Hepatitis B Virus Genotyping in a Cohort of Chronic Hepatitis B Patients from Saudi Arabia: Relationships with Clinical and Laboratory Features

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Abstract

Chronic hepatitis B (CHB) is a leading cause of liver cirrhosis and its complications globally. An estimated 350-400 million people are chronically infected with hepatitis B virus (HBV). HBV genotype has been linked to HBV patient outcome and treatment response, with 8 HBV genotypes of variable distribution around the world.

Methods: A prospective study was performed to determine the genotypes of HBV present among a cohort of patients randomly selected from the King Abdulaziz University Hospital Hepatology Outpatient Department from January 2012 to December 2012. We collected demographic data (age, sex, and nationality) and obtained serum alanine aminotransferase (ALT) and bilirubin levels. HBV genotyping was performed using molecular tests (INNO-LiPA HBV Genotyping A to H).

Results: A total of 62 patients were enrolled, of which the majority were female (32, 51.2%) and Saudi (43, 69.4%), and the mean age was 43.61 years with a standard deviation (SD) of 12.04 (20-72). The most common genotype was genotype D or genotype D mixed with other genotypes (54, 87.1%).

The HBV DNA levels were significantly lower in genotype D patients compared with the other genotypes, even after exclusion of one genotype C patient with a very high viral titer that skewed the results (mean=73,161 IU/ml, SD=372,731.8 IU/ml versus mean=782,873 IU/ml, SD=1,376,852.8 IU/ml, respectively; P<0.001).

One out of three patients with decompensate cirrhosis showed HBV genotype D, and 5 of 6 patients with ultrasound-evident portal hypertension also presented genotype D (P=0.005). Treatment with oral antivirals was initiated in 13 (21%) patients; 4 received only lamivudine, 7 received entecavir and 2 received lamivudine followed by entecavir. The mean duration of treatment was 39±27.24 (12-105) months.

In conclusion, our report showed that genotype D was the most common in our cohort of patients from western Saudi Arabia.

INTRODUCTION

Chronic hepatitis B (CHB) is a leading cause of liver cirrhosis and its associated complications globally [1-4]. It has been estimated that 350-400 million people are chronically infected with hepatitis B virus (HBV), with an estimated 15-40% of patients developing serious liver disease-related outcomes [2,5]. The HBV double-stranded DNA level is an essential determinant of the outcome of HBV infection [6]. The HBV genotype is another determinant of HBV outcome [1,7-13] as well as an important predictor of response to interferon and oral antiviral therapy [2,3,11]. There are at least ten different methods used to detect HBV genotypes [14-17]. Eight HBV genotypes have been recognized (A through H) [1,7,9,11,12], and these genotypes show different distributions in different parts of the world [1,10,17-27]. Only a few reports from Saudi Arabia have provided data on HBV genotyping [15,28,29], and the most frequently reported genotype among CHB patients is genotype D [18,29,30]. The aim of this study was to determine the genotypes of HBV and its associated clinical features among CHB patients at the hepatology clinic at the largest tertiary university hospital in western Saudi Arabia (King Abdulaziz University Hospital in Jeddah).

METHODS

Objectives

The study period spanned from January to December 2012.
Study design

This was a prospective cohort study. The study cohort was randomly selected from patients with CHB. Inclusion criteria: patients who had CHB and regular outpatient visits to the Hepatology Outpatient Department at King Abdulaziz University Hospital every 3-4 month over a period of at least three years including regular checkup for HBV PCR and serum ALT.

Exclusion criteria: Patients with hepatitis C virus or human immunodeficiency virus (HIV) co-infection were excluded, as were patients who refused to provide blood samples and those who did not have detectable viremia according to the polymerase chain reaction (PCR) method.

Medical college ethical committee approval was obtained for the publication of this work. Additionally, the molecular biology lab agreed to conduct the study and provided the reagents needed for testing the HBV genotypes. All included patients gave their approval to participate in the study before blood testing was performed.

From each patient, we collected demographic data, including age, sex, and nationality. We also reviewed the patient files for clinical data, including a diagnosis of compensated or decompensated liver disease.

