Original Article

Clinical features of hepatitis B virus genotypes in Turkish patients

Sevgi Ciftci,[1] Fahriye Keskin,[2] Selim Badur[3]

Department of Microbiology, Faculty of Dentistry,[1,2] Department of Microbiology and Clinical Microbiology, Faculty of Medicine,[3] Istanbul University, Istanbul, Turkey.

Corresponding Author: Sevgi Ciftci. Email:sevgiciftci@gmail.com

Abstract

Objective: To investigate the relationship between hepatitis B virus (HBV) genotypes and hepatitis B early antigen (HBeAg) as well as the hepatitis C antibody, alanine aminotransferase (ALT) levels and liver inflammation and damage in chronic patients of hepatitis B.

Methods: The study was conducted at the Department of Microbiology, Istanbul University, Istanbul, Turkey, between June 2002 and February 2003. Randomly selected 63 patients of chronic hepatitis B represented the study population. Biochemical and virological activities, and histopathological stages were determined in all the patients. All serum samples were investigated for the presence of hepatitis B virus DNA by nested polymerase chain reaction (PCR). The product of the reaction was sequenced by using Long Read Tower automated DNA sequencer. Clinical characteristics and laboratory features of the patients were compared using Mann-Whitney Rank Sum test, Kruskal Wallis test and Chi Square test for statistical analysis.

Results: Genotype D was the only type found in all patients. Of the 63 patients studied, 16 (25.4%) tested positive for HBeAg. Hepatitis B antigen-negative patients were more elderly than those who were positive ($p<0.05$). The degree of hepatic inflammation, fibrosis stage and alanine aminotransferase levels were similar in both the groups ($p>0.05$). Liver cirrhosis was present in 2 (4%) of the patients in the negative group. In terms of gender, the serum alanine aminotransferase levels were significantly higher in male patients than in females ($p<0.05$).

Conclusion: There was no relationship between the genotype of the patients and their response to the therapy since there was only one genotype detected in the study. The results showed that the hepatitis e antibody-negativity was not associated with good prognosis, while less necrotic activity, and alanine aminotransferase levels were not correlated with the severity of the disease.

Keywords: Hepatitis B virus, Relationship genotypes, Chronic cases, Clinical features. (JPMA 62: 759; 2012)

Introduction

Chronic hepatitis is considered an important health problem due to the fact that it can cause serious hepatic diseases such as cirrhosis and hepatocellular carcinoma (HCC).[1] Patients with significant hepatic inflamation and fibrosis are at the highest risk of these complications.[2] The clinical course of HBV infection depends on many factors, including age at infection, level of ALT, and genetic variability of the virus; such as influencing the expression of viral antigens.[3,4] Eight HBV genotypes have been defined from A to H according to their S gene part.[5-7] Recently, however, HBV genotype I has also been described in northwestern China.[8] HBV displays diverse genotypic distribution in different geographical parts in the world. For example, the frequency of genotype D is higher in the Mediterranean region, India and Southern Europe.[7-9]

The outcome of infection depends on many factors. In recent years, the impact of the variability of the virus on the clinical course of disease has been investigated. However, the effect of HBV genotypes on clinical course and response of the chronic HBV infection patients is stil not well defined. Previous studies have reported that the difference in genotype may be associated with the clinical course.[3,10,11]

In this study, we investigated the HBV genotype distribution in HBV strains obtained from HBV chronic patients and its correlation with clinical and histopathological features.

Patients and Methods

The study population consisted of 63 chronically infected HBV patients who were randomly selected at the Department of Microbiology, Istanbul University, Istanbul, Turkey between June 2002 - February 2003. Informed consent was obtained from all patients. Liver biopsy samples of the patients were examined at the Department of Pathology for histological activity indexes (HAI) and fibrosis stages were evaluated according to Knodell.[12] HAI was assessed as mild activity (A1), moderate to severe activity (A2); and fibrosis stages were evaluated as stage 1,2,3 and 4, respectively. HBsAg, HBeAg and anti-HBe statuses were determined by enzyme immunassay (Organon Teknika BV, Boxtel, Netherlands) in all serum samples. HBV-DNA level was determined by Digine Hybride Capture System (Beltsville USA; sensitivity limit 5 pg/ml). HBV-DNA was extracted from serum samples by using the silica method. First, 50 µl extract was obtained from 100 µl serum.[13] The S gene was amplified by PCR on 5µl extracted DNA with S gene-specific primers: HBVF1; 5-YCCTGCTGGTGGCTCCAGT TC-3, HBVR2; 5-AAGCCANCAYTG GGGGAAAGC-3.45µl reaction mix was made with 0.25 µl Taq DNA polymerase. Amplification was performed for 30 cycles with denaturation at 94°C for 20 sec, annealing at 50°C for 45 sec and elongation at 72°C for 60 sec. Samples negative in first-round PCR were further amplified with nested PCR for 30 cycles in the sameprofile:HBVF2;5-CTAGACTCGTGGTGGACTTCTC-3,HBVR2;5-AAGCCANACAYTGGGGGAAAG C-3. Then, 5µl of the PCR product was analysed by electrophoresis in 1% agarose gels, stained with ethidium bromide and visualised under ultraviolet light. In order to avoid contamination, a maximum physical separation between the pre- and post-amplification steps was done. The PCR products were purified before sequencing by using the High Pure PCR Product Purification Kit (Roche, Germany) and then denaturation was applied. The PCR products were sequenced in both directions using the Cy5/5.5 Dye Primer kit (Visible Genetics, Inc., Toronto, Canada) on a Long Read Tower automated DNA sequencer (Visible Genetics, Inc.) according to the manufacturer's instructions. The sequencing primers were labelled upstream and downstream PCR primers. Visible Genetics computer software was used to align the forward and reverse sequences to ensure reliability of generated sequences and resolve possible ambiguous nucleotides on a Long Read Tower automated DNA sequencer (Visible Genetics, Inc.). The nucleotide sequences were aligned using the Clustal X programme. Distances between pairs of sequences were calculated by using the DNADIST module in Phylogeny Inference Package (PHYLIP) version 3.572 (J. Felstein, Department of Genetics, University of Washington, Seattle, WA, USA). PHYLIP was used to construct phylogenetic trees by means of the neighbour-joining method (Saitou, 1987). The obtained HBV sequences were alligned with reference nucleotide sequences from Genbank. In order to assess the robustness of the groupings obtained, a bootstrap neighbour-joining analysis with 1,000 replications was also performed using Molecular Evolutionary Genetics Analysis (MEGA) version 2.1 (Bioinformatics).

