4 4 3 5 3 4 1 2 4 4 6 1	SSSS
			1A	12		477777777413031	EAI5	355	2 4 5 4 4 3 5 3 4 1 2 4 4 6 1	SSSS
10	F	32	4B	0	Endo	477777777413071	EAI3_IND	11	2 4 5 4 4 3 6 3 5 1 2 4 4 6 1	RRSS
			4B	18		477777777413071	EAI3_IND	11	2 4 5 4 4 3 6 3 5 1 2 4 4 6 1	RRSS
11	M	60	2D	0	Endo	474377777413671	ORPHAN		2 4 5 3 4 3 10 3 6 1 2 5 4 6 1	SSSS
			2D	12		474377777413771	EAI5	340	2 4 5 3 4 3 10 3 6 1 2 5 4 6 1	SSSS
12	M	24	14K	0	Endo	703777740003771	CAS1_DELHI	26	4 2 2 4 6 4 4 2 4 4 7 4 6 7 4	SRSS
			14K	12		703777740003771	CAS1_DELHI	26	4 2 2 4 6 4 4 2 4 4 7 4 6 7 4	SRSS
13	M	30	1A	0	Endo	477777777413031	EAI 5	355	8 2 4 4 3 4 2 4 6 1 9 1 3 6 6	SSSS
			1A	7		477777777413071	EAI3_IND	11	8 2 4 4 3 4 2 4 6 1 9 1 3 6 6	SSSS
14	F	22	10F	0	Endo	700777740003771	CAS1_DELHI	26	2 5 3 6 3 5 4 2 4 2 3 3 2 4 8	SSSS
			10F	8		700377740003771	CAS1_DELHI	26	2 5 3 6 3 5 4 2 4 2 3 4 2 4 8	SSSS

Table 2. ***(Continued.)***

Patient	Sex	Age	RFLP	Month	Exogenous or Endogenous	Spoligotyping	Spoligo	ST	MIRU	SHRE
15	M	20	13AC	0	Endo	777777777760771	T1	53	2 5 1 3 1 3 2 4 3 2 4 2 3 2 5	SSSS
			13AC	7		777777777760771	T1	53	2 5 1 3 1 3 2 4 3 2 4 2 3 2 5	SSSS
16	M	55	1A	0	Endo	477777777413031	EAI 5	355	5 2 4 6 3 4 2 4 6 1 4 1 3 6 6	SSSS
			1A	15		477777777413031	EAI 5	355	5 2 4 6 3 4 2 4 6 1 4 1 3 6 6	RRSS
17	M	45	1A	0	Endo	474000377413031	ORPHAN		5 2 4 4 3 4 2 4 5 1 4 1 3 6 7	SSSS
			1A	10		474000377413031	ORPHAN		5 2 4 4 3 4 2 4 5 1 4 1 3 6 7	SSSS
18	F	32	1A	0	Endo	477777777413031	EAI 5	355	5 2 4 4 3 4 2 4 6 1 2 1 3 6 6	SSSS
			1A	9		477777777413031	EAI 5	355	5 2 4 4 3 4 2 4 6 1 2 1 3 6 6	SSSS
19	M	48	12AI	0	Endo	777777763560371	ORPHAN		2 5 2 4 3 2 3 5 2 1 2 2 2 3 6	SRSS
			12AI	7		777777663560371	ORPHAN		2 5 2 4 3 2 3 5 2 1 2 2 2 3 6	SRRS
20	M	18	1A	0	Endo	477777777403071	ORPHAN		5 2 4 4 3 4 2 4 6 1 4 1 3 6 6	SSSS
			1A	8		477777777413071	EAI3_IND	11	5 2 4 4 3 4 2 4 6 1 4 1 3 6 6	SSSS
21	M	44	11Y	8	Endo	777777770020771	H3	742	2 5 2 2 3 3 0 4 3 2 3 2 3 3 6	SSSS
			11Y	0		777777770020771	H3	742	2 5 4 2 3 3 0 4 3 2 3 2 3 3 6	SSSS
22	M	52	10AK	0	Endo	777777777700771	U	124	2 5 3 5 3 3 2 3 3 4 3 3 5 3 5	SSSS
			10AK	9		777777777700771	U	124	2 5 3 5 3 3 2 3 3 4 3 3 5 3 5	SSSS
23	M	23	10AK	0	Endo	777777777700771	U	124	2 5 3 5 3 3 2 3 3 4 3 3 1 3 3	SSSS
			10AK	8		777777777700771	U	124	2 5 3 5 3 3 2 3 3 4 3 3 6 3 3	SSSS

