IS6110 Fingerprinting of Sensitive and Resistant Strains (1991-1992) of Mycobacterium tuberculosis in Colombia

Jorge Enrique Gómez-Marin/+, Clara Inés León Franco*, Martha Inirida Guerrero*, Leen Rigouts**, Françoise Portaels**

Centro de Investigaciones Biomedicas, Facultad de Medicina, Universidad del Quindio, Armenia (Q), Colombia *Laboratorio de Micobacterias, Subdirección de Investigación y Desarrollo, Instituto Nacional de Salud, Bogotá, Colombia **Laboratory of Mycobacteriology, Institute of Tropical Medicine, Antwerpen, Belgium

The standardized method to study the polymorphism of IS 6110 was used to characterize 53 isolates of Mycobacterium tuberculosis obtained during 1991-1992 from 14 regions in Colombia. In Valle region cluster rate was 25% (4/16). The mean number of IS6110 band was 10 ± 3. Similarity between strains was of 60% in 81% of strains and this tended to be correlated with geographic origin. For the first time M. tuberculosis without IS6110 bands in restriction fragment length polymorphism analysis was found in Colombia. Additional studies are necessary in order to best characterize the situation in relation to human immunodeficiency virus epidemic and recent changes in tuberculosis control program.

Key words: IS6110 - Mycobacterium tuberculosis - Colombia

In Colombia are reported near of 11,000 new cases of tuberculosis each year (Victoria 1999). The incidence of new cases has been shown to be in decrease, however this can be explained by a reduction in the search of new case (Victoria 1999). There are an urgent need to best define effective measures in order to stop transmission in particular conditions where restriction fragment length polymorphism (RFLP) analysis can play a helpful role. RFLP is based on the detection of the species specific polymorphism (RFLP) analysis can play a helpful role. For the first time M. tuberculosis without IS6110 bands in restriction fragment length polymorphism analysis was found in Colombia. Additional studies are necessary in order to best characterize the situation in relation to human immunodeficiency virus epidemic and recent changes in tuberculosis control program.

Key words: IS6110 - Mycobacterium tuberculosis - Colombia

Screening of all isolates from a particular geographical area would identify related isolates and provide the starting point for contact investigations. In 1993, international consensus was reached on a standardized method of M. tuberculosis RFLP analysis opening the way to interlaboratory comparison (van Embden et al. 1993). However, in a Medline search using MESH term IS6110, appeared 77 papers dealing with use of RFLP for IS6110 in Europe, 103 from United States, 35 from Africa and only 17 articles from Latin America. Some papers from Latin America characterized the polymorphisms in general and human immunodeficiency virus (HIV) population (Escalante et al. 1998, Ferrazoli et al. 2000, Suffys et al. 2000, Díaz et al. 2001, Yang et al. 2001). In Colombia, two previous published works (Gomez-Marin et al. 1995, Henao et al. 1999) reported RFLP patterns of IS6110 in M. tuberculosis strains in two particular regions: Quindio (center west region department) and Guaviare (eastern region department). The objective of the present work was to enlarge and complete these previous studies and to characterize IS6110 polymorphism of M. tuberculosis strains obtained in different geographical areas in Colombia. The group was a sample of 53 from a total of 121 strains collected during a survey on initial drug resistance in different regions of Colombia performed at the National Institute of Health of the Republic of Colombia during 1991-1992. All the patients were smear positive. The methodology used to select this group of patients has been described in detail previously (Laszlo & Kantor 1995).

Samples were decontaminated using the Kudoh’s method and cultured on Kudoh’s modified Ogawa medium and Giraldo’s modified Stonebrink medium at 37°C (Orozco et al. 1985). Culture identification was made by the CDC’s criteria (Kent & Kubirci 1985) and drug susceptibility testing by the proportion method of Cannetti et al. (1969) for isoniazide (INH, 0.2 µg/ml), rifampicin (RPM,
40 µl/ml), ethambutol (EMB, 2 µg/ml) and streptomycin (SM, 4 µg/ml). Resistance was defined according to the past history of treatment referred by the patient. Some of the results of the drug sensitivity testing were confirmed in the WHO-Collaborator Center in Ottawa (Canada) or in the Institute of Tropical Medicine in Antwerpen. Strains were sent to the Institute in Antwerpen on L-J medium for fingerprinting.

The standardized method (van Embden et al. 1993) was applied to all isolates. Briefly about 10 mg of bacteria were suspended in 500 µl TE buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA). After lysis with lysozyme, proteinase K and SDS (Sodium Dodecyl Sulfate), the DNA was extracted with CTAB (cetyltrimethylammoniumbromide). The DNA was digested with \textit{PvuII}, and the fragments were separated by electrophoresis on a 0.8% agarose gel followed by a DNA transfer to a charged nylon membrane by vacuum-blotting. The membrane was hybridized with a 245 bases pair long probe which is directed against the segment at right of cut site of \textit{PvuII} in the IS6110. The presence of the peroxidase labeled probe was realized by the Enhanced Chemoluminescence System (ECL, Amersham, UK) and registered on hypersensitive films.

