



MULTIDRUG RESISTANT TUBERCULOSIS OUTBREAK IN BUENOS AIRES

DNA FINGERPRINTING ANALYSIS OF ISOLATES

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Summary In order to determine the possible relationship among HIV patients coinfecting with multidrug resistant tuberculosis strains who had been receiving clinical assistance in our Hospital, clinical and epidemiological information from 28 patients was collected. DNA fingerprinting by restriction fragment length polymorphism (RFLP) pattern was performed on the mycobacterial isolates from these patients, using the restriction enzyme PvuII and IS 6110 as genetic marker. A unique RFLP pattern was found in 10 isolates from 10 different patients who had a disease caused by a single strain. Our findings confirm RFLP as a reliable and useful tool to analyze TB transmission.

Key words: tuberculosis, multidrug resistant, DNA fingerprinting.

It has been well established that those individuals infected with *Mycobacterium tuberculosis* (MT) who acquire an HIV infection, have an extraordinarily high risk of developing clinical tuberculosis (TB) due to the increasing impairment in the immunologic mechanisms involved in controlling TB infection. The same impaired mechanisms may not be able to control progression to the disease due to a new TB infection acquired from the community. This situation is worse in patients who harbour multidrug-resistant MT strains (MDR-TB) which cause treatments to fail, and often threaten their lives^{1, 2, 3}. A MDR-TB is defined as simultaneous resistance to isoniazid (H) and rifampin (R) with or without resistance to other drugs.

During the last two years (1992-1994) an increasing number of AIDS patients with TB disease due to MDR strains have been diagnosed and treated in our Hospital, located in North Buenos Aires suburbs⁴.

In order to determine the possible relationship among these cases, clinical and epidemiological information was collected from AIDS/TB out and in-patients who presented TB due to drug susceptible or to MDR-TB strains, and from every HIV negative MDR-TB case who had received clinical assistance at the Hospital during this period.

Medical records were reviewed from a total of 28 patients. Of these, 23 were AIDS cases: 14 harboured MDR-TB, 2 had TB isolates resistant to only one or two drugs (not the combination H-R), and 7 had susceptible TB strains. The remaining 5 patients were HIV negative infected with MDR-TB strains.

Recorded epidemiological data included age, sex, nationality, risk behaviours for HIV infection, contact with TB cases in the Hospital or in the community and relevant clinical data such as admissions to the Hospital, status of smear and culture examination for TB, dates when their positivity was recorded, drug susceptibility of different isolates obtained from each patient, associated diseases, and date of death of each AIDS MDR-TB case.

M. tuberculosis strains were isolated on Lowenstein-Jensen (L-J) medium. Susceptibility

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Patients



Fig. 1.— Sequence in time: diagnosis and medical assistance given to AIDS patients in the Hospital (HC), from April 1991 to September 1994.

Patients: 1 to 10

^: date of first positive smear examination for AFB or culture for *M. tuberculosis*.

†: date of death.

testing by the proportion method on L-J medium was performed on the isolates to determine resistance to H, R, streptomycin (SM), para-amino salicylic acid (PAS) and ethambutol (E)⁵.

DNA fingerprinting by restriction fragment length polymorphism (RFLP) was carried out on the mycobacterial isolates using IS 6110 as genetic marker on PvuII restriction fragment^{6, 7}.

The average age of AIDS patients was 27 years (22-45). There were 26 males and 2 females. All of them resided in the Northern suburbs of Buenos Aires City.

Each one of the 19 MDR-TB cases had received several antituberculosis treatments. Only a pair of AIDS patients were relatives, and two other patients had shared their home. The only apparent occasions for contact among the rest of them were the coincidental periods of time elapsed at the Hospital either as out or in-patients.

