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RECURRENT TUBERCULOSIS FROM 1992 TO 2004 IN THE METROPOLITAN AREA OF MADRID, SPAIN

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Short title: RECURRENT TUBERCULOSIS IN AN AREA OF MADRID, SPAIN

Abstract

The proportion of recurrent tuberculosis cases caused by reinfection has varied widely in previous study. Our aim was to determine the relative frequency of relapse and exogenous reinfection in patients with second episodes of tuberculosis, by means of DNA fingerprinting.

A population-based retrospective longitudinal descriptive study was conducted in Madrid from 1992 to 2004. The study consisted of 645 patients with culture-confirmed tuberculosis. Twenty (3.1%) patients were retained because they presented a second isolate of *M. tuberculosis*. Finally, twelve of these cases were excluded because they did not complete the full treatment prescribed. All strains were typed by restriction fragment length polymorphism (RFLP) and some of them by mycobacterial interspersed repetitive units (MIRU-VNTR). The patients with recurrent tuberculosis were compared with patients without recurrent tuberculosis.

For seven of the eight patients, the RFLP patterns of the *M. tuberculosis* strains from the episode of recurrent disease had identical initial and final genotypes, indicating relapse, the remaining recurrente case showed different genotypes, suggesting exogenous reinfection. Reinfection is possible among people in developed countries, but the rates are lower than those in high-risk areas. The risk factors of recurrent tuberculosis might be taken into account in following up of treatment and tuberculosis control strategies.

Keywords: epidemiology; recurrence; reinfection; relapse; RFLP; tuberculosis

Introduction

The role of reinfection compared with relapse in recurrence of tuberculosis in general is still unclear and has potential implications for public health (1, 2). The relative contributions of reinfection and relapse are likely to depend on the epidemiological context. In populations at high risk of infection, there is a substantial chance of repeated infection, and hence reinfection may be a major contributor to the overall rate of tuberculosis in adults. However, in populations with a low risk of infection, there is little probability of repeat infection, and thus most cases of second episodes of tuberculosis in adults are probably the result of relapse (3). With the introduction of short-course combination therapy, the relapse rate has dropped from 21 percent to 1 to 2 percent (4), questioning, in an era of effective treatment regimens, the notion that multiple episodes of tuberculosis in one patient are almost always caused by endogenous reactivation. The *Mycobacterium tuberculosis* genotype can now be characterized by DNA fingerprinting, this shows whether a new episode of the disease was caused by infection with the same strain that caused a previous episode or a different strain.

In this study we used DNA fingerprinting to determine the relative frequency of relapse and exogenous reinfection in patients with second episodes of tuberculosis. We aimed to determine the importance of this distinction in terms of the efficacy of current treatment regimens, and the control of tuberculosis.

Material and Method

Study population and data collection. This was a population-based retrospective longitudinal descriptive study. The cohort of tuberculosis patients included those whose diagnosis was confirmed from 1 January 1992 through 31 December 2004 in the 10th health district of Madrid of the Autonomous Community of Madrid.

Patients who met the following criteria were included in the analysis: (1) patients suffering from an episode of tuberculosis with a positive culture for *Mycobacterium tuberculosis*; (2) patients suffering from a subsequent episode of tuberculosis with an isolate of *M. tuberculosis* after completing the full anti-tuberculosis treatment. Treatment regimens used included: (1) two months of H, R, and Z followed by at least 4 months of H and R; or (2) two months of H, R, Z, and E followed by at least 4 months of H and R. Patients who had a second isolated of *M. tuberculosis,* but who did not complete the full treatment prescribed were defined as non-compliant and excluded from the study.

Patient information was obtained from the Regional Tuberculosis Case Register, which contains information on demographics, treatment, bacteriology, and outcome for all suspected and confirmed cases of tuberculosis.

Procedures. All *M. tuberculosis* strains were sent to the laboratory of the Mycobacterium Genetic Group of the Faculty of Medicine of Saragossa University and subjected to standardized IS6110-based RFLP typing, as described elsewhere (5). Since differentiation of *M. tuberculosis* strains carrying few IS6110 copies is poor, all strains with $\leq$4 IS6110 copies were subjected to subtyping by means of spoligotyping analysis (6). Similarity among strains was compared using the BioNumerics v4.5 software (Applied Maths Kortrijk, Belgium).

All patients in clusters were investigated for possible cross-contamination. Laboratory cross-contamination was considered if: (i) the patient had no clinical evidence of tuberculosis, (ii) each had a single positive culture but a negative smear for acid-fast bacilli (iii) the sample was processed on the same day as other patients' cultures and showed identical RFLP patterns.

