Genotypes and Viremia of Hepatitis B and D Viruses Are Associated With Outcomes of Chronic Hepatitis D Patients

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Background & Aims: **Genotypes and viremia of hepatitis D virus (HDV) and hepatitis B virus (HBV) may be associated with outcomes. This study evaluated the impact of viral genotypes and viremia on outcomes of dual HBV and HDV infection.** ***Methods:*** **Viremia and viral genotypes were analyzed in 194 consecutive chronic hepatitis B patients with HDV superinfection and correlated with outcomes.** ***Results:*** **The numbers of HBV genotype A, B, C, and nonclassified were 4, 57, 23, and 110, respectively. There were 51 genotype I HDV, 74 genotype II HDV, 8 genotype IV HDV, and 61 nonclassified HDV genotype. In a median follow-up of 135 months, 24 progressed to cirrhosis and 41 developed hepatocellular carcinoma. Patients infected with genotype I HDV had a lower remission rate (15.2% vs 40.2%; $P = .007$) and more adverse outcomes (cirrhosis, hepatocellular carcinoma, or mortality) (52.2% vs 25.0%; $P = .005$) than those with genotype II HDV. Patients infected with genotype C HBV had a lower remission rate (0 vs 32.1%; $P = .005$) and more adverse outcomes (70.0% vs 33.9%; $P = .005$) than those with genotype B HBV. The presence of HBV or HDV viremia was associated with lower remission rates compared with those negative for both (26.4% and 24.3% vs 69.2%; $P < .001$). In multivariate analysis, age, genotype C HBV, and genotype I HDV were independent factors associated with adverse outcomes.** ***Conclusions:*** **In chronic HBV and HDV dual infections, older age, genotype I HDV, and genotype C HBV correlated with adverse outcomes.**

Hepatitis B virus (HBV) infects more than 2 billion people in the world, and 350 million of these people are chronic carriers of the virus.[1–3] HBV infection could cause a wide clinical spectrum, ranging from acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carrier status, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).[4] Based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into 8 genotypes: A–H.[3,5,6] In Asia, genotypes B and C are predominant in patients with chronic hepatitis B. Recent studies revealed a correlation between HBV genotypes and clinical manifestations and therapeutic effect.[7–19] Briefly, HBV genotype C is associated with a lower rate of hepatitis B e antigen (HBeAg) seroconversion, more serious liver disease, a higher serum HBV DNA level, worse response to antiviral therapy, and poorer clinical outcome than genotype B.

Hepatitis D virus (HDV) is a defective, single-stranded RNA virus that needs the supply of hepatitis B surface antigen for its assembly and transmission.[20,21] HDV superinfection in patients with chronic hepatitis B may also cause various clinical manifestations and prognoses.[22–24] It is an important etiology of fulminant or subfulminant hepatitis in Taiwan.[25] Three genotypes of HDV had been identified previously: genotype I, widely distributed around the world; genotype II, mainly found in Asia; and genotype III, isolated from South America.[26–32] Recently, 4 additional new genotypes have been classified by Radjef et al: genotype IV (genotype IIb by old nomenclature),[33] distributed in Taiwan and Okinawa islands, and genotypes V–VII, found in west and central Africa.[34] Our previous study showed that genotype I is relatively more pathogenic than genotype II.[27]

The role of HBV, hepatitis C virus (HCV), and HDV in dual or triple infections was studied in the past.[35–37] Nevertheless, the integrated impact of HBV and HDV genotypes on disease outcomes of HDV infection is still obscure. Taiwan is a unique area in that both genotype B and C HBV as well as genotype I and II HDV could be found in the population.[3,7,27] In this study, 194 HDV superinfected patients were

Abbreviations used in this paper: BCP, basic core promoter; PCR, polymerase chain reaction.

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analyzed to investigate the clinical significance of HBV and HDV genotypes in a longitudinal manner. The influence of HBV precore, basic core promoter (BCP), and other mutations on prognosis was also determined.

Materials and Methods

Patients

From 1983 to 2003, consecutive Taiwanese patients with chronic HBV and HDV dual infections who visited Taipei Veterans General Hospital were included in this study. All patients had underlying chronic hepatitis B, which was diagnosed by a history of positive hepatitis B surface antigen for more than 6 months and a negative immunoglobulin (Ig) M antibody against hepatitis B core antigen. These patients were all positive for antibodies to HDV (anti-HDV). Patients positive for antibodies to HCV or human immunodeficiency virus were excluded from this study. Acute hepatitis A and E were also excluded by assays of serum IgM antibody to hepatitis A virus and serum hepatitis E virus RNA using reverse-transcription polymerase chain reaction (PCR) as previously described.[38] A total of 194 patients were recruited (68 outpatients and 126 inpatients), and none of them had alcoholism, autoimmune hepatitis, Wilson's disease, hemochromatosis, or drug-induced hepatitis.