NOTE. MIRU, mycobacterial interspersed repeat unit; RFLP, restriction fragment length polymorphism; SHRE, streptomycin, isoniazid, rifampin, and ethambutol; ST, spoligotype.

recurrence isolates among HIV-uninfected and HIV-infected patients, respectively. Recurrences among HIV-infected patients tended to have more clustering. The dendrograms in Figure 5*A* and 5*B* show the clustering pattern of HIV-infected and HIV-uninfected patients with tuberculosis by usable DNA recovered. The distance between the isolates represents the similarity index of individual isolates calculated by Jaccard coefficient.

DISCUSSION

The fact that persons previously infected with *M. tuberculosis* could be exogenously reinfected has been well documented [20–23]. However, this was thought to occur rarely because of the immunity conferred by primary infection. The pandemic of HIV has modified the epidemiological profile, pathogenesis, and clinical manifestations of tuberculosis [24, 25]. Distinguishing between recurrence due to endogenous reactivation or to exogenous reinfection is essential to accurately determine the efficacy of tuberculosis treatment regimens. The contribution of recurrence to the epidemiological profile and pathogenesis of tuberculosis also has important implications for vaccine design, chemoprophylaxis, and design of national tuberculosis control programs. This distinction is particularly important in settings with high tuberculosis prevalence. In the absence of clinical or epidemiological pointers to the origin of a tuberculosis episode being due to recent or reactivated infection, molecular epidemiological analysis has played a substantial role in clarifying the issue. In this study, we have com-

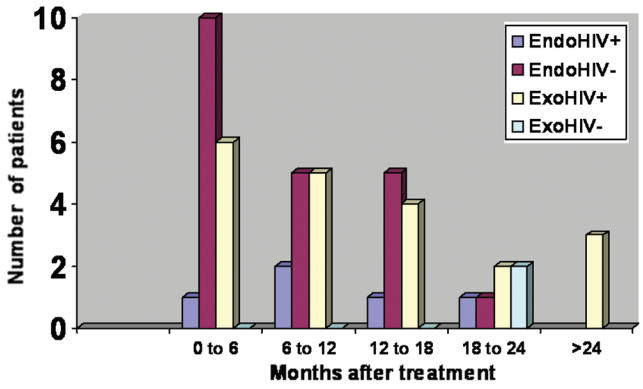

Figure 2. Time of endogenous reactivation and exogenous reinfection among recurrence isolates. Tuberculosis recurrence by time period and human immunodeficiency virus (HIV) status.

pared pretreatment isolates of *M. tuberculosis* with isolates at the time of recurrence among HIV-infected and HIV-uninfected patients with tuberculosis treated with short-course antituberculosis regimens in South India. Our objectives were to examine the role played by HIV infection in determining the type of tuberculosis recurrence (ie, relative frequency of endogenous reactivation versus that of exogenous reinfection) in a high tuberculosis prevalence setting, as well as to study the performance of 3 molecular typing methods (IS6110 RFLP, spoligotyping, and analysis of MIRU-VNTR).

In previous studies from TRC using a combination of DR- and IS6110-RFLP typing, a high rate of endogenous reactivation was reported among HIV-uninfected patients with tuberculosis [26]. In contrast, in this study conducted in the same setting, we have clearly shown that the majority (88%) of recurrences among HIV-infected patients with tuberculosis are due to reinfection with a new strain of *M. tuberculosis*. Among HIV-uninfected patients with tuberculosis during the same period, ~9% of recurrences were due to exogenous reinfection, whereas the majority (>90%) were due to endogenous reactivation. These results show that reinfection is much more common in patients with tuberculosis who are coinfected with HIV, particularly in the presence of immunosuppression. A study among South African mine workers also showed predominance of reinfection among HIV-infected (62%) and more of endogenous reactivation among HIV-uninfected patients with tuberculosis (94%) [27], which is similar to our findings. There has been wide variability in studies involving patients with negative or unknown HIV status, with reinfection accounting for 18%–80% of recurrences in countries with low incidence of tuberculosis and 6%–80% in countries with high incidence of tuberculosis [7, 28, 29].

