Quantitative Detection of Hepatitis B Virus DNA in Sera from Patients with Acute Hepatitis B

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Two hundred forty-four serial serum samples from 30 adults hospitalized with benign (nonfulminant) acute hepatitis B were tested for the presence of hepatitis B virus (HBV) DNA by a quantitative solution hybridization assay using a 125I-labeled DNA probe complementary to HBV-DNA sequences. Acute hepatitis B was self-limiting in 28 and progressed to chronicity in the remaining two patients. Of the 28 patients with self-limiting hepatitis, 21 (75%) were hepatitis B e antigen (HBeAg) positive, 26 (93%) were HBV-DNA positive, and one patient (3.6%) was negative for both markers on admission to the hospital. HBV-DNA cleared after HBeAg clearance in 20 (71.4%), before HBeAg clearance in five (17.9%) and simultaneously with the loss of HBeAg in the remaining two (7.1%) of the 27 initially HBV-DNA- and/or HBeAg-positive patients. Moreover, HBV-DNA remained detectable in serum for 13.3 ± 6.6 (range: 4–22) days after the appearance of anti-HBe in 71.4% of these patients. In contrast, HBV-DNA and HBeAg remained persistently positive in the two patients who developed chronic HBV infection. These data show that: (1) viremia frequently persists after disappearance of HBeAg and (2) appearance of anti-HBe does not indicate the cessation of HBV replication in adults with acute self-limiting hepatitis B.

KEY WORDS: acute hepatitis B; hepatitis B virus DNA; solution hybridization; hepatitis B e antigen; viral replication.

Hepatitis B e antigen (HBeAg) is secreted from infected hepatocytes, shares considerable amino acid sequences with hepatitis B virus (HBV) core antigen, and has been used as an indirect serological marker of viral replication and infectivity in HBV infection (1). Seroconversion to the corresponding antibody (anti-HBe) is considered to be associated with cessation or low-level replication of HBV, although there is experimental (2) as well as clinical evidence (3, 4) of HBV transmission from HBsAg-positive individuals with anti-HBe. However, the application of molecular hybridization techniques to the study of HBV infection has greatly elucidated the natural history of HBV-related disease. Serum HBV-DNA represents a more sensitive and direct index of HBV replication irrespective of the HBeAg/anti-HBe status (5–7).

Serum HBV-DNA has been primarily measured by molecular hybridization of a 32P-labeled HBV-DNA probe to extracts of sera (8). This method is time-consuming and complex in terms of the numbers of steps, reagents, and apparatus required. Moreover, interpretation of autoradiographic sig-
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tens is often subjective and semiquantitative. Recently, a quantitative solution phase hybridization assay using a $^{125}$I-labeled DNA probe has been developed (9). This assay is simple and represents a standardized molecular hybridization test for HBV-DNA in serum.

In the present study, we have quantitatively analyzed serial serum samples for HBV-DNA from 28 patients with acute self-limiting hepatitis B and two patients with acute hepatitis B that progressed to chronicity.

MATERIALS AND METHODS

From a total of 190 cases of acute hepatitis B (HBsAg positive) consecutively admitted to our Liver Unit between February and June 1986, 28 patients were selected who had acute self-limiting hepatitis and from whom a series of evenly spaced serum samples were available. Two patients whose acute hepatitis B progressed to a chronic HBsAg carriership, admitted to our Liver Unit during 1986, were also studied. The average number of serum samples available for DNA analysis from each of the 30 patients was 8.1 (range: 7–10). Of the 244 serum samples, 184 sera were collected during the acute phase (4–71 days after the onset of hepatitis) and 60 were collected during the convalescent phase (89–387 days after the onset of hepatitis). Our basic schedule was to collect acute-phase serum samples on admission, three days subsequent to admission, and at the end of weeks 1, 2, 3, 4, and/or 5 after hospitalization. The mean age of the 30 patients (22 males, 8 females) was 33 ± 12.5 (range: 18–59) years; they were hospitalized 12.3 ± 7 (range: 4–30) days after the onset of symptoms compatible with acute hepatitis. The possible source of HBV infection was parenteral drug abuse in six, blood transfusions in 12, dental or surgical procedures in seven, and unknown in five patients. Acute phase of the disease was defined as the first 10 weeks after the onset of symptoms and/or signs of hepatitis.