The diagnostic criteria for various groups of HDV-infected patients have been described previously.[23,39] Acute hepatitis D was defined as de novo seroconversion or increasing titer of anti-HDV from a low titer $<$1:100 and serum alanine aminotransferase (ALT) levels more than 10 times the upper normal value.[23,39] In patients with acute nonfulminant hepatitis, 17 had de novo seroconversion of anti-HDV and the remaining 41 had a positive test result for anti-HDV in the initial serum sample and were diagnosed based on increasing anti-HDV titer from a low titer $<$1:100. The diagnosis of fulminant or subfulminant hepatitis was based on acute liver failure with hepatic encephalopathy in the absence of preexisting symptomatic liver disease or cirrhosis within 2 weeks or between 2 weeks and 3 months after the onset of jaundice.[23,25,40] In this study, 13 patients presented with fulminant or subfulminant hepatitis. Chronic hepatitis was diagnosed by elevated ALT levels lasting for more than 6 months. Of the 41 patients with chronic hepatitis at the time of diagnosis, cirrhosis was excluded by needle biopsy in 25 patients[25] and the remaining 16 patients were excluded by the absence of cirrhotic signs, esophageal varices, splenomegaly, thrombocytopenia ($<$100,000/mm^3), and image findings. Cirrhosis (23 patients) was verified based on the result of needle biopsies for 11 patients and clinical sonographic findings for the remaining 12 patients.[25] HCC (34 cases) was confirmed histologically in 15 cases and based on α-fetoprotein levels of $>$400 ng/mL and/or positive findings on at least 2 image examinations (sonography, computed tomography, magnetic resonance imaging, and hepatic angiography) in addition to progressive course for the remaining 19 patients.[25] Biochemical remission was defined as normal ALT levels on at least 3 separate samples lasting for at least 12 months without histologic or clinical evidence of cirrhosis or HCC.[25]

These patients were followed up regularly at least every 3–6 months after enrollment. The survival status of the studied patients was obtained from hospital records and further verified by the mortality databank established by the Statistics Office, Department of Health, Republic of China (Taiwan). The mortality databank is based on data from the certificate of death, which contains time, place, and cause of death and details of the person who issued the document.

Serologic Viral Markers and Biochemistry Tests

Serum hepatitis B surface antigen, HBeAg, antibody to hepatitis B e antigen, IgM antibody against hepatitis B core antigen, IgM antibody to hepatitis A virus, and anti-HDV were tested by radioimmunoassay kits (Ausria II-125, HBeAg-RIA, CORAB-M, HAVABM, anti-Delta; Abbott Laboratories, North Chicago, IL). An enzyme immunoassay kit was used to detect IgM anti-HDV (Sorin Biomedica Diagnostics, Saluggia, Italy), while antibody to HCV was detected by a second-generation enzyme immunoassay (Abbott Laboratories). Serum albumin, ALT, and total bilirubin levels were measured with a systemic multiautoanalyzer (Technicon SMAC; Technicon Instruments Corp, Tarrytown, NY).

Detection, Genotyping, and Sequencing

Serum HBV DNA was detected by PCR using primers synthesized according to the consensus sequence of the precore region as described.[41] Moreover, serum HBV DNA was also detected by a spot hybridization technique as described.[42] The quantification of HBV DNA was performed based on a Cobas Amplicor HBV Monitor (Roche Diagnostic Systems, Basel, Switzerland). The limit of detection for this assay was 200 copies/mL. Genotyping of HBV was performed by PCR restriction fragment length polymorphism of the surface gene of HBV.[43,44] Serum HDV RNA was detected using reverse transcription followed by PCR as described.[23] The sensitivity of our assays was about 100 viral genomes/mL.[25] HDV genotypes were also determined by restriction fragment length polymorphism as described previously.[27] In this study, HDV genotypes were classified according to a new genotyping system proposed by Radjef et al.[34] Moreover, serum HDV RNA was also detected by Northern blot hybridization as described.[21,23,24] To confirm the genotyping results, sequencing of PCR products was also performed.[27,44] Sequences of the PCR products of HBV or HDV were determined by the dye terminator cycle sequencing reaction according to the standard protocol provided by the manufacturer (Dye Terminator Cycle Sequencing Core Kit 402117; Perkin-Elmer Cetus Corp, Norwalk, CT). The sequencing products were precipitated with