The dendrogram shows 5 major clusters among tuberculosis isolates (baseline and recurrence) from HIV-infected patients and 2 clusters among HIV-uninfected patients, suggesting that the former are more heterogeneous in nature, probably because exogenous reinfection was more common. We considered the possibility that different fingerprints at various time points observed among HIV-infected patients with tuberculosis in this study could have arisen from either true new infection or misclassification. Over time, mutations or transpositions can alter the fingerprint pattern of a strain, resulting in overestimation of reinfection. We addressed this potential error by examining more than 1 isolate from the same patient at different time points after recurrence and confirming that they were identical. We also compared the DNA fingerprints of samples in 20% of the cases processed in the laboratory on the same day to rule out laboratory cross-contamination. Furthermore, strains that were clustered by MIRU were differentiated by spoligotyping and RFLP, and similarly isolates that were clustered by spoligotyping and RFLP were differentiated by MIRU. Hence, a reliable estimate of exogenous and endogenous infection could

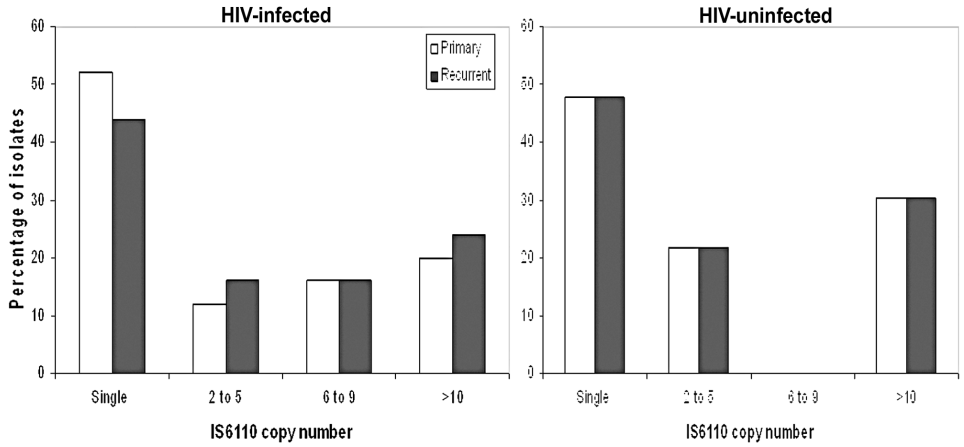

Figure 3. IS6110 restriction fragment length polymorphism (RFLP) of tuberculosis isolates from human immunodeficiency virus (HIV)–infected and HIV-uninfected patients, at baseline and recurrence.

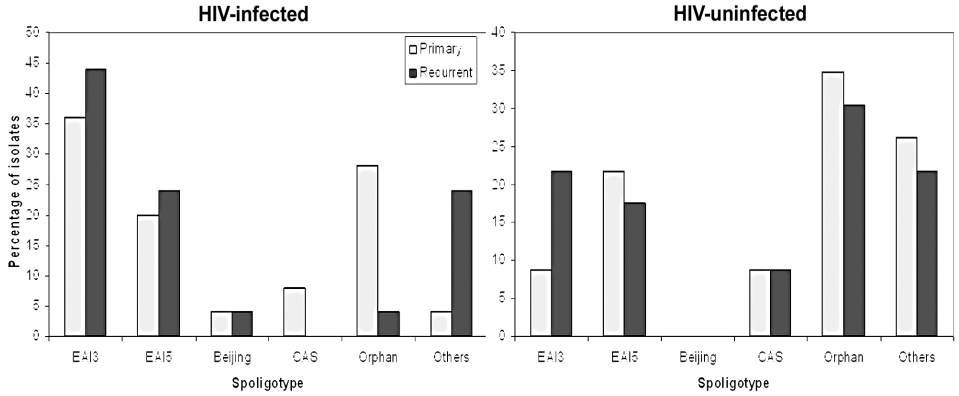

Figure 4. Spoligotyping pattern of data of tuberculosis isolates from human immunodeficiency virus (HIV)–infected and HIV-uninfected patients, at baseline and recurrence.

be obtained by using multiple genotyping methods to fingerprint tuberculosis clinical isolates.