We also obtained laboratory test results for serum alanine aminotransferase (ALT) and serum bilirubin at the time of HBV genotype testing. We reviewed the HBV immunological serum markers available from the hospital information system to obtain the antigen status for all patients. Each patient received HBV DNA testing using the HBV PCR assay (TaqMan Roche Diagnostic, Basel, Switzerland). Specifically, genotype testing was performed using the INNO-LiPA HBV genotyping assay, which was designed to identify HBV genotypes A through H through the detection of type-specific sequences in the polymerase gene domains B to C.

For those patients who received treatment, the treatment duration and the medication regimen were recorded. The duration of treatment was calculated in months. Patients with lamivudine resistance were identified.

Statistical method

We performed statistical analysis using the IBM Statistical Package for Social Sciences (SPSS) version 20. We obtained frequencies and descriptive statistics and used a t-test to compare the mean HBV DNA levels between males and females. We used one-way analysis of variance (ANOVA) and Tukey’s post-hoc test to compare variables between patients with normal abdominal ultrasound, those with asymptomatic portal hypertension evident on ultrasound (dilated portal vein and or its territories and collaterals formation) and patients with ascites.

RESULTS

During the study period, we tested 62 CHB patients for HBV genotype according to the inclusion criteria.

The majority of patients were female (n=32, 51.2%) and Saudi (n=43, 69.4%), and the mean age was 43.61 years (range=20-72; SD=12.04). The demographic data are provided in Table 1. Only 2 patients (3.1%) tested positive for HBV e antigen the remaining 60 patients (97%) tested negative for the e antigen.

The mean serum ALT and platelet counts are shown in Table 1. There was no difference in the serum ALT levels between males and females. The platelet counts in male patients were significantly lower than those in females (Table 1).

One female patient with a very high titer of HBV DNA (1.7x10^9 IU/ml) was excluded due to skewing of the HBV-PCR results (mean=154,603 IU/ml; SD=603,584 IU/ml).

The most common genotype was genotype D (either alone or mixed with other genotypes) (n=54, 87.1%) (Table 2,3) There were no differences in the serum ALT values between the different genotypes.

The HBV DNA levels were significantly lower in genotype D patients compared with the other genotypes, even after exclusion of the one genotype C patient with a very high viral titer that skewed the results (mean=73,161 IU/ml, SD=372,318 IU/ml versus mean=782,873 IU/ml, SD=1,376,852 IU/ml, respectively; P<0.001).

Patients who had no clinical or radiological evidence...
of cirrhosis were significantly younger than those who had ultrasound evidence of portal hypertension and those with clinically evident decompensate cirrhosis and ascites (difference in age of 12.1 and 19.7 years, respectively; P=0.029 and 0.008, respectively).

Similarly, platelet counts were significantly lower in decompensate patients compared to patients with asymptomatic portal hypertension and patients with normal ultrasound of the abdomen (difference of 90 k/µl and -127.7 k/µl, respectively; P=0.048 and <0.001, respectively). Moreover, the serum bilirubin levels were higher in symptomatic decompensate patients compared to normal and asymptomatic patients with radiological evidence of portal hypertension (difference of 21 µmol/L and 22.5 µmol/L and P=0.008 and 0.017, respectively).

In contrast, the HBV DNA levels were significantly higher in patients with clinically evident, decompensate cirrhosis compared to both patients with only radiological evidence of portal hypertension and patients without radiological or clinical evidence of cirrhosis (mean differences of 1,944,107.3 and 1,926,193.05, respectively; P<0.001 for both).

Treatment with oral antivirals was initiated in 13 patients (21%), with 4 patients receiving only lamivudine (100 mg), 7 patients receiving entecavir (0.5 mg), and 2 patients receiving lamivudine followed by entecavir. The mean duration of treatment was 39±27.24 months (range=12-105 months). Eleven patients maintained sustained viral suppression. Two patients were shifted from lamivudine to entecavir treatment (1 mg per day) after developing lamivudine resistance. One of these patients received lamivudine treatment for approximately 4-5 years before developing resistance; both patients achieved sustained viral suppression on entecavir. Currently, all treated patients have undetectable HBV-DNA or levels less than 12 IU/ml, which we defined as an appropriate reference for response.