Isolates taken from seven of the eight patients that belong to the recurrent tuberculosis group could be typing by means of variable numbers of tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR) system based on 15 loci (7).

Drug susceptibility testing for isoniazid, rifampin, streptomycin and ethambutol was performed by the proportion method and, from 2004, using another system (BACTEC MGIT 960; Becton Dickinson Microbiology Systems, Sparks, Md.).

Definition of relapse and reinfection. A patient whose isolates of *M. tuberculosis* from the first and second episodes of tuberculosis were identical on RFLP analysis with each DNA sample was considered to have tuberculosis due to relapse. A patient whose isolates from the first and second episodes of tuberculosis were different was considered to have tuberculosis due to a new, exogenous infection.

Statistical analysis. The epidemiological variables were subjected to univariate analysis. Patients who had a reinfection or relapse were compared with patients with no reinfection or relapse on the basis of selected demographic, epidemiological, clinical, and diagnostic features. The chi-square test or Fisher's exact test were performed to test the association of recurrent tuberculosis with predictor variables. Predictor variables that were associated with recurrent tuberculosis to a significant degree ($p < 0.005$) were included in a bivariate correlation model and in linear regression analysis to calculate the odds ratios with their intervals of confidence.

Results

Of the total of 645 patients with positive cultures during the study period, 20 (3.1%) patients were studied because they had a second isolate of *M. tuberculosis* after receiving treatment. After a careful review of all clinical charts, eight (1.2%) of these cases were included as they completed the full treatment prescribed and were assigned to the recurrent tuberculosis group; twelve were excluded from the study as they did not complete the full treatment prescribed and were assigned to the non-compliance group. None of the positive cultures for *M. tuberculosis* from the eight patients were due to cross-contamination.

During the study period (January 1992 through December 2004), DNA from cultures of *M. tuberculosis* was available for at least one RFLP analysis for 529 out of 633 patients (83.6%). Fingerprinting results were available for all eight patients with at least two cultures positive by RFLP analysis and for seven of the eight patients by MIRU-VNTR analysis. All isolates from these patients showed five or more copies of *IS6110*.

The demographic and clinical characteristics of the eight patients with recurrent tuberculosis are shown in Table 1. The mean age of these eight patients was 38.6 ($\pm$9.5) years, the median 36.5 years; the majority of patients (6) were male. All the patients were born in Spain and four patients were infected with HIV; five had a history of drug use; five were alcohol abusers and another seven were smokers. None of the patients had a medical history of diabetes, end-stage renal disease or cancer, or had been treated with immunosuppressant drugs. Seven patients had pulmonary disease only and one had both pulmonary and extrapulmonary disease. Chest x-rays revealed evidence of cavitary disease in 5 patients during the first episode of tuberculosis and in 2 during the second episode of tuberculosis. Seven patients had a positive acid-fast direct smear during the first or second episodes.

The mean interval between cure and subsequent diagnosis (isolation of a subsequent culture-positive specimen) was 54.4 months ($\pm$37), and the median 64.5 moths (range 9 to 113).

Age, sex, and positive acid-fast direct smear were not found to be risk factors for recurrent tuberculosis. However, we identified drug and alcohol abuse, HIV infection, smoking and chest x-ray with cavitary lesion as significant risk factors for recurrent tuberculosis, as shown in Table 2.

Isolates taken from the patients during the first and second episodes of disease were susceptible to all the antimicrobial agents tested.

For seven of the eight patients, the RFLP patterns of the strains of *M. tuberculosis* from the episode of recurrent disease had identical initial and final genotypes, indicating relapse. The remaining recurrent case showed different genotypes in the respective episodes, suggesting

exogenous reinfection (Table 3 and Figure 1). The same results were obtained when MIRU-VNTR analysis was used.

Isolates from the eight patients with recurrent tuberculosis were studied in relation to the complete RFLP database for our area (covering 1992 through 2004 and including 541 patients). It was established that five patients belonged to a cluster of strains present in the community (Table 4). No matching strain was identified in the database for the remaining patients. Both isolates from the patient with exogenous reinfection belonged to different clusters: the first strain belonged to a cluster containing only two other strains (his son and the index case), the second strain to a cluster with six other strains from a restaurant outbreak. The strains from the four patients with relapse belonged to three clusters: two patients (patients 7 and 8) were brothers and belonged to a long-standing cluster (12 years) with only their strains; another patient (patients 3) belonged to a medium-sized cluster (8 patients); and the other patient (patients 6) to a large (14 patients) and long- standing (11 years) cluster.