We have previously reported that ~40% of *M. tuberculosis* strains in South India carry a single copy of IS6110, making it difficult to use this as the sole method for typing [10]. In this study also, 45%–47% of patients were infected with a single-copy strain, regardless of HIV status. MIRU-VNTR typing is a promising method with high discriminative characteristics that

Table 3. IS6110 Restriction Fragment Length Polymorphism (RFLP) and Spoligotyping Clusters Differentiated by Mycobacterial Interspersed Repeat Unit (MIRU)

Patient ID	IS6110 RFLP	Spoligotyping	MIRU
Cluster 1			
V037	1A	477777777413031	2 4 2 4 4 3 3 3 6 1 2 4 4 4 1
V040	1A	477777777413031	2 4 2 4 4 3 6 3 6 1 2 4 4 0 1
J001	1A	477777777413071	2 4 5 4 4 3 3 2 6 1 2 5 4 0 1
V008	1A	477777777413071	2 4 5 4 4 3 4 2 4 1 2 4 4 7 1
V044	1A	477777777413071	5 2 3 4 3 4 2 4 6 1 4 1 3 6 6
J030	1A	477777777413071	5 2 4 4 3 4 2 4 6 1 4 1 3 6 2
J030	1A	477777777413071	5 2 4 4 3 5 2 4 6 1 2 1 3 6 5
J034	1A	477777777413071	5 2 4 4 3 5 2 4 6 1 2 1 3 7 6
J083	1A	477777777413071	7 1 5 4 3 4 2 4 6 1 4 1 3 7 7
Cluster 2			
V008	1A	477777777413771	2 4 4 4 4 3 4 3 6 1 2 6 4 6 1
J001	1A	477777777413771	2 4 5 4 4 3 6 0 0 1 2 5 4 0 1
J001	1A	477777777413771	2 4 5 4 4 3 6 2 0 1 2 5 4 0 1
L025	1A	477777777413771	5 2 2 4 3 5 2 4 6 1 4 1 3 6 2
Cluster 3			
L048	1A	477777777413031	5 2 4 4 3 4 2 4 6 1 2 1 3 6 6
L150	1A	477777777413031	5 2 4 6 3 4 2 4 6 1 4 1 3 6 6
L174	1A	477777777413031	8 2 4 4 3 4 2 4 6 1 9 1 3 6 6
Cluster 4			
L138	1A	477777777413071	5 2 3 4 3 4 2 4 6 1 4 1 3 6 5
L095	1A	477777777413071	5 2 4 4 3 4 2 4 6 1 4 1 3 6 6
L163	1A	477777777413071	5 2 4 4 3 4 2 4 6 1 4 1 3 8 6
L130	1A	477777777413071	5 2 4 4 3 5 2 4 6 1 4 1 3 10 6
L121	1A	477777777413071	5 2 5 4 3 4 3 4 6 1 4 1 3 6 6
L174	1A	477777777413071	8 2 4 4 3 4 2 4 6 1 9 1 3 6 6

NOTE. RFLP, restriction fragment length polymorphism.

Table 4. Clustering of Relapse Isolates of Human Immunodeficiency Virus–Negative Patients with Tuberculosis

	IS6110 RFLP	Spoligotyping	MIRU	All 3 genotyping
Total no. of isolates	23.0	23.0	23.0	23.0
No. of isolates in cluster	13.0	15.0	3.0	0.0
No. of clusters	2.0	5.0	1.0	0.0
Range	2–11	2–5	3.0	0.0
Percentage	47.8	43.5	8.7	0.0

NOTE. MIRU, mycobacterial interspersed repeat unit; RFLP, restriction fragment length polymorphism.

can be used to differentiate IS6110 single-copy isolates. Genotypic strategies using 2 or more PCR-based methods have been proposed for investigations into tuberculosis epidemiological profile and phylogeny [30, 31]. Spoligotyping showed that the EAI3 and EAI5 clades of *M. tuberculosis* are most prevalent in this region, with the Beijing strain being rare. This is similar to previous reports from South India [32] but different from clades commonly observed (type 26, Delhi type, type 54) in North India [33].

In many countries, facilities for care and treatment of patients with HIV/AIDS and tuberculosis overlap, creating opportunities for nosocomial transmission and cross-infection. This may be one of the reasons for the high rate of exogenous reinfection seen in our study, because many of our patients had spent time in hospital wards where they could have been exposed to infectious patients with tuberculosis. Unfortunately, because of the large number of in-patients and high turnover in these hospitals, we were unable to actually prove nosocomial transmission. There is indirect evidence for this because clustering of recurrence isolates was greater among HIV-infected than HIV-uninfected patients (Tables 4 and 5). Recurrences among HIV-uninfected patients with tuberculosis appear to be due mainly to reactivation of mycobacteria in lesions that have been incompletely sterilized by the antituberculosis drugs, a phenomenon that is incompletely understood. In this study, half the patients had received a trial regimen of 4 or 5 months duration (with ofloxacin), whereas the other half had received the standard 6-month short-course regimen. Regardless of the regimen, almost all the recurrences were due to reactivation.

