Characterization of hepatitis B virus genotypes in chronically infected patients

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SUMMARY
Genomic mutations occurring during reverse transcription of hepatitis B virus (HBV) could explain its genetic diversity and account for 8 genetically distinct genotypes that are geographically distributed quite differently. The main objectives of this study were to determine the prevalence of hepatitis B virus genotypes in patients with chronic hepatitis B and to see if there was a relationship between genotypes and risk factors for transmission based on HBeAg status. A total of 14 serum samples were analyzed using INNO-LIPA HBV genotyping assay. Genotype D was the most prevalent (64.3%) followed by genotype A (28.6%). There was one case of co-infection (D/E genotypes) that was confirmed by PCR sequencing. All patients except one were HBeAg-negative and anti-HBe-positive. The risk factors for HBV transmission were unknown in half of the cases; in the other half, sexual, transfusion, maternal or interfamilial transmission were observed. The results show that genotype D is the most prevalent genotype in our hospital, followed by genotype A. On the other hand, no relationship was found between HBeAg status and genotype.

Key words: Hepatitis B virus - Genotype - Co-infection - Seroconversion - Risk factors for transmission

Caracterización de los genotipos del virus de la hepatitis B en pacientes infectados crónicamente

RESUMEN
Las mutaciones genómicas presentes durante la transcripción inversa del virus de la hepatitis B (VHB) podrían explicar su variabilidad genética y ser la causa de la existencia de ocho genotipos distintos que presentan una distribución geográfica diferente. Los objetivos principales del presente trabajo fueron determinar la prevalencia de los genotipos del virus de la hepatitis B en pacientes con hepatitis crónica B y buscar la relación entre los genotipos y los factores de riesgo de transmisión con respecto al estado del HBeAg. Se analizaron 14 muestras de suero utilizando el kit INNO-LIPA HBV Genotyping assay. El genotipo D fue el más prevalente (64.3%), seguido del genotipo A (28.6%). En una muestra se detectó una coinfección (genotipos D/E) que fue confirmada mediante secuenciación. Todos los pacientes, excepto uno, presentaron HBeAg negativo y anti-HBe positivo. Con respecto a los factores de riesgo de transmisión del VHB, en la mitad de los casos se desconocían, en la otra mitad se observaron transmisiones sexuales, mediante transfusión, verticales e intrafamiliares. Estos resultados demuestran que el genotipo D es el más prevalente en nuestro hospital, seguido del genotipo A. Por otra parte, no hemos encontrado relación entre el estado del HBeAg y el genotipo implicado en la infección.

Palabras clave: Virus de la hepatitis B - Genotipos - Coinfección - Seroconversión - Factores de riesgo de transmisión
INTRODUCTION

Hepatitis B virus (HBV) infection is a major cause of acute and chronic liver disease worldwide. More than 350-400 million people are chronically infected with HBV (1) and are at a very high risk for developing severe liver diseases, including cirrhosis and hepatocellular carcinoma. Every year, approximately one million people die due to HBV infection, with 33% of cases caused by hepatocellular carcinoma and the rest by terminal complications of liver disease (2).

Genomic mutations occurring during reverse transcription could explain the genetic diversity of HBV and account for 8 genetically distinct genotypes, designated A to H. Most genotypes are widely distributed throughout the world (3).

During the natural course of chronic HBV infection, HBeAg antigen (HBeAg) is considered as a marker for viral replication; seroconversion to anti-HBe antibody is generally associated with absence of detectable viral replication and a return of alanine aminotransferase (ALT) levels to normal or nearly normal (4). Some genotypes, such as genotypes A and C, are more prevalent compared to genotype B and D in HBeAg-positive than in anti-HBe-positive patients (5).

Recognized risk factors for hepatitis B include blood transfusions, needle sharing by intravenous drug users, recycling of contaminated needles for acupuncture, piercing and tattoos, sexual transmission, perinatal transmission, homosexual men, promiscuous heterosexuals, immunosuppressed patients, patients on dialysis, transplant patients, and health care transmission (6).

The aim of the present study was to determine the prevalence of HBV genotypes in a group of patients with chronic hepatitis B at our hospital, and to investigate the relationship between those genotypes and the risk of transmission based on HBeAg status.

PATIENTS AND METHODS

Patients

Serum samples were collected from 14 chronic HBV patients (9 men, 5 women) from the Digestive Service in Baseruto’s Hospital in Bilbao. None of these patients had any evidence of other liver disease. The mean age was 46.57 years (range 14-61 years). All patients were tested for liver profile (serum albumin, ALT, alkaline phosphatase and bilirubin levels). All participants in the study had given their consent.

Serological testing

Hepatitis B virus surface antigen (HBsAg), antibody against HBsAg (anti-HBs), hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) were determined by ADVIA Centaur (Bayer Healthcare LLC, Diagnostic Division, Tarrytown, NY).

