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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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 [125I]DNA probe with a minimum specific activity of 10⁶ 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 x 10⁴ 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 HBeAg-negative/HBV-DNA-positive cases had 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

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