One of the limitations of our study is that not all recurrences that occurred during this period could be included, usually because either the baseline or recurrence isolate was unavailable for genetic analysis or all 3 techniques had not been used. Thus, a potential bias could have occurred as a result of selectivity of specimens studied. Furthermore, the number of patients with recurrences studied is not an indication of the overall efficacy of the regimens under trial. In fact, it is well known that recurrence rates are higher among HIV-infected patients with tuberculosis [34, 35]. In a pilot study of patients with advanced HIV and tuberculosis treated with a 6-month regimen, we observed a favorable response of 72% at the end of treatment and during 2 years of follow-up, a mortality rate of 35% and tuberculosis recurrence rate of 39% [36]. Some of the patients in the present report were participants in a randomized clinical trial comparing 6 and 9 months antituberculosis treatment, results of which have been submitted for publication separately [13]. There, we describe the outcome of tuberculosis treatment, including failures and recurrences and mention of DNA fingerprinting results. Here, we report the details of strain typing using robust molecular methods among recurrences that occurred in the HIV clinical trial and have used data from concurrently treated HIV-uninfected patients with tuberculosis for comparison. Hence, we are not comparing tuberculosis outcomes between these 2 very different groups but only the nature of recurrences.

In the ofloxacin trial among HIV-negative patients, recurrence rates ranged from 2% to 13% for the different regimens, whereas the rate for patients treated using the standard short-course regimen in the tuberculosis control program has been reported to be in the range of 11%–12% [14, 37]. We believe the fact that half the HIV-uninfected patients received a nonstandard antituberculosis regimen (with ofloxacin) does not change the interpretation of the results because of the overwhelming preponderance of reactivation in this group, regardless of regimen received. The strengths of this study are the concurrent nature of the 2 treatment trials and the identical setting under which they were conducted (ensuring comparability), as well as the excellent follow-up and documentation of treatment outcomes. Furthermore, we have taken care to distinguish true reinfection from other potential causes of strain differences.

Our results suggest that different strategies would be required to reduce tuberculosis recurrences in these 2 groups of patients. For HIV-uninfected patients (still the majority of tuberculosis cases in India), these may include vaccines to boost cell-mediated immune responses, intensification of regimens to ensure

Table 5. Clustering of Relapse Isolates of Human Immunodeficiency Virus–Positive Patients with Tuberculosis

	IS6110 RFLP	Spoligotyping	MIRU	All 3 genotypes
Total no. of isolates	25.0	25.0	25.0	25.0
No. of isolates in cluster	16.0	19.0	6.0	3.0
No. of clusters	3.0	5.0	3.0	1.0
Range	2–11	2–9	2.0	3.0
Percentage	52.0	56.0	12.0	8.0

NOTE. MIRU, mycobacterial interspersed repeat unit; RFLP, restriction fragment length polymorphism.

