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

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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 Australian 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.

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) A(G/A) AAG GGA CTC AGA CAA AAG AAA ATT GG-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) GTG A(T/C) CAC CAC GCC GCT GAA AAG GGT ATC A/CA G/G ATG-3', respectively)9. A final fragment of 341 base pairs, encoding aminoacids 89 to 211 from the HBsAg molecule, was finally obtained and detected by agarose gel electrophoresis. Viral DNA was subsequently quantified by a molecular hybridisation test (Digene Hybrid Capture II, Diagene Corp., Gaithersburg, MD, USA) on all the n-PCR-positive samples. Since the nested primers in the n-PCR test were bimitted, the final amplification products from all these samples were then labelled and could be directly tested for identification of HBV A-G genotypes by a reverse hybridisation test that uses a collection of genotype-specific probes adsorbed on nitrocellulose strips (Line Probe Assay, INNO-LiPA HBV Genotyping, Innogenetics, Belgium).
TABLE 1. HBV genotypes found among 278 chronic carriers positive for HBV DNA in serum in regard to the HBeAg/anti-HBe status and the level of viral DNA

<table>
<thead>
<tr>
<th>HBeAg</th>
<th>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>Negative</td>
<td>&gt; 1,000</td>
<td>106</td>
<td>34 (32.1)</td>
<td>3 (2.8)</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>
</tr>
<tr>
<td></td>
<td>&lt; 1,000</td>
<td>21</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>127</td>
<td>37 (29.3)</td>
<td>3 (2.4)</td>
<td>9 (7.1)</td>
<td>50 (39.1)</td>
<td>6 (4.7)</td>
<td>5 (3.9)</td>
<td>3 (2.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>&gt; 1,000</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 1,000</td>
<td>115</td>
<td>1</td>
<td>1</td>
<td>119</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>127</td>
<td>1</td>
<td>1</td>
<td>119</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total studied</td>
<td>278</td>
<td>68 (24.4)</td>
<td>3 (1.1)</td>
<td>7 (2.5)</td>
<td>181 (65.1)</td>
<td>11 (4.0)</td>
<td>4 (1.4)</td>
<td>4 (1.4)</td>
<td></td>
</tr>
</tbody>
</table>

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

Discussion

The results obtained in this study confirm the dominance of HBV strains from genotypes A and D in Spain, as well as the circulation of genotype F strains among the Spanish population, as already suggested by the prior observation of HBV strains from the antigenic subtype adw420. In addition, the significantly higher prevalence of genotype D found among the anti-HBe-positive carriers agrees with prior data suggesting that strains of this genotype may show a pronounced trend to establish HBsAg-negative chronic infections due to selection of precore-defective mutants. HBV genotype D strains exist in two main, separate antigenic subsets, namely ayw2 and ayw3, which present a distinct pattern of geographical distribution. Both types of strains are common in the Western world, but D/ayw3 strains are also highly prevalent in Asia and could have been introduced recently into Europe and North America through the intravenous drug abuse. Whether or not both antigenic groups share the same ability to establish precore-defective chronic infections is unknown and could be a matter of future investigations.

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 immigrants and shows that, as formerly happened with genotype 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 coming 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 infection in most urban and rural areas unrelated with the Amazonian Basin.41

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 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 given geographical area is necessary. The results obtained in this study extend the data available from Spain and evidence an epidemiological reality that seems to be more complex than previously thought.

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References