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.

Genotypes of the virus of the hepatitis B detected by hybridisation reverse in tira (LiPA) en portadores crónicos residentes en España

Se ha encontrado una alta prevalencia de los genotipos A y D del virus de la hepatitis B al estudiar 278 portadores residentes en España. Además, el 9% de las infecciones se asociaron a los genotipos B, C, E y F, incluyendo 2 casos de infección por genotipo E en portadores españoles que nunca habían visitado África. Estos resultados indican que el genotipo E ha comenzado ya a circular entre la población española, como ya lo hizo el genotipo F en el pasado.


Introduction

Molecular studies performed on hepatitis B virus (HBV) genomes have rendered the identification of six major genotypes, namely genotypes A-F, whose complete sequences have already been obtained. An additional HBV genotype, the genotype G, has been found among a few chronic carriers from France and the United States and has shown to belong to an independent phylogenetic branch. Recently, a novel genotype (genotype H), closely related with genotype F, has been found in Central America.

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 centers from Spain. Since these samples were sent for study just for diagnostic purposes and without a specific request, they are not representatives 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) A(A/T)A AAG GGA CTC A/C-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 GGT GAC TCA AGA AGA GGT ATG TTC GCC GGT GTT ACC-3' and 5'-GTT AAT /AA GGT ATG AGA GGT A/GCA GG CTC A/G/ C/A-3', respectively)9,10. A final fragment of 341 base pairs, encoding aminoacids 89 to 211 from the HBsAg molecule, was finally obtained from all the HBsAg-positive samples. Since the nested primers in the n-PCR test were bimolecular and could be directly tested for the identification of HBV A-G genotypes by a reverse hybridization test that uses a collection of genotype-specific probes adsorbed on nitrocellulose strips (Line Probe Assay, INNO-LiPA HBV Genotyping, Innogenetics, Belgium), the genotypes A-G have been identified by hybridization reverse in tira (LiPA).
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>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>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></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>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>&gt;1,000</td>
<td>13</td>
<td>1</td>
<td></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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></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></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.

Results

Samples from 278 carriers (38.5%) were positive in the nPCR 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-Ón, 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, 65.1%). However, the prevalence of genotype D was significantly lower among the HBeAg-positive carriers (47.2 vs. 89.5%) and genotype D was the most prevalent (181 cases, 65.1%); contrary to Spain from Latin American countries in the last 20 years. This finding agrees with prior data suggesting that strains of this genotype may show a pronounced trend to establish precore-defective chronic infections due to selection of precore-defective mutants8. 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.

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 population9, as already suggested by the prior observation of HBV strains from the antigenic subtype adwe8. 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 HBeAg-negative chronic infections due to selection of precore-defective mutants8. 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.

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 genotypes A and D, 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 Basin11.

Although the investigations regarding the influence of the HBV genotypes on the events of the viral persistence 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 arise, 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.
Acknowledgments

The authors wish to thank the following hospitals and transfusion centres for sending the samples from HBV carriers involved in this study: Hospital de Poniente and Hospital de La Inmaculada, Almería; Hospital Infanta Helena, Huelva; Hospital de La Línea, Cádiz; Hospital San Juan de la Cruz, Albacete; Hospital de Santa Bárbara, Hospital Mancha-Centro and Complejo Hospitalario de Ciudad Real, Ciudad Real; Hospital General Universitario, Guadalajara; Hospital Universitario, Toledo; Hospital General Yagüe, Burgos; Hospital del Bierzo, León; Banco de Sangre de Palencia, Palencia; Hospital General de Segovia, Segovia; Hospital General de Soria, Soria; Centro de Damián de Sangre de la Cruz Roja, Coruña; Hospital de Llerena, Hospital Infanta Cristina, Hospital de Mérida, Hospital Juan Sánchez Cortes and Banco de Donaciones de Extremadura, Badajoz; Hospital Ciudad de Coria, Hospital Campo Aranazalo and Hospital San Pedro de Alcázar, Cáceres, Hospital Arquitecto Marcide, A Coruña; Hospital da Costa, Lugo; Complexo Hospitalario de Pontevedra and Hospital de Valdeorras, Pontevedra; Fundación Hospital de Alcorcón, Hospital de La Princesa, Hospital Príncipe de Asturias, Hospital de Móstoles, Hospital Reina Sofia, Cáceres; Hospital General de Alicante, Hospital Marina Baixa and Hospital General de Elche, Alicante.

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