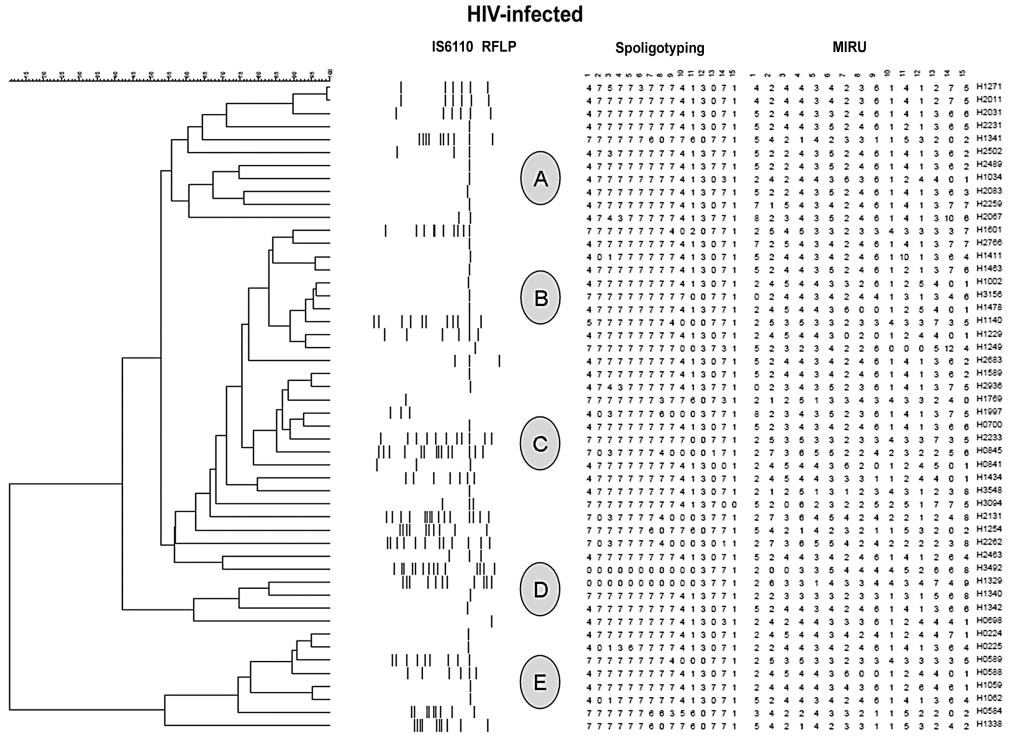

Figure 5*A*.

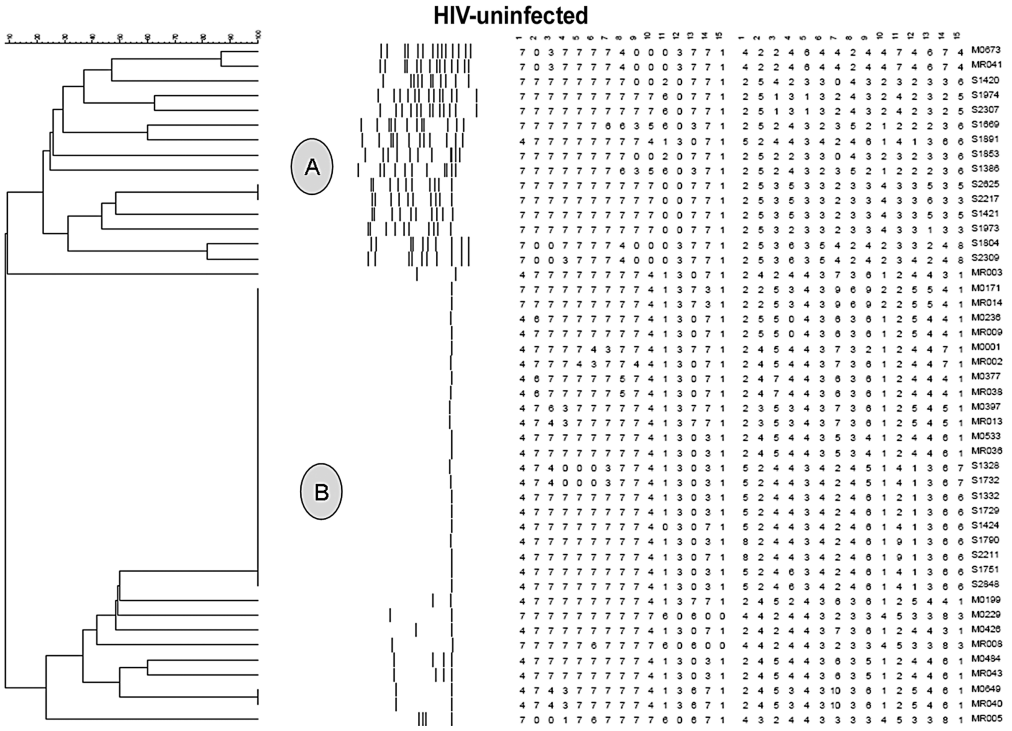

Figure 5*B*.