To obtain accurate standardization of RFLP results two internal molecular weight markers (supercoiled DNA ladder/\textit{PvuII} and \textit{PhiX174HaeIII}) were included in each sample and two external markers on each membrane. The last consisted of lambda\textit{HindIII} to check the migration during electrophoresis and DNA of Mt14323 \textit{M. tuberculosis} reference strain with a known fingerprint. These internal markers were used during the computer assisted analyses.

The DNA fingerprint patterns of the mycobacterial analysis were analyzed and compared by a computer assisted system (Gelcompar 4.2 software, Kortrijk, Belgium) in order to construct dendograms based on similarity coefficients. IS6110 groups were formed based on a similarity coefficient greater than 60%.

In total, 53 isolates of \textit{M. tuberculosis} derived from 53 unrelated patients from 14 regions in Colombia were studied. The results of IS6110 characterization are shown in the Figure. We found that two couples of isolates from Valle region had identical IS6110 pattern. Thus, 25% (4/16) of the strains in Valle department originated two clusters that probably were originated from two index cases of recent transmission. However, we were unable to obtain the epidemiological data concerning these cases. By using the criteria of similarity coefficient greater than 60% it were formed seven groups of IS6110 related strains (Figure). The distribution of groups by department and type of resistance is shown in the Table. Group A have a large number of strains located in western departments (Valle, Risaralda and Antioquia), only one isolate from this group was obtained in Caribbean region. Also, a large group of strains from Antioquia were in group F. In total, 43 of 53 isolates (81%) were grouped based on this similarity coefficient. No correlation was found between RFLP types and the phenotypes of resistance to various antitu-

![Dendrogram of IS6110 groups](image-url)
berculous drugs. Each group included resistant and sensitive isolates.

The mean number in all strains that showed some IS6110 band was 10 ± 3. The number of copies ranged from 0 to 18. Four isolates were found without IS6110 copies and were originating from four different regions in Colombia (Antioquia, Valle, Choco and Tolima). The number of isolates with low number of copies (less than 4) was 6 of 53 isolates studied (11%).

The two clusters of strains with identical fingerprinting in present study occurred in Valle, the region with the greater number of isolates included. The percent of isolates that were related with probably recent transmission (25%) in Valle strains is near to the 14% found in Quindio (Gomez-Marin et al. 1995). However, the percentage is in contrast with the rate found in Guaviare where were found 31 isolates that formed cluster between 55 isolates (56%) that were studied (Henao et al. 1999). The situation in Guaviare is particular because most of cases were originated from indigenous people that have a rate of incidence of 558 cases/100,000 habitants (Pineda-Garcia et al. 1997). We selected a coefficient of similarity greater than 90% with an incidence of tuberculosis (TB) of 84/100,000 habitants (Pineda-Garcia et al. 1997). It have been showed for others authors that isolates with low number of copies (less than 4) was 11%, that is a percent intermediate between regions of the world with an important proportion of isolates with absence or low number of copies as 21% in Vietnam (Yuen et al. 1993) or 24% in Guadeloupe, French West Indies (Sola et al. 1997) or 26% in Tanzania (Yang et al. 1995) and regions with only 8% in Denmark (Yang et al. 1994) or 5% in Tunisia (Hermans et al. 1995) or 4% in Cuba (Diaz et al. 2001). It have been showed for others authors that isolates with IS6110 copies lower than four require the use of additional genetic markers, such as PGRS and DR, to establish the genetic relatedness (van Soolingen et al. 1993). It was observed that in some groups DNA fingerprint patterns tended to be in accordance with geographical origins of the isolates. This was, obviously, more evident in groups formed by a number of isolates greater than two or three. Thus, in Antioquia region 5 of 11 isolates (45%) were classed in Group F. This is evidence that coefficient similarity can be used to reflect the common past of IS6110 exchange between diverse migratory and selective forces. These factors can acts as positive selection factors. Evolutionary forces in Colombia can include, among others, people of diverse ethnic origin, migratory movements and a long history of vaccination with BCG vaccines that is compulsory in our country in all newborns. In this context, it will be important to compare results of present study performed during 1992 and isolates of recent years where national BCG production and rates of vaccination have been reduced.

The number of IS6110 copies was evenly distributed within a range of 0 to 18. The most frequent number of bands was 9. Notoriously, for the first time in Colombia we found some isolates without IS6110 bands. The total of isolates with low number of copies (less than 4) was 11%, that is a percent intermediate between regions of the world with an important proportion of isolates with absence or low number of copies as 21% in Vietnam (Yuen et al. 1993) or 24% in Guadeloupe, French West Indies (Sola et al. 1997) or 26% in Tanzania (Yang et al. 1995) and regions with only 8% in Denmark (Yang et al. 1994) or 5% in Tunisia (Hermans et al. 1995) or 4% in Cuba (Diaz et al. 2001). It have been showed for others authors that isolates with IS6110 copies lower than four require the use of additional genetic markers, such as PGRS and DR, to establish the genetic relatedness (van Soolingen et al. 1993).