Acute viral hepatitis was diagnosed on the basis of typical features, compatible liver function tests [alanine aminotransferase (ALT) activity more than eight times the upper limit of normal], and, where appropriate, the exclusion of other causes of jaundice. Selection for the study required: (1) presence of HBsAg and IgM antibody to hepatitis B core antigen (IgM anti-HBc) determined by an enzyme immunoassay (Corzyme-M), (2) absence of IgM antibody to hepatitis A virus (IgM anti-HAV) and antibody to hepatitis delta virus (anti-HD), and (3) benign clinical course.

Commercially available enzyme immunoassays (Abbott Laboratories, North Chicago, Illinois) were used for the detection of HBsAg, antibody to HBsAg (antib-HBs), anti-HBc, HBeAg, anti-HBe, anti-HD, IgM anti-HAV, and IgM anti-HBc. The commercial solid-phase enzyme immunoassay for IgM anti-HBc is designed to detect high

ters of IgM anti-HBc such as occur during the acute phase of type B hepatitis. It has been found to be highly sensitive (98.4–100%) and specific (96.6–100%) (10, 11). Therefore, it may differentiate between acute and chronic (HBsAg positive) HBV infection (10). In addition, serum samples obtained on admission and one month and three months after onset of acute hepatitis were retrospectively tested for antibodies to hepatitis C virus (anti-HCV) by a second-generation enzyme immunoassay (Abbott Labs). Supplementary testing with recombinant immunoblot assays (RIBA-2, Ortho Diagnostic Systems, Raritan, New Jersey) was done in all anti-HCV-positive cases. Anti-HCV was considered detectable only if confirmed by RIBA-2.

Serum levels of HBV-DNA were measured by a solution hybridization assay that employed a single-stranded $^{125}$I-DNA probe with a minimum specific activity of 10$^9$ cpm/µg (9). Solubilized HBV-DNA in 100 µl of serum was hybridized to the DNA probe over 16–18 hr at 65°C. The hybridization mixture was then applied to a column containing a gel matrix that separated hybridized from nonhybridized probe, and the activity of the hybridized DNA was analyzed in a gamma counter. The samples were quantitated relative to the positive control and expressed in picograms per milliliter. The detection limit of the assay is 0.15 pg HBV-DNA or approximately 4 × 10$^5$ HBV genomes (9). Sensitivity and specificity of the assay were described previously (9).

Statistical analysis was carried out by using the chi-square method and Student's t test.

RESULTS

Acute Self-Limiting Hepatitis. HBV-DNA was detected in 27 (96.4%) of the 28 acute self-limiting hepatitis B patients. HBV-DNA was not demonstrated in any tested serum (five acute-, two convalescent-phase) samples of a 19-year-old homosexual man who had been admitted to the hospital 15 days after the onset of symptoms. Of the 27 HBV-DNA-positive cases, 26 were found to be positive on admission to the hospital. The 27th patient became seropositive three days after hospitalization. Absence of HBeAg from serum, on admission to the hospital, was not accompanied by clearance of HBV-DNA since six of seven HBeAg-negative patients were HBV-DNA positive. Two of these six patients were already seroconverted to anti-HBe.

Anti-HCV was detectable in serum of four (67%) of the six parenteral drug abusers on admission to the hospital. HBV DNA cleared after HBeAg clearance in three anti-HCV-positive subjects and before HBeAg in the fourth anti-HCV-positive subject. All the remaining patients were anti-HCV negative.

HBV-DNA levels in serum samples collected on admission to the hospital are given in Table 1. Eight