Hepatitis B virus genotypes identified by a Line Probe Assay (LiPA) among chronic carriers from Spain

José María Echevarría y Pilar León

Methods

From May, 2001 to August, 2002, single serum samples taken from 722 HBV surface antigen (HBsAg) carriers were sent to our laboratory from different health care centres from Spain. Since these samples were sent for study just for diagnostic purposes and without a specific request, they are not representative of the population of HBV carriers from these regions. HBV DNA was tested by a nested, polymerase chain reaction (n-PCR) assay, targeted on the P-S region of the HBV genome, in all samples. Outer primers HBPr134 and HBPr135 (5’-TGC TGC TAT GCC TCA TCT TC-3’ and 5’-CA(A/G) AGA CAA AAG GTA GTT TTC-3’, respectively) were used in the first reaction for obtaining a fragment that was amplified again in a second reaction by using nested primers HBPr75 and HBPr94 (5’-CA(A/G) AGA CAA AAG GTA GTT TTC-3’ and 5’-GGT A(A/T) AAG GGA CTC-3’, respectively)9. A final fragment of 341 base pairs, encoding aminoacids 89 to 211 from the HBsAg molecule, was finally sequenced.

On the basis of molecular clock studies, the genotype F has been identified as the closest to the putative HBV virus ancestor. Since this genotype is characteristic from the human populations original from America, it is thought that HBV emerged as a human virus in that continent and was brought to other geographical regions, evolving locally to generate the remaining genotypes. Genotypes B and C are characteristic from the Far East, but a particular subset of genotype C strains, found among Australian Aborigines, seems to be genetically divergent from the Chinese strains. Genotype E is prevalent in the Subsaharian Africa, whereas the genotype A prevails in the North of Europe, North America and among the American population of European origin. The genotype D is spread worldwide, but it is characteristic from the Mediterranean region, the Middle East and India. Introduction of exotic genotypes by immigrants coming to Western Europe has been, however, already documented and such introduction may be influencing the molecular epidemiology of the HBV infection in the region.

Data regarding the distribution of HBV genotypes in Spain are still very scarce, but reveal the circulation of strains from genotypes A, D and F. With the aim of extending such data, the genotypes present in serum samples from 278 HBV DNA-positive chronic carriers residing in Spain have been examined.

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N. V., Gent, Belgium) Finally, HBV “a” antigen (HBsAg) and anti-body to HBsAg (anti-HBs) were determined in all samples by an au-
tomated immunoassay test (Vitros ECi, Ortho Clinical Diagnostics, Raritan, NJ, USA).

Results

Samples from 278 carriers (38.5%) were positive in the n-
PCR assay. Of them, 33 were foreigners, coming from the
Far East, Africa and Eastern Europe, and 248 were
Spaniards who lived in 11 different regions of Spain,
namely Andalucía, Baleares, Castilla-La Mancha, Castil-
la-León, Ceuta, Extremadura, Galicia, Madrid, Murcia,
Navarra and Valencia. Eighty-one were women and 197 men,
including a two months-old infant born from a
carrier mother, seven children aged six to 14 years and
270 adults (age range, 15-79 years, mean age, 45.3 years).

The results obtained from genotyping the HBV strains
detected among these carriers are summarised in table 1. Four
strains (1.4%) did not react with any of the probes
and could not be, therefore, typed by the LPA test. Geno-
types A and D were the most commonly found (249 cases,
89.5%) and genotype D was the most prevalent (181 cases,
65.1%). However, the prevalence of genotype D was signif-
icantly lower among the anti-HBe-positive carriers (47.2 vs.
65.1%).

The finding of a significant proportion of HBV strains
from genotypes B, C and E indicates that exotic HBV
genotypes are being introduced in Spain by the immi-
grants and shows that, as formerly happened with geno-
type F, some of them are beginning to circulate among the
autochthonous population. Noteworthy, no carriers of
genotype F coming from Latin America were detected in
this study, besides the high number of immigrants com-
ing to Spain from Latin American countries in the last
20 years. This finding agrees with the data obtained in
that region, which show a low endemicity of the HBV in-
fection in most urban and rural areas unrelated with the
Amazonian Basin11.

Although the investigations regarding the influence of
the HBV genotypes on the events of the viral persistency
and the chronic liver infection are still scarce, evidence
suggesting the clinical and public health relevance of
despite these issues are already emerging. Most of the issues
risen by these investigations are still controversial and
these genotypes is already emerging. Most of the issues
risen by these investigations are still controversial and
further studies in relation with these matters should be,
therefore, performed. In order to provide a better basis
for interpreting the results that such studies may rise-up,
an assessment of the distribution of HBV genotypes
among the population of chronic HBV carriers from a giv-
en geographical area is necessary. The results obtained in
this study extend the data available from Spain and evi-
dence an epidemiological reality that seems to be more
complex than previously thought.

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TABLE 1. HBV genotypes found among 278 chronic carriers positive for HBV DNA in serum in regard to the HBsAg/anti-HBs status
and the level of viral DNA

<table>
<thead>
<tr>
<th>HBsAg Anti-HBe</th>
<th>Viral DNA (pg/ml)</th>
<th>Number of cases</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
<th>D (%)</th>
<th>E (%)</th>
<th>F (%)</th>
<th>NT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>&gt; 1,000</td>
<td>57</td>
<td>13</td>
<td>2</td>
<td>3</td>
<td>32</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt; 1,000</td>
<td>49</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>18</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>34 (32.1)</td>
<td>6 (5.6)</td>
<td>50 (47.2)</td>
<td>6 (5.6)</td>
<td>4 (3.8)</td>
<td>3 (2.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>&gt; 1,000</td>
<td>13</td>
<td>1</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 1,000</td>
<td>159</td>
<td>33</td>
<td>1</td>
<td>119</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>34 (19.8)</td>
<td>1 (0.6)</td>
<td>131 (76.1)</td>
<td>5 (2.9)</td>
<td>1 (0.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total studied</td>
<td>278</td>
<td>68 (24.4)</td>
<td>3 (1.1)</td>
<td>170 (61.1)</td>
<td>11 (4.0)</td>
<td>4 (1.4)</td>
<td>4 (1.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NT: strains that could not be typed by the genotyping test.
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References