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

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Genotypes A and D of the hepatitis B virus were found to be prevalent among 278 chronic carriers residing in Spain, and genotypes B, C, E and F were detected with significant frequency (9%). Two genotype E infections corresponded to carriers born in Spain who had never traveled to Africa. These results indicate that genotype E is beginning to circulate in the Spanish population in the same way that genotype F did in the past.

Key words: Hepatitis B virus. Genotypes. Epidemiology. Line probe assay.

Introduction

Molecular studies performed on hepatitis B virus (HBV) genomes have revealed the identification of six major genotypes, namely genotypes A-F, whose complete sequences have rendered the identification of six major genotypes from different regions. HBV DNA was tested by a nested, polymerase chain reaction (n-PCR) assay, targeted on the P-S region of HBV for obtaining a fragment that was amplified again in a second reaction for obtaining a fragment that was amplified again in a second reaction by using nested primers HBPr75 and HBPr94 (5'-CAA GGT GAC 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'-CAA GTG ACG TTO GTC GGT TGT TCC C-3' and 5'-GTT A(A/T)A AAG GGA CTC A(G/C)G ATG-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'-CAA GTG ACG TTO GTC GGT TGT TCC C-3' and 5'-GTT A(A/T)A AAG GGA CTC A(G/C)G ATG-3', respectively) were used in the first reaction for obtaining a fragment that was amplified again. Viral DNA was subsequently quantified by a molecular hybridization test (Dupont Hybrid Capture II, Dupont Corp., Gaithersburg, MD, USA) on all the n-PCR-positive samples. Since the nested primers in the n-PCR test were bimodal, the final amplification products from all these samples were biotin-labelled and could be directly tested for hybridization with a collection of genotype-specific probes adsorbed on nitrocellulose strips (Line Probe Assay, INNO-LiPA HBV Genotyping, Innogenetics) on all the n-PCR-positive samples. Since the nested primers in the n-PCR test were bimodal, the final amplification products from all these samples were biotin-labelled and could be directly tested for hybridization with a collection of genotype-specific probes adsorbed on nitrocellulose strips (Line Probe Assay, INNO-LiPA HBV Genotyping, Innogenetics).
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>Negative</td>
<td>Positive</td>
<td>&gt; 1,000</td>
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</tr>
</tbody>
</table>
| NT: strains that could not be typed by the genotyping test.

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, Castilla-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 LiPA test. Genotypes A and D were the most commonly found (249 cases, 89.1%) and genotype D was the most prevalent (151 cases, 65.1%). However, the prevalence of genotype D was significantly lower among the HBeAg-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 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 endemcity of the HBV infection in most urban and rural areas unrelated with the Amazonian Basin.

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 detection of HBV strains from the antigenic subtype ayw3. In addition, the significantly higher prevalence of genotype D found among the anti-HBe-positive carriers agrees with data suggesting that strains of this genotype may show a pronounced trend to establish HBeAg-negative chronic infections due to selection of pre-core-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 India 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.

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