Molecular epidemiology of tuberculosis in Elche, Spain: a 7-year study

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The epidemiology of tuberculosis in Elche (Spain) was studied by restriction fragment-length polymorphism (RFLP) typing of clinical isolates of Mycobacterium tuberculosis over a 7-year period. A total of 165 isolates was typed and the clinico-epidemiological data of the patients were studied retrospectively. An overall cluster aggregation of 52.4% was found, rising to 71.43% in HIV-positive patients. There was greater aggregation in younger patients, but no statistically significant differences when other variables were analysed. The percentage of aggregation was higher than in other studies in Spain and this may be due to the longer time period of this study. The high percentage of aggregation in young patients and in those who were HIV-positive suggests increased recent transmission in both groups.

Introduction

It is estimated that 25–29% of the population of Spain is infected by Mycobacterium tuberculosis. When the curves for age are analysed, it can be seen that the situation in this country with respect to tuberculosis is similar to that in developing countries, with a predominance of the disease in young people. Therefore, tuberculosis is a serious health problem in Spain and this situation is especially worrying in AIDS patients because, according to published data, 41.9% of these patients have tuberculosis [1–3].

Although tuberculosis is a serious health problem, our understanding of this disease is far from complete [4]. The standardised technique of restriction fragment-length polymorphism (RFLP) of the insertion sequence IS6110 has been applied to resolve many outstanding epidemiological questions and thanks to this technique important advances are being made in the study of this disease [5]. Application of RFLP has improved knowledge of disease transmission within a community and between communities [6,7], enabled outbreaks of nosocomial infections to be characterised [8], enabled re-activations and exogenous infections to be differentiated [9,10], revealed laboratory contaminations [11], helped to achieve a better understanding of the particular characteristics of tuberculosis in certain groups of patients [12] and provided data on the genetic evolution of M. tuberculosis [13].

Studies have shown the existence of important differences as a function of economic, geographic, socio-sanitary factors and those associated with the pathogenesis of tuberculosis [14]. Therefore, it is of interest to communicate the data obtained in the first study performed in the province of Alicante (situated in the south-east of Spain, on the Mediterranean coast) with strains isolated over a period of 7 years.

Material and methods

Population studied

The study included 165 clinical isolates from 147 patients, representing 59.34% of the patients microbiologically diagnosed with tuberculosis from 1993 to 1999 in the Elche Health District (population 250 000), situated in the province of Alicante, and therefore belonging to the Autonomous Region of Valencia, on the Mediterranean coast of south-east Spain.

Microbiological data

In all, 13 089 respiratory and 3292 urine samples were inoculated on to Löwenstein-Jensen's medium with pyruvate and Cotleito's medium (Biomedics, Spain).
Also, 882 pleural fluid samples, 489 tissue biopsies, 406 CSF and 197 blood samples were cultivated in a semi-automated procedure with liquid medium (ESP-II, Difco, USA). The strains were identified by means of a genomic probe (Gen Probe, USA). The identification was confirmed and sensitivity to isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide was determined in the Carlos III Institute (Majadahonda, Madrid, Spain) by the proportion method. Direct microscopy of the respiratory and urine samples was performed with auramine-phenol; the remaining samples were stained by the Ziehl Neelsen technique.

**IS6110 RFLP**

This technique was used following a standardised protocol [15, 16] and the patterns obtained were analysed with the GelCompar system (Comparative analysis of electrophoresis patterns, version 4.1; Applied Maths, Kortijk, Belgium) and also by visual examination of the patterns.

A cluster of *M. tuberculosis* isolates was defined as two or more isolates for which RFLP revealed an identical number and location of IS6110 sequences when five or more copies of IS6110 were present.

**Epidemiological data**

A descriptive, retrospective study was performed by analysing the data from the computer files of the Microbiology Laboratory and from the clinical history of each patient. The data were collected by a protocol designed to include the following parameters: age, sex, HIV serology, result of direct staining, localisation of the disease and sensitivity of the isolate to isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide.

**Contamination**

An isolate was considered to be a cross-contaminant if direct staining of the sample was negative, few colonies were isolated in only one of the samples from the patient and another positive sample was processed on the same day showing the same RFLP pattern. These isolates were not included in the calculation of percentages of aggregation.

**Statistical analysis**

All the variables considered in the study were qualitative except the age (classified in groups). Contingency tables and the χ² test were used.

**Results**

**Patients and isolates**

It was possible to type 96.36% of the isolates, as only six presented with less than five bands. The most frequent numbers of bands in the isolates analysed were 8–15 (Fig. 1).

**Grouping in clusters**

The overall percentage of aggregation of the isolates studied was 52.40% forming 25 clusters. The characteristics of the clusters are shown in Table 1.

![Graph](image-url)

**Fig. 1.** Number of copies of IS6110 in *M. tuberculosis* clinical isolates in the Elche Health District 1993–1999.
Table 1. Characteristics of the clusters

<table>
<thead>
<tr>
<th>Cluster no.</th>
<th>Number of isolates</th>
<th>Interval (months)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.53</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
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<td>9</td>
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<td>0</td>
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<td>10</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>0.83</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>0.83</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
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<td>0</td>
</tr>
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<td>4</td>
<td>9.33</td>
</tr>
<tr>
<td>21</td>
<td>5</td>
<td>12.26</td>
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<td>14.60</td>
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<td>7</td>
<td>40.70</td>
</tr>
<tr>
<td>24</td>
<td>8</td>
<td>21.46</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>39.13</td>
</tr>
</tbody>
</table>

*Difference in months between the time the first and last strains in the cluster were isolated.

Distribution as a function of the characteristics of the patients

Clinical isolates from 103 men and 44 women were studied. The percentage of aggregation in isolates from men (53.39%) was greater than that in isolates from women (47.72%). Furthermore, the percentage of aggregation decreased as the age of the patient increased (p = 0.018; Table 2).