Discussion

The possibility of persons previously infected with *M. tuberculosis* being exogenously reinfected has been debated for decades. However, it was supposed that this rarely occurred given the immunity conferred by initial infection. In this study, 1.2% of all tuberculosis patients had a recurrent episode. This percentage is similar to the rates found in previous studies from areas with a low and moderate incidence of tuberculosis: 1.5% in Northern Italy (8), 2.4% in Gran Canaria (9), and is slightly lower than studies from the United States and Canada (6.8%) (10), and Madrid (7%) (11). As most patients are monitored closely, this could explain the low percentage of recurrent tuberculosis in our area.

The extent to which exogenous reinfection occurs depends on the prevalence of disease, the higher the prevalence, the greater the likelihood of exogenous reinfection (3). Studies from environments with a high incidence produce conflicting evidence as the proportion of cases attributable to reinfection varies from 12% to 75% (4, 12-15). On the few occasions on which exogenous reinfection has been documented in areas with a low incidence of the disease, it has usually involved a selected population, for instance alcoholic residents of a homeless shelter or patients with advanced HIV infection (16-19). In scarce population-based studies, the percentage of exogenous reinfection varies from study to study. In our study, one of the eight patients had exogenous reinfection, on a par with the results of a study from Northern Italy (8), where they found five of the 32 patients (16%); however, other studies showed higher

percentages: 44% in Gran Canaria (9), 33% in Madrid (11) and 24-31% in Texas (20). These differences compared with the exogenous reinfection rates determined in other studies may be explained by criteria applied when selecting the patients with tuberculosis recurrence, since we excluded non-compliant patients; furthermore, the rate of patients with mixed infection is difficult to prove, as demonstrated by van Rie et al (21). Differences in methods can contribute, to varying degrees, to the heterogeneity of the study results. It is very important that the same timing and bacteriological definition of recurrence be applied.

Two patients (patients 3 and 6) who were found to have the disease due to relapse were infected with individual strains that belonged to a cluster circulating within the communities during these patients' respective disease-free intervals (22); another two patients (patients 7 and 8) who were brothers and belonged to the same long-standing cluster were considered to have been reactivated; it is possible that some of them had contracted a new, exogenous infection with the same strain. Our results may thus underestimate the extent of exogenous reinfection. We therefore consider it necessary to continue with the study.

There are no empirical data reflecting the changes in the level of immunity after primary infection, but it is assumed that reinfection is rare in immunocompetent persons during the first two to five years after a first infection (4). In our study, the only patient with exogenous reinfection (patient 5) was an immunocompetent person whereby there were six years between the two episodes. Unlike other studies (23,24), this patient only reported a history of alcohol abuse. These results suggest that reinfeccion and progression to active disease is possible once treatment has been discontinued in immunocompetent persons living in an area with a moderate incidence of tuberculosis. The majority of our cases of relapse tended to occur four years after the previous episode had been cured. However, two patients, one with HIV infection and the other with severe underlying lung disease, suffered reactivation at 16 and 9 months respectively.

In our study, we found significant differences between the recurrent and no-recurrent groups in the distribution of drug and alcohol abuse, HIV infection, smoking and chest x-ray with cavitary lesion for tuberculosis. Despite our study being limited to a small number of patients with recurrent tuberculosis, these risk factors might be taken into account during follow-up treatment and when reviewing tuberculosis control strategies.

The controversy with regard to endogenous as opposed to exogenous pathogenesis of tuberculosis is of importance when planning clinical trials and national tuberculosis control programs. Where episodes of pulmonary tuberculosis following previous cure are the result of exogenous reinfection, the effectiveness of the drug regimen cannot be evaluated on the basis

of the relapse rate without the additional information provided by an RFLP analysis of bacterial isolates. In the evaluation of national tuberculosis control programs for a specific area, RFLP analysis can prove the effectiveness of the treatment regimens currently used.
In conclusion, our data seem to confirm that reinfection is possible among people in developed countries, but at a lower rate than in high-risk areas. Relapse of a previous infection remains the more probable cause of recurrence in our region. However, this scenario could change in future on the grounds of social, microbiological, and epidemiological factors. Reinfection may be a major contributor to the overall rate of tuberculosis in adults in immigrant populations from high-risk area in particular, especially those living in poor socio-economic conditions, These events should be considered when planning clinical trials and national tuberculosis control programs.