Serum HBV nucleic acid was isolated with an automated DNA extraction on the COBAS AmpliPrep and quantified using a real time PCR assay COBAS TaqMan HBV test (Roche Diagnostics, Germany).

HBV genotyping

HBV DNA was isolated from serum by using the commercially available QUIamp DNA mini-kit (Qiagen, Hilden, Germany). Amplification was carried out by nested PCR assay using primers from the HBV P gene region as described previously (7). Amplification products were hybridized using a line probe assay designed to identify hepatitis B virus genotypes A to H by the detection of type-specific sequences in the HBV polymerase gene domain B to C (INNO-LIPA HBV Genotyping Assay; Innogenetics, Belgium) according to the manufacturer’s instructions. The presence of mixed genotype infection was verified by sequencing. For that purpose, the PCR product was purified with the QUIAquick PCR purification kit (Qiagen, Hilden, Germany) and was sequenced using the BigDye Terminator Cycle Sequencing Kit on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, USA).

RESULTS

The INNO-LIPA assay is a convenient tool for detecting HBV genotypes. All samples could be identified. In most cases, only a single-step PCR and hybridization was necessary for HBV genotype detection. A nested PCR and subsequent hybridization was necessary in only two serum samples (14.3%) for genotype determination.

Determination of HBV genotypes showed that in our study population genotype D was dominant in 9/14 cases (64.3%) and genotype A was observed in 4 patients (28.6%). Mixed genotype was detected in only one patient who had a genotypes D/E co-infection. This genotype co-infection was confirmed by sequencing PCR. The distribution of HBV genotype according to the HBeAg status showed that all patients except one were HBeAg-negative and anti-HBe-positive. The only patient testing HBeAg-positive showed genotype D and had previously had a seroconversion but later experienced a new reactivation (Table 1).

With respect to risk factors for HBV acquisition, the most common were unknown in 7/14 (50%) samples, followed by sexual (21.4%), transfusion (14.3%), maternal (7.1%) and interfamilial (7.1%) transmission (Table 2).
Due to its high replication capacity with a release of up to $10^{13}$ viral particles per day and the high error rate of viral reverse transcriptase, hepatitis B virus is able to adapt to the host’s environment (8). Therefore, hepatitis B virus sequence has different types of variability (9). The first type of variability reflects the HBV genotype or local strain. In addition, a specific genotype is stably transmitted within the host population and is always present from the beginning of infection in a patient.

These genotypes show a distinct geographic distribution (3, 10, 11): genotype A is most prevalent in Northern and Middle Europe, the U.S. and South Africa; genotypes B and C are found in the Far East; genotype D is predominant in the Mediterranean area, and in the Middle and Near East; genotype E is typical of Africa; genotype F is predominant in South and Central America; genotype G is found in Europe and North America; and finally, all strains of the recently identified genotype H are found in North and Central America. The prevalence of genotype D in the Mediterranean area, Southern and Eastern Europe and the Middle East was confirmed in the present study (64.3%), followed by genotype A (28.6%), which is commonly found in South and Eastern Europe.

Genotypes may result from evolutionary drift of viral genome, from recombination, or as a consequence of a long-term adaptation of the virus to genetic determinants of specific host populations. Genotype co-infection raises the possibility of recombination between genotypes. In our study group, there was one female patient with D/E genotypes co-infection that was caused by an unknown risk factor. During her childhood she had lived in an orphanage and had blood transfusions. Genotype co-infection is considered to be a rare event, probably due to suppression of the replication of the second strain, or masked by the very high virus load of the first strain (12).

Different risk factors of HBV acquisition have been described (6). However, in analyzed patients unknown transmission is the most prevalent, followed by sexual transmission. Some studies have indicated that genotype A is more efficiently transmitted sexually than genotype D (13). However, based on our data and the small number of patients, we could not corroborate those findings.

On the other hand, some authors suggest that clearance of HBeAg may occur by different mechanisms in patients with hepatitis B caused by different genotypes (14). In that regard, genotype B and D are associated with a higher rate of seroconversion from HBeAg to anti-HBe and the predominance of genotype A in HBeAg-positive cases has been described in several studies (5, 15). This could be indirect evidence of the differences in the incidence rates of HBeAg loss and seroconversion among those genotypes. However, our results showed that all patients infected with genotype A had a seroconversion and only one patient with genotype D was HBeAg-positive. This could be due to the small number of patients tested, which resulted in fewer genotypes A being analyzed.

In conclusion, the results of our study confirm that genotype D is the most prevalent, followed by genotype A, in chronically infected patients in our hospital. Moreover, there was no association between HBeAg status and identified genotypes. However, more studies with a larger number of patients are necessary to determine a possible association.

**ACKNOWLEDGEMENTS**

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<th>HBeAg-negative</th>
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<td>8</td>
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**Table 2. Risk factors for HBV acquisition.**

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<th>Genotypes D/E</th>
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<tr>
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REFERENCES