Dendrograms are used to establish IS6110 degrees of similarity between isolates. Thus, in a study in Tanzania 45% of non-identical isolates were included in three big clusters with similarity coefficients greater than 90% with an incidence of tuberculosis (TB) of 84/100,000 habitants (Pineda-Garcia et al. 1997). We selected a coefficient of similarity greater than 60% to establish groups of IS6110 patterns. Although the stability of IS6110 element is high, it is by nature transposable and the highly related but non identical patterns reflect the result of evolution of IS6110 through generations of tuberculosis infections in the country. When groups of isolates were located geographically, it was observed that in some groups DNA fingerprint patterns tended to be in accordance with geographical origins of the isolates. This was, obviously, more evident in groups formed by a number of isolates greater than two or three. Thus, in Antioquia region 5 of 11 isolates (45%) were classed in Group F. This is evidence that coefficient similarity can be used to reflect the common past of IS6110 exchange between diverse migratory and selective forces.

### TABLE

Geographic origin of 53 Colombian strains *Mycobacterium tuberculosis* isolated at the “Instituto Nacional Salud” (Bogotá, 1991)

<table>
<thead>
<tr>
<th>Department</th>
<th>TB incidence X 100,000 ( a )</th>
<th>Number of strains</th>
<th>Code (resistance) ( b )</th>
<th>Cluster group of IS6110 ( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choco</td>
<td>38</td>
<td>1</td>
<td>9602</td>
<td>-</td>
</tr>
<tr>
<td>Tolima</td>
<td>25</td>
<td>2</td>
<td>9598 (PZ), 9599</td>
<td>B (1)</td>
</tr>
<tr>
<td>Guajira</td>
<td>59</td>
<td>2</td>
<td>9605 (PZ), 9613</td>
<td>C (1)</td>
</tr>
<tr>
<td>Cesar</td>
<td>30</td>
<td>2</td>
<td>9603 (SM-PZ), 9606</td>
<td>G (1)</td>
</tr>
<tr>
<td>Quindio</td>
<td>36</td>
<td>1</td>
<td>9608 (PZ)</td>
<td>C (1)</td>
</tr>
<tr>
<td>Valle</td>
<td>19</td>
<td>16</td>
<td>9635 (PZ), 9620, 9621, 9623, 9639, 9640, 9643, 9644, 9642, 9641, 9655 (EMB), 9634, 9622, 9646, 9647, 9654</td>
<td>A (4), C (1), D (1), E (1), G (4)</td>
</tr>
<tr>
<td>Risaralda</td>
<td>22</td>
<td>4</td>
<td>9645, (PZ) 9629, 9606, 9628</td>
<td>A (1), D (1)</td>
</tr>
<tr>
<td>Cauqueta</td>
<td>38</td>
<td>1</td>
<td>9614</td>
<td>-</td>
</tr>
<tr>
<td>Santander</td>
<td>19</td>
<td>1</td>
<td>9600</td>
<td>C (1), G (1)</td>
</tr>
<tr>
<td>Bolivar</td>
<td>10</td>
<td>2</td>
<td>9610, 9618</td>
<td>C (1), E (1)</td>
</tr>
<tr>
<td>N. Santander</td>
<td>36</td>
<td>5</td>
<td>9611, 9612, 9624, 9625, 9626</td>
<td>C (3), F (1), G (1)</td>
</tr>
<tr>
<td>Atlántico</td>
<td>35</td>
<td>3</td>
<td>9616, 9619 (SM-INH), 9630</td>
<td>B (1), C (1), F (1)</td>
</tr>
<tr>
<td>Huila</td>
<td>32</td>
<td>2</td>
<td>9604 (SM-INH-EMB-RM), 9627</td>
<td>C (1)</td>
</tr>
<tr>
<td>Antioquia</td>
<td>15</td>
<td>11</td>
<td>9632, 9633, 9648 (SM), 9643, 9631, 9617, 9638 (SM-INH), 9650, 9651, 9637, 9652</td>
<td>A (2), D (1), F (5), D (1)</td>
</tr>
</tbody>
</table>

\( a \): obtained from Victoria et al. (1999); \( b \): resistance patterns in parenthesis; SM: streptomycin, PZ: pirazinamide, EMB: ethambutol, RM: rifampin, INH: isoniazid; \( c \): determined by the clustering of IS6110 polymorphisms as showed in the Figure. The number of strains is indicated in parenthesis; TB: tuberculosis.
seems that PvuII site of restriction in IS6110 can be methylated in some strains and this can explains that can be missed during Southern blotting (van Soolingen et al. 1996).

In conclusion, this study showed that similarity between strains was of 60% in 81% of strains and this tended to be correlated with geographic origin. However, due to the small number of strains studied a new work is needed to include a representative number of strains from all isolates that are found each year in Colombia. Also it would be necessary to complement epidemiological investigation of cases with identical fingerprinting that we were unable to perform due to the retrospective character of present work. For the first time M. tuberculosis without IS6110 bands in RFLP was found in Colombia and 11% of isolates had less than four bands. Additional studies are necessarily in order to characterize the situation in relation with HIV epidemic and recent changes in tuberculosis control program as the reduction in BCG vaccination.

REFERENCES