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References

1. Lambert M-L, Hasker E, van Deun A, Roberfroid D, Boelaert M, and van der Stuyft P. Recurrence in tuberculosis: relapse or reinfection?. Lancet Infect Dis 2003; 3:282-287.
2. Chiang CY, Riley LW. Exogenous reinfection in tuberculosis. Lancet Infect Dis 2005; 5:629-636.
3. Fine PEM and Small PM. Exogenous reinfection in tuberculosis. N Engl J Med 1999; 341:1226-1227.
4. van Rie A, Warren R, Richardson M, Victor TC, Gie RP, Enarson DA, Beyers N, and van Helden PD. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N Engl J Med 1999; 341:1174-1179.
5. Van Embden JDA, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Herrmans P, Martín C, McAdam R, Shinnick TM, and Small PM. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting recommendations for a standardized methodology. J Clin Microbiol 1993; 31:406-409.
6. Kremer K, van Soolinger D, Frothingham R, Haas WH, Hermans WM, Martín C, Palittapongarnpim P, Plikaytis BB, Riley LW, Yakrus MA, Musser JM, and van Embden JD. 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.
7. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rusch-Gerdes S, Willery E, Savine E, de Haas P, van Deutekom H, Roring S, Bifani P, Kurepina N, Kreiswirth B, Sola C, Rastogi N, Vatin V, Gutierrez MC, Fauville M, Niemann S, Skuce R, Kremer K, Locht C, van Soolingen D. Proposal for standardization of optimised mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol 2006; 44:4498-4510.
8. Bandera A, Gori A, Catozzi L, Esposti AD, Marchetti G, Molteni C, Ferrario G, Codecasa L, Penati V, Matteelli A, and Franzetti F. Molecular epidemiology study of exogenous reinfection in an area with a low incidence of tuberculosis. J Clin Microbiol 2001; 39:2213-2218.

9. Caminero JA, Pena MJ, Campos-Herrero MI, Rodríguez JC, Afonso O, Martín C, Pavón JM, Torres MJ, Burgos M, Cabrera P, Small PM, and Enarson DA. Exogenous reinfection with tuberculosis on a European island with a moderate incidence of disease. Am J Respir Crit Care Med 2001; 163:717-720.
10. Jasmer RM, Bozeman L, Schwartzman K, Cave MD, Saukkonen JJ, Metchock B, Khan A, Burman WJ, and the Tuberculosis Trials Consortium. Recurrent tuberculosis in the United States and Canada. Relapse or reinfection?. Am J Respir Crit Care Med 2004; 170:1360-1366.
11. García de Viedma D, Marín M, Hernangómez S, Díaz M, Ruiz Serrano MJ, Alcalá L, Bouza E. Tuberculosis recurrences. Reinfection plays a role in a population whose clinical/epidemiological characteristics do not favor reinfection. Arch Intern Med 2002; 162:1873-1879.
12. Das S, Chan SL, Allen BW, Mitchison DA, Lowrie DB. Application of DNA fingerprinting with IS986 to sequential mycobacterial isolates obtained from pulmonary tuberculosis patients in Hong Kong before, during and after short-course chemotherapy. Tuber lung Dis 1993; 74:47-51.
13. Sahadevan R, Narayanan S, Paramasivan CN, Prabhakar R, and 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.
14. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet 2001; 358: 1687-1693.
15. Verver S, Warren RM, Beyers N, Richardson M, van der Spuy GD, Borgdorff MW, Enarson DA, Behr MA, and van Helden PD. Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. Am J Respir Crit Care Med 2005; 171:1430-1435.
16. Chaves F, Dronda F, Alonso-Sanz M, and Noriega AR. Evidence of exogenous reinfection and mixed infection with more than one strain of *Mycobacterium tuberculosis* among Spanish HIV-infected inmates. AIDS 1999; 13: 615-620.
17. Nardell E, McInnis B, Thomas B, and Weidhaas S. Exogenous reinfection with tuberculosis in a shelter for the homeless. N Engl J Med 1986; 315:1570-1575.