After statistical analysis, baseline information was summarised as mean ± SD for continuous variables, and frequencies for discreate variables. HBeAg prevalence, liver inflamation and damage, ALT level, age and gender was compared for statistical significance using the Mann-Whitney Rank Sum test, Kruskal Wallis test and Chi-Square test.

The study was approved by the Ethical Committee of Istanbul University Groups.

Results

The study included 63 patients with chronic hepatitis B infection. There were 50 (79.3%) males and 13 (30.7%) females. The mean age was 36.2±10.2 years. After demographics, clinical characteristics and laboratory features of the patients (Table) were noted, no significant difference was detected according to gender in age distribution ($p>0.05$). Phylogenetic analysis based on the HBV-S gene revealed that

Table: Characteristics of Patients with Chronic Hepatitis B(n: 63).

	HBeAg+ (n:16)	anti-HBe + (n:47)
Age (year)*	30.3 ± 10.2	38.7 ± 9.4
Gender(F/M)	13	50
ALT (IU/mL)*	78.8±107.9	86.2 ± 131.9
HAI		
A1(mild)	10	30
A2 (moderate+severe)	6	17
Fibrosis stages		
F1(mild)	6	16
F2(moderate)	7	20
F3 (severe)	3	9
F4(cirrhosis)	0	2

*mean ± standard deviation.
F: Female, M: male, ALT: alanine aminotransferase, HBeAg: hepatitis B e antigen, anti-HBe: hepatitis B e antibody, HAI: Histology Activity Index,, F: Fibrosis stage, IU: ?nternational Unit, mL: milliliter, +: positive.

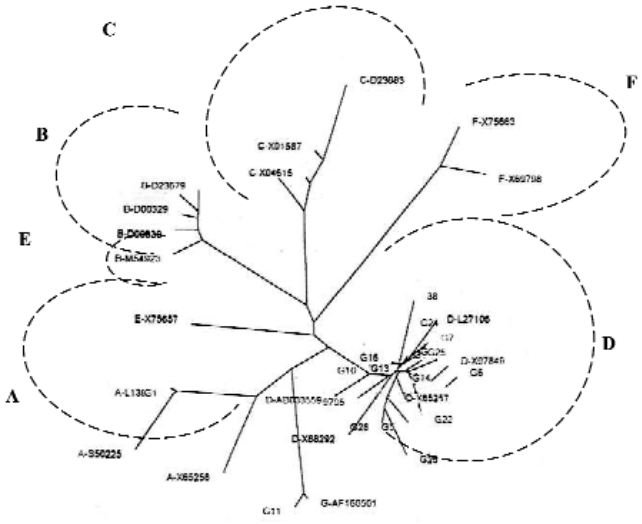

Figure-1: Phylogenetic tree analysis in the S region of the HBV genome.

all the 63 patients were infected with HBV genotype D (Figure-1).

Genotype D was also the only type found in both HBeAg positive and negative patients, independently of ALT levels, histopathological stage, age and gender. The relationship between genotype D with gender, age and clinical features could not be determined since all of the patients in the study had the same genotype.

Of all the patients,16 (25.4%) were HBeAg positive, while 47 (74.6%) were negative. The mean age was significantly higher in the latter group (38.7±9.4 years) compared to the former (30.3±10.2 years, $p<0.05$).

No significant differences were observed between the HbeAg status and the clinical parameter as ALT levels, HAIinfl scores, HAIfibr stages and gender in either group ($p>0.05$).