Figure 5. Combined dendrogram based on the similarities of the isolates, using all 3 genotyping methods: IS6110 restriction fragment length polymorphism (RFLP), spoligotyping, and mycobacterial interspersed repeat unit (MIRU).

complete sterilization of lesions, and measures to improve treatment adherence. The American Thoracic Society guidelines recommend that, for those patients with cavitation on baseline chest X-ray or positive sputum cultures at 2 months, treatment should be extended to 9 months [37]. However, the Indian Tuberculosis Control program recommends 6-month treatment for all new patients with tuberculosis disease. Recent efforts in drug development suggest that it may be possible to develop treatment regimens (eg, using fluoroquinolones) that are more effective (as well as of shorter duration) than those that are currently available [38]. A real challenge is to ensure that patients complete 6 months of regular treatment because recurrence rates have been shown to correlate with adherence [37]. For HIV-infected patients, strategies to minimize recurrences would include longer treatment regimens, post-treatment prophylaxis (with 1 or 2 drugs), or initiation of antiretroviral treatment, thereby improving immune status and reducing susceptibility to new infections [39, 40]. Our study also highlights the importance of infection control in both the hospital and community settings, particularly in areas with high HIV prevalence.

In summary, our study has documented the differences in the nature of recurrences observed among HIV-infected and HIV-uninfected patients after successful treatment of tuberculosis disease in South India. Previous reports have been mainly from Africa; to the best of our knowledge, our data are the first from a tuberculosis-endemic country in Asia. These findings have implications for policy makers, as well as clinicians who manage patients with tuberculosis.

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References

1. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. Lancet **2003**; 362:887–899.
2. Harries AD, Hargreaves NJ, Kemp J, et al. Deaths from tuberculosis in sub-Saharan African countries with a high prevalence of HIV-1. Lancet **2001**; 357:1519–1523.
3. UNAIDS/WHO. Report on the global AIDS epidemic. July **2008**. http://data.unaids.org/pub/GlobalReport/2008/JC1511_GR08_ExecutiveSummary_en.pdf. Accessed 3 March 2009.
4. World Health Organization. WHO report **2008**.Global tuberculosis control: surveillance, planning, financing. http://www.who.int/tb/publications/global_report/2008/pdf/fullreport.pdf. Accessed 3 March 2009.
5. Sharma SK, Alladi Mohan, Tamilarasu Kadhiravan. HIV-TB co-infection: epidemiology, diagnosis, and management. Indian J Med Res **2005**; 121:550–567.
6. Swaminathan S, Ramachandran R, Baskaran G, et al. Risk of development of tuberculosis in HIV-infected patients. Int J Tuberc Lung Dis **2000**; 4:839–844.
7. El-Sadr WM, Perlman DC, Denning E, Matts JP, Cohn DL. A review of efficacy studies of 6-month short-course therapy for tuberculosis among patients infected with human immunodeficiency virus: differences in study outcomes. Clin Infect Dis **2001**; 32:623–632.
8. Lambert M-L, Hasker E, van Deun A, Roberfroid D, Boelaert M, van der Stuyft P. Recurrence in tuberculosis: relapse or re-infection? Lancet Infect Dis **2003**; 3:282–287.
9. Cacho J, Pérez Meixeira A, Cano I, et al. Recurrent tuberculosis from 1992 to 2004 in a metropolitan area. Eur Respir J **2007**; 30:333–337.
10. Narayanan S, Das S, Garg R, Hari L, Bhaskara Rao V, Frieden TR, Narayanan PR. Molecular epidemiology of tuberculosis in a rural area of high prevalence in South India: implications for disease control and prevention. J Clin Microbiol **2002**; 40:4785–4788.
11. Charalambous S, Grant AD, Moloi V, et al. Contribution of reinfection to recurrent tuberculosis in South African gold miners. Int J Tuberc Lung Dis **2008**; 12:942–948.
12. Golub JE, Durovni B, King BS, et al. Recurrent tuberculosis in HIV-infected patients in Rio de Janeiro, Brazil. AIDS **2008**; 22:2527–2533.
13. Swaminathan S, Narendran G, Venkatesan P, et al. Efficacy of 6-month vs 9-month intermittent treatment regimens in HIV-infected tuberculosis patients: a randomized clinical study. Am J Respir Crit Care Med doi:10.1164/rccm.200903-0439OC. Published 3 December **2009**.
14. Tuberculosis Research Centre. Shortening short course chemotherapy: a randomized clinical trial for the treatment of smear positive pulmonary tuberculosis with regimens using ofloxacin in the intensive phase. Indian J Tuberc **2002**; 49:27–38.
15. Swaminathan S, Paramasivan C N, Ponnuraja C, Iliayas S, Rajasekaran S, Narayanan P R, Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. Int J Tuberc Lung Dis **2005**; 9:896–900.
16. Baess I. Isolation and purification of deoxyribonucleic acid from Mycobacteria. Acta Pathol Microbiol Scand [B] Microbiol Immunol **1974**; 82:780–784.
17. Van Embden JD, Cave MD, Crawford JT, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol **1993**; 31:406–409.
18. Kremer K, van Soolingen D, Frothingham R, et al. Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. J Clin Microbiol **1999**; 37:2607–2618.
19. Supply P, Allix C, Lesjean S, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol **2006**; 44:4498–4510.
20. Concheiro M, Samper S, Gavin P, Martin C. Recurrent tuberculosis from 1992 to 2004 in a metropolitan area. Eur Respir J **2007**; 30:333–337.
21. Bandera A, Gori A, Catozzi L, et al. Molecular epidemiology study of exogenous reinfection in an area with a low incidence of tuberculosis. J Clin Microbiol **2001**; 39:2213–2218.
22. Small PM, Shafer RW, Hopewell PC, et al. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. N Engl J Med **1993**; 328:1137–1144.
23. Caminero JA, Pena MJ, Campos-Herrero MI, et al. Exogenous reinfection with tuberculosis on a European island with a moderate incidence of disease. Am J Respir Crit Care Med **2001**; 163:717–720.
24. Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990–2000. Bulletin of the World Health Organization **1994**; 72:213–220.
25. Hopewell PC. The impact of human immunodeficiency virus infection