TABLE 1.— DNA Fingerprinting results: Restriction fragment length polymorphism (RFLP) patterns found in *Mycobacterium tuberculosis* isolates from patients with and without AIDS

| PATIENTS IDENTIFICATION | HIV STATUS | DRUG RESISTANCE | RFLP PATTERN |
|-------------------------|------------|-----------------|--------------|
| 6 XR | (+) | H-R-SM | II |
| * 1 VR | (+) | H-R-SM | II |
| 3 MW | (+) | H-R-SM | II |
| * 4 PJ | (+) | H-R-SM | II |
| BR | (+) | R | III |
| PA | (-) | H-R-SM | V |
| 5 OC | (+) | H-R-SM | II |
| FB | (+) | H-R | VI |
| YC | (+) | H-R-SM | VII |
| SM | (+) | H-R-SM | VIII |
| * 10 GJ | (+) | H-R-SM | II |
| * 8 AJ | (+) | H-R-SM | II |
| RA | (+) | H-R-SM | VIII |
| * 9 MJ | (+) | H-R-SM | II |
| AE | (+) | S | IX |
| AA | (+) | S | IX |
| * 7 CJ | (+) | H-R-SM | II |
| DM | (-) | H-R-SM | X |
| *** MN | (+) | S | XI |
| *** MN | (+) | R | XI |
| * 3 MW | (+) | H-R-SM | II |
| 3 MW | (+) | H-R-SM | II |
| AJ | (-) | H-R-SM | XII |
| AR | (+) | H-R-SM | XIII |
| LD | (+) | H-R-SM | XIV |
| LW | (+) | S | XV |
| ** SD | (+) | S | XVI |
| ** SF | (+) | S | XVI |
| AC | (+) | S | XVII |
| * 2 TL | (+) | H-R-SM | II |
| BC | (-) | H-R-SM | XVIII |

* MDR-TB isolates with identical RFLP pattern (II). The numbers following asterisks identify patients indicating their sequential TB diagnosis in time (Figure 1).

** An identical RFLP pattern was found in two drug susceptible isolates from two HIV(+) patients.

*** Two isolates with similar RFLP pattern from the same patient (the second isolate was resistant to R).

S: Susceptible.

A unique RFLP pattern was found in 10 isolates obtained from 10 different patients who therefore had a TB disease due to a single MDR-TB strain identified by us as pattern II (Table 1) with 7 bands situated approximately at 1.0, 1.7, 2.0, 2.4, 3.0, 4.0 and 4.4 Kb positions. These patients had been in-patients at the Hospital for different periods of time until death (Fig. 1).

Another DNA pattern designated as VIII was found in 2 isolates (MDR-TB) from 2 members of a same family (Table 1, patients SM and RA).

Pattern IX was identified in 2 drug susceptible isolates from two brothers (AIDS cases) (AA and AE in Table 1).

Pattern XVI belongs to a drug susceptible strain isolated from a couple of homosexual AIDS patients (SF and SD in Table 1).

The strain designated pattern XI was isolated twice from the same patient (MM in Table 1). It was drug susceptible in the first isolation and resistant to R in the second one. The same strain with pattern II was also identified in 3 isolates obtained at different times during the process of disease from a same patient (identified as patient 3 in Table 1 and Fig. 1).

Our results confirm that RFLP is a useful and reliable tool to analyze TB transmission. A MDR-TB outbreak among patients whose only relationship was to be assisted in the same Hospital, was here demonstrated using this method. Since containment of TB infection depends on cellular immunity, in cases where HIV is destroying this defensive mechanism, breakdown from TB infection to disease occurs rapidly and frequently. The risk of developing clinical TB in this situation has been estimated in about 8-10% per year⁸.

These findings show the existing limitations in strategies and resources assigned for prevention of TB transmission in immunosuppressed patients in the nosocomial environment. Prompt and thorough investigation of contacts of each newly diagnosed TB case is exceedingly important, mainly among HIV infected persons and it must be performed as soon as possible in order to control TB transmission.

Resumen

Brote de tuberculosis multirresistente en Buenos Aires. Análisis de DNA fingerprinting en los aislamientos de micobacterias

Con el propósito de determinar la posible relación entre pacientes HIV positivos coinfectados con *Mycobacterium tuberculosis* multirresistente (TB-MR) atendidos en nuestro Hospital, hemos recolectado información clínica y epidemiológica de 28 pacientes. Sobre los aislamientos de micobacterias se realizó el *finger-printing* del DNA empleando la enzima de restricción PvuII e IS 6110 como sonda genética. Un único patrón de RFLP fue encontrado en 10 cepas TB-MR analizadas y aisladas de 10 pacientes HIV positivos diferentes. Nuestros resultados confirman al RFLP como una técnica útil y confiable para el análisis de la transmisión de la tuberculosis y ponen de manifiesto la necesidad de reforzar estrategias para su control.

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