DISCUSSION

Our data showed that the most common HBV genotype in our cohort was genotype D, which is consistent with previously reported data from Saudi Arabia in the studies by Abdo et al., (81.4% of patients in Riyadh) and Hajeer et al., (93.9% of CHB patients with genotype D). Similarly, a recent study by Bajrai et al., which was performed at the King Fahad Research Center at our university, showed that HBV genotype D was the most common genotype among asymptomatic blood donors from the western region of Saudi Arabia. This report compared two different methods other than the method used in our study (sequencing and restriction fragment polymorphism) for the detection of HBV genotypes and reported similar results [15]. However, our report is the first from the western region of Saud Arabia to link genotyping results to clinical outcomes and laboratory features of CHB patients. Other regional data from Egyptian and Turkish patients with CHB showed a similar predominance of genotype D [17,20,24]. Genotype D mixed with other genotypes was the second most frequent genotype found in our cohort. Similarly, in the report of Abdo et al., 10% of patients presented this genotype, and Chen et al., reported the presence of mixed genotypes in intravenous drug users [30]. However, the reason for the presence of mixed genotypes in our report and the report of Abdo et al., is unclear because most CHB in Saudi Arabia is due to vertical transmission from mothers to children [31]. Thus, the presence of mixed genotypes in our population requires further investigation, although it may be related to the methods used for genotyping or the result of co-infection with multiple genotypes.

The majority of the patients in our cohort were Saudi. In this country, there has been an effective vaccination program for HVB since 1989, which has led to a significant reduction in the prevalence of CHB [31-33].

However, CHB is still frequently diagnosed in adults born prior to the establishment of the HBV vaccination program.
in Saudi Arabia [31,32], which is supported by the age of the patients in our cohort.

The predominance of females in our cohort may have been due to selection bias. We have shown in previous data on CHB from Saudi Arabia that males are more commonly affected than females. This finding has also been shown in international data of CHB patients younger than 60 years of age [6,28,31,34].

Most of the patients in our cohort with CHB were negative for the HBV e antigen. This result is similar to previous results reported by Abdo et al., for CHB patients from the same region of Saudi Arabia as well as the central region [28,31,34].

Some patients presented abnormal lab results due to advanced liver disease, although the mean serum ALT level, platelet count and prothrombin time fell within normal limits, indicating that most of the patients in our cohort had stable compensated liver disease. This finding is similar to that reported by Yalcin et al. from Turkey [20]. Although our results showed that evidence of portal hypertension was higher in genotype D patients, it was difficult to demonstrate a clear association between genotype D and severe disease because of the small number of other genotypes. However, several international reports have suggested that HBV genotype D might be associated with more advanced disease and may predict the development of hepatocellular carcinoma (HCC) at a young age [2,11,35].

Patients with higher viral loads were more likely to have evidence of portal hypertension and decompensate liver disease. Indeed, it has been shown in multiple previous reports on CHB that HBV viremia is a major factor associated with the progression of liver disease and the development of HCC [2,3,6]. Furthermore, older patients were more likely to have decompensate disease compared to younger patients, and several reports have shown that progression to fibrosis and the development of HCC are increased in patients older than 40 years of age [2,28,34].

The 3 patients with decompensate liver disease were male, which is consistent with our previous report on CHB and other international reports [30,31,36]. It has been speculated that estrogen may have a protective effect in females against the progression of liver disease, making them less likely to advance to liver disease compared to male patients with similar causes of liver disease [37].

Lamivudine resistance is a well-known complication of the prolonged use of lamivudine [2,3,38]. In our cohort, 2 patients who developed lamivudine resistance after 2 and 5 years of treatment were shifted to 1 mg of entecavir, and both patients achieved optimal control of HBV viremia. Entecavir is a potent first-line antiviral therapy that carries only a small risk of resistance during long-term use and is effective for the treatment of lamivudine resistance [2,29,40]. All entecavir-treated patients in our cohort showed sustained responses without the development of resistance.

CONCLUSION

The commonest hepatitis genotype among HBV infected patients in our cohort was genotype D. Although we have shown that genotype D patients are more likely to have advanced liver disease, the small number of patients with other genotypes makes it difficult to link the severity of liver disease to HBV genotype.

FUNDING

This article did not require funding because all laboratory testing, including HBV genotype testing, was performed at the hospital while conducting this study.

CONFLICT OF INTEREST

Dr. Hind Fallatah, Prof Hisham Akbar, Dr. Ahmad A Ghamdi, Faten Qattan and Maha Al Rumani have neither conflicts of interest to be declared in relation to the article nor funding or personal relationships with people or organizations that could inappropriately influence this article.

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