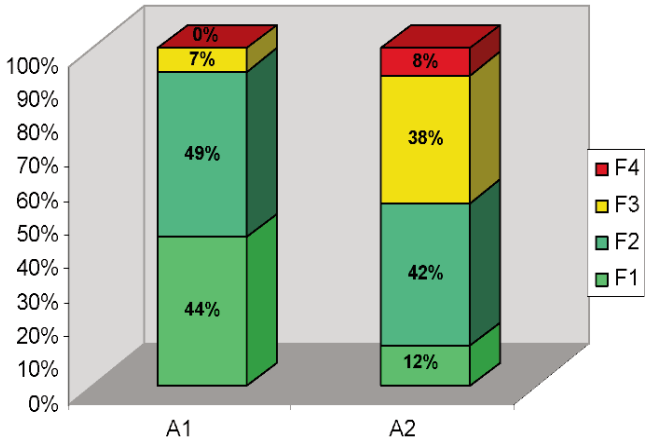

Figure-2: Distribution of fibrosis scores on liver biopsies in chronic hepatitis B patients.
A1: histology activity index-mild, A2: histology activity index-moderate+severe, F: fibrosis stage (F1: mild, F2: moderate, F3: severe, F4: cirrhosis).

Serum ALT levels were significantly higher in male patients (median:86, range: 13-547, $p<0.05$) than in females (median:62, range:11-157). No statistically significant difference was found between ALT levels and other parameters in both groups like HBeAg positivity, HAIinfl scores, HAIfibr stages and age.

The prevalence of A1 inflammation was higher in both groups compared to the prevalence of A2 inflammation ($p>0.05$).

None of the patients in the HBeAg positives had stage F4 fibrosis, but 2 (4%) patients had cirrhosis in the HBeAg negatives. The stage F2 fibrosis was the commonest finding in both groups.

The results of liver biopsies of the patients showed a similar trend that was comparable to the grades of inflammation and degrees of fibrosis. In general, with increase of inflammatory activity, the degree of fibrosis also rose, except for F2 (Figure-2). No patient with F4 fibrosis had A1 inflammation.

Discussion

The geographical distribution of HBV genotypes differs in patients infected with HBV.[5-7,14] In this study, the only genotype determined was genotype D which was present in all the patients. Genotype D is particularly prevalent in countries in the Middle East, Mediterranean region and Central Asia.[7,9] Studies from Turkey have also reported the most prevelant genotype to be genotype D.[14-16] Our results also correlate with all other Turkish studies. This is an important finding because, in Europe, most HBV infections are genotype A and D, and the likelihood of developing chronic liver disease is significantly higher in genotype A

carriers compared to those with genotype D.[14]

Several studies have reported the relation between HBeAg status and natural seroconversion to anti-HBe antibody and HBV genotypes.[2,17] It has been reported that HBeAg positivity existed frequently in patiens infected with genotype A, and the anti-HBe antibody with genotype D.[10,18]

In our patients, anti-HBe positivity (74.6%) was found higher than the HBeAg negativity (25.4%). These findings may explain the high prevalence of genotype D found in patients with chronic hepatitis B and anti-HBe in Mediterranean countries.[19] In recent years, the prevalence of HBeAg-negative chronic hepatitis B has increased worldwide.[20]

Generally, the HBeAg/anti-HBe seroconversion develops during adolescence or early adulthood in individuals infected with genotype D.[20,21] Furthermore, according to literature, progression and genotype correlate with the age of the patient. A study in India suggested that genotype D in young patients may lead to HCC in these patients, but this finding has not been confirmed by any other Indian study.[22,23] In our study, the mean age was higher in anti-HBe positive patients ($p<0.05$) and severe liver damage was less common in early adulthood patients (between 15 and 24; 75%, above 25 years; 25%). Additionally, in female and male patients, an almost similar proportion was observed in HBeAg positivity and negativity (25.0% and 75.0% vs. 21.3% and 78.7%). In contrast, in a Portuguese study HBeAg negativity was more prevalent in female patients with genotype D than in genotype A.[24]

ALT is an enzyme used as an indicator in the evaluation of liver damage. Interestingly, in the present study, there was no significant correlation between ALT levels and histological lesions. In studies taking gender into consideration, ALT was found to be an independent risk factor for HBV infection because abnormal ALT levels have been reported more frequently in males.[25,26] We have also found similar results for gender and ALT levels ($p< 0.05$).

The relationship between the HBV genotypes and the liver damage has been investigated in many studies, but no clear association has been found till now.[27] Since all the patients in this study were of genotype D, it is not possible to comment on any relation between response to treatment and genotype. It is known that HBeAg/anti-HBe seroconversion is not associated with good prognosis, less necrotic activity or less replication and infectivity. In fact, 16 (34%) of our HBeAg-negative patients had no or mild fibrosis in liver biopsies; 20 (43%) had moderate; 9 (19%) had severe; and 2 (4%) had cirrhosis.

Conclusion

According to the study results, anti-HBe positivity was not associated with good prognosis, while less necrotic activity and ALT levels were not correlated with the severity of the liver disease. Such studies are important and necessary both for epidemiological reasons and for planning rational treatment strategies. Large-scale longitudinal studies are recommended for the future.

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