on the epidemiology, clinical features, management, and control of tuberculosis. Clin Infect Dis **1992**; 15:540–547.
26. Sahadevan R, Narayanan S, Paramasivan CN, Prabhakar R, Narayanan PR. Restriction fragment length polymorphism typing of clinical isolates of *Mycobacterium tuberculosis* from patients with pulmonary tuberculosis in Madras, India, by use of direct-repeat probe. J Clin Microbiol **1995**; 33:3037–3039.
27. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and re-infection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet **2001**; 358:1687–1693.
28. Nahid P, Gonzalez LC, Rudoy I, et al. Treatment outcomes of patients with HIV and tuberculosis. Am J Respir Crit Care Med **2007**; 175:1199–1206.
29. Chiang CY, Riley LW. Exogenous re-infection in tuberculosis. Lancet Infect Dis **2005**; 5:629–636.
30. Sun YJ, Lee AS, Ng ST, et al. Characterization of ancestral *Mycobacterium tuberculosis* by multiple genetic markers and proposal of genotyping strategy. J Clin Microbiol **2004**; 42:5058–5064.
31. Sola C, Filliol I, Legrand E, et al. Genotyping of the *Mycobacterium tuberculosis* complex using MIRUs: association with VNTR and spoligotyping for molecular epidemiology and evolutionary genetics. Infect Genet Evol **2003**; 3:125–133.
32. Narayanan S, Gagneux S, Hari L, et al. Genomic interrogation of ancestral *Mycobacterium tuberculosis* from south India. Infect Genet Evol **2008**; 8:474–483.
33. Singh UB, Suresh N, Bhanu NV, et al. Predominant tuberculosis spoligotypes, Delhi, India. Emerg Infect Dis **2004**; 10:1138–1142.
34. Korenromp EL, Scano F, Williams BG, Dye C, Nunn P. Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifamycin based treatment: an analytical review. Clin Infect Dis **2003**;37: 101–112.
35. Sterling TR, Alwood K, Gachuhi R, et al. Relapse rates after short course (6-month) treatment of tuberculosis in HIV-infected and uninfected persons. AIDS **1999**; 13:1899–1904.
36. Swaminathan S, Deivanayagam CN, Rajasekaran S, et al. Long-term follow up of HIV-infected TB patients treated with 6-month intermittent short course chemotherapy. Natl Med J India **2008**; 21:3–8.
37. Thomas A, Gopi PG, Santha T, et al. Predictors of relapse among pulmonary tuberculosis patients treated in aDOTS programme in South India. Int J Tuberc Lung Dis **2005**; 9:556–561.
38. Spiegelman MK. New tuberculosis therapy: a growing pipeline. J Infect Dis **2007**; 196:S28–S34.
39. Harries AD, Zachariah R, Lawn SD. Providing HIV care for co-infected tuberculosis patients: a perspective from sub-Saharan Africa. Int J Tuberc Lung Dis **2009**; 13:6–16.
40. Churchyard GJ, Fielding K, Charalambous S, et al. Efficacy of secondary isoniazid preventive therapy among HIV-infected Southern Africans: time to change policy? AIDS **2003**; 17:2063–2070.