Direct staining of the sample was positive in 54 patients, with a percentage of aggregation (53.70%; CI 52.80%, 54.60%) greater than that in the case of patients with negative direct staining (42.34%).

The 21 patients with HIV had a higher percentage of aggregation (71.43%) than that in the general population (p = 0.017). As regards the clinical characteristics of the illness, there was greater aggregation (56.19%, CI 55.37%, 57.01%) in the 105 isolates from pulmonary tuberculosis than in the isolates from extrapolmonary tuberculosis (45.0%).

Nine strains were resistant to anti-tuberculosis drugs.

The percentage of aggregation of resistant strains was 33.33%.

Sources of isolates

Altogether, 18 patients had 2 isolates, the second was isolated between 1 and 13 months after the first. In all cases the pattern of bands was identical.

Isolates from different sites in two patients had an identical band pattern.

Four isolates (2.42% of the total) could have been due to cross-contamination.

Discussion

This technique enabled 96.36% of the clinical isolates to be typed, a slightly lower percentage than that reported in other studies carried out in Spain [16], but higher than that reported for patients in Asia, where the isolates have a small number of copies. Most of the isolates studied had 8–15 bands, which is in agreement with the findings of other studies in Europe [17].

It has been reported that there is a greater proportion of association in Africa (more recent transmission), whereas in countries with few cases of tuberculosis, such as Holland, there is more heterogeneity (more reactivations) [18] and although the percentage of aggregation found in the present study is greater than that reported in Zaragoza (39%) [16] and Seville (38%) [19], this is probably due to the fact that the present study covered a longer period of time. However, these data may be affected by other factors, such as the type of population studied (54% aggregation was detected in a 1-year study of a very poor urban population) [20].

The present study detected the presence of three clusters of more than six strains, which together included 32.47% of the strains; 50.65% of the aggregated strains were associated in small clusters of two or three patients. This contrasts with the data from Berne [7], in which 46% of the cases of tuberculosis belonged to a single cluster and only 21% were associated in small clusters. Therefore, tuberculosis in this region of Spain is generally characterised by the existence of small independent outbreaks or micro-epidemics.

Table 2. Percentage of tuberculosis clustering in patients stratified by age and gender: data from Elche (1993–1999)

<table>
<thead>
<tr>
<th>Age</th>
<th>Percent of isolates in clusters (number in clusters/total number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>0–20 years</td>
<td>66.66% (4/6)</td>
</tr>
<tr>
<td>20–40</td>
<td>61.11% (33/54)</td>
</tr>
<tr>
<td>40–60</td>
<td>55.55% (10/18)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>27.78% (5/18)</td>
</tr>
<tr>
<td>Unknown</td>
<td>42.85% (3/7)</td>
</tr>
</tbody>
</table>
In many studies, the grouping of isolates in clusters has been associated with recent transmission, although there are some studies that question this interpretation because this association may be due to other factors, such as the existence of strains that are endemic in the area. The predictive value of the association between the grouping in clusters and recent infection is greater in unstable populations, in which new strains occur frequently [21, 22].

In countries with low rates of tuberculosis, such as Holland, it is observed that isolates which are not epidemiologically associated have different band patterns, which may indicate that endemic strains do not exist [23].

In the health region studied, there was a lot of migratory movement 30–40 years ago, but more recently the population has remained fairly stable. However, tourism is a major industry in this region and so the existence of endemic strains should not be ruled out. The presence of three large clusters suggests the possibility that supertransmitters may exist in the area [24]. However, further studies are necessary to confirm both these hypotheses.

Various studies are in agreement with the data from the present study in showing that there is greater aggregation in HIV patients [7, 22, 24], although other studies indicate the opposite [16, 20]. This discrepancy may be related to the existence of nosocomial transmission, to the prevailing socio-economic situation and the effect of rapid diagnosis and effectiveness of treatment in these patients. The data support the great importance of recent infection in HIV patients, and indicate that reactivations are less frequent, in spite of the fact that the high prevalence of tuberculosis in Spain suggests the opposite. Neither may the presence of endemic strains be ruled out in this population [25], associated with geographical and socio-economic factors.

The present study found no differences between serial isolates from the same patient, which is in agreement with the findings of other authors [26]. These authors, when studying serial strains obtained from a patient over a period of 6–24 months, found no changes in the patterns. However, variations in the pattern of bands have been reported to be associated with the natural evolution of the micro-organism [27].

Some of the clusters detected consist of clinical isolates obtained over several years (up to 5 years), which supports the possibility, already discussed, that there are endemic strains which are transmitted within the community [25].

In contrast to other reports [28], the present study did not detect any transmission of resistant strains in this health area and the resistant strains associated with clusters were included with the sensitive strains, a finding that is in agreement with other reports [25]. This confirms that acquisition of resistance by a strain in a particular patient does not alter its pattern of bands. The percentages of cross-contamination are very variable, ranging from 1.8 to 26% [11] and this technique is very important to optimise the diagnostic procedures for this illness and to clarify doubtful cases (negative staining and low clinical suspicion).

The strains obtained from young patients had a greater percentage of association, which indicates greater transmission among the young, associated with pathogenic characteristics in the re-activation of tuberculosis [25].

It has been reported that extrapulmonary tuberculosis is associated with a lower number of grouped strains, which coincides with the data from the present study [22]. Therefore, these manifestations are more associated with processes of re-activation of the illness, despite occurring more often in HIV-positive patients.

This technique provides a lot of data that are useful for developing a better understanding of tuberculosis in this particular setting and improving the control of this illness. However, there are some unanswered questions regarding the interpretation of these findings which make it necessary to perform further detailed studies.

References

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