18. Small PM, Shafer RW, Hopewell PC, Singh SP, Murphy MJ, Desmond Ed, Sierra MF, and Schoolnik GK. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. N Engl J Med 1993; 328:1137-1144.
19. Rivero A, Márquez M, Santos J, Pinedo A, Sánchez MA, Esteve A, Samper S, and Martín C. High rate of tuberculosis reinfection during a nosocomial outbreak of multidrug-resistant tuberculosis caused by *Mycobacterium bovis* strain B. Clin Infect Dis 2001; 32:159-161.
20. El Sahly HM, Wright JA, Soini H, Bui TT, Williams-Bouyer N, Escalante P, Musser JM, Graviss EA. Recurrent tuberculosis in Houston, Texas: a population-based study. Int J Tuberc Lung Dis 2004; 8:333-340.
21. van Rie A, Victor TC, Richardson M, Johnson R, van der Spuy GD, Murray EJ, Beyers N, van Pittius NCG, van Helden PD, and Warren RM. Reinfection and mixed infection cause changing *Mycobacterium tuberculosis* drug-resistance patterns. Am J Respir Crit Care Med 2005; 172:636-642.
22. Cacho Calvo J, Astray Mochales J, Pérez Meixeira A, Ramos Martos A, Hernando García M, Sánchez Concheiro M, Domínguez Pérez JR, Gómez AB, Samper S, Martín C. Ten-year population-based molecular epidemiological study of tuberculosis transmission in the metropolitan area of Madrid, Spain. Int J Tuberc Lung Dis 2005; 9(11):1236-1241.
23. Shafer RW, Singh SP, Larkin C, and Small PM. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in an immunocompetent patient. Tuber lung Dis 1995; 76:575-577.
24. Turett GS, Fazal BA, Justman JE, Alland D, Duncalf RM, and Telzak EE. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis*. Clin Infect Dis 1997; 24:513-514.

Table 1. Demographic, epidemiological, clinical and diagnostic characteristics by recurrent tuberculosis in eight patients.

Characteristic	No. of patients in cohort (n=8)
Demographic	
Mean age (yr) (±SD)	38.6 (±9.5)
Sex (%)	
Male	6 (75)
Female	2 (25)
Epidemiological (%)	
Spain-born	8 (100)
Drug abuse	5 (62.5)
Persons with AIDS	4 (50)
Alcohol abuse	5 (62.5)
Smoking	7 (87.5)
Clinical (%)	
Disease site	
Pulmonary only	7 (87.5)
Extrapulmonary only	0
Both	1(12.5)
Diagnostic (%)	
Chest x-ray with cavitary lesion	5 (62.5)
Positive acid-fast direct smear	7 (87.5)

Table 2. Odds ratio adjusted by linear regression analysis of the risk factors for recurrent tuberculosis.

Characteristic	Odds ratio adjusted	Lower limit confidence interval	Upper limit confidence interval	*P* value*
Demographic				
Age	1.9	0.9	1.0	N.S.
Sex	1.7	0.2	17.2	N.S.
Epidemiological				
Drug abuse	13.2	5.9	29.9	< 0.001
Persons with AIDS	2.8	1.5	5.4	< 0.001
Alcohol abuse	8.6	4.1	17.8	< 0.001
Smoking	49.1	19.8	125.6	< 0.001
Diagnostic				
Chest x-ray with cavitary lesion	1.9	1.0	3.5	< 0.05
Positive acid-fast direct smear	0.8	0.4	1.5	N.S.

* NS, not statistically significant.

Table 3. Epidemiologic and clinical characteristics and genotypic classification of eight patients with postprimary pulmonary tuberculosis after previous cure.

Patient No.	Age (yr)/Sex	Interval between episodes mths	Positive acid-fast direct smear		IS6110 RFLP	
			Previous episode	Subsequent episode	Pattern	Mechanism
1	58/M	9	Yes	Yes	Same	Relapse
2	42/M	17	Yes	Yes	Same	Relapse
3	41/M	57	No	Yes	Same	Relapse
4	30/M	16	No	Yes	Same	Relapse
5	43/M	73	Yes	No	Different	Reinfection
6	26/F	72	No	No	Same	Relapse
7	31/M	78	Yes	No	Same	Relapse
8	26/F	113	Yes	Yes	Same	Relapse

Table 4. Characteristics of the clusters belonging to eight patients recurrent tuberculosis.

Patient No.	Clusters (No. of cluster)	No. of patients per cluster	Mean duration (yrs)
1	No	-	-
2	No	-	-
3	Yes (31)	8	8
4	No	-	-
5*	Yes (41/77)	3/7	4/2
6	Yes (24)	14	11
7†	Yes (75)	2	12
8 †	Yes (75)	2	12

*, patient with exogenous reinfection who belong to two different clusters, the first is the initial isolate and the second is the subsequent isolate; †, patients who belong to the same cluster

Figure 1. RFLP patterns of the first and second episode of bacterial isolates from eight patients. The first of each pair is the initial isolate and the second is the subsequent isolate.