Influence of Human Immunodeficiency Virus Type 1 Infection on the Natural Course of Chronic Parenterally Acquired Hepatitis C


The aim of the present study was to investigate the possible role of human immunodeficiency virus (HIV) infection in the natural course of chronic hepatitis C. Seventy-six adult patients with chronic parenterally acquired hepatitis C virus (HCV) infection examined from 1989 to 1993 were enrolled; of these 32 (42.1%) were HIV positive and 44 (57.9%) were HIV negative. Serum HCV RNA quantitation was carried out by polymerase chain reaction in a well-characterized group (n = 20; 11 HIV positive and 9 HIV negative). Distribution of histological findings in liver biopsies from both HIV-infected and noninfected patients was similar. However, within 15 years after initial HCV infection, 8 of 32 (25%) HIV-positive patients developed cirrhosis, in comparison with only 2 of 31 (6.5%) patients in the HIV-negative group (p < 0.05); similar incidences of cirrhosis were found in both patient groups within 5 and 10 years after HCV infection. Most of the HIV-negative cirrhotic patients (9 of 11) developed cirrhosis in a time interval longer than 15 years. Finally, HCV load was almost ten times higher (10-fold dilution) in the HIV-positive group, but this difference did not reach statistical significance in this small study population. These results suggest that HIV infection can alter the natural course of chronic parenterally acquired hepatitis C, causing an unusually rapid progression to cirrhosis.

Hepatitis C virus (HCV) infection is common in intravenous drug abusers and blood-transfusion recipients and can be associated with human immunodeficiency virus (HIV) infection (1, 2). However, the possible role of HIV infection in the natural course of chronic hepatitis C remains controversial (3–5). Several studies have suggested that chronic hepatitis C could be more severe in HIV-infected patients (3, 4), but other studies have failed to find evidence to support this hypothesis (5). Unfortunately, no reports of which we are aware have taken into account the duration of HCV and HIV coinfection, and only small numbers of patients were enrolled in those studies published to date.

HCV replication may play an important role in liver injury and progression of liver disease. Some recent studies have suggested that HIV-related immunodeficiency may be associated with higher levels of HCV viremia (6, 7). Thus, HIV infection could modify the clinical and histological outcome of chronic hepatitis C. The aim of the present study was to investigate the possible role of HIV infection on the natural course of nonsymptomatic chronic type C liver disease. We also examined the influence of HIV infection on HCV viremia levels.

Materials and Methods

Patients. All adult patients with chronic parenterally acquired HCV infection examined at Virgen del Rocio University Hospital, Seville, Spain, from 1989 to 1993 were eligible for enrollment in the study. Patients were referred to our unit, the Viral Hepatitis and AIDS Study Group, for assessment of possible viral hepatitis either because they were found to have elevated ALT levels in routine medical examinations or because they belonged...
to a risk group for parenterally sexually transmitted viral diseases. Seventy-six patients met the following inclusion criteria: (1) asymptomatic liver disease; (2) persistent ALT elevations (> 40 U/l) for more than one year; (3) absence of other toxic, metabolic, vireologic, or immunologic causes of liver damage; (4) availability of liver biopsy; (5) provision of informed consent; (6) Centers for Disease Control (CDC) classification stage II or III in HIV-infected patients; and (7) availability of information about the approximate duration of HCV infection, estimated as the interval (in years) between either blood transfusion or, in drug abusers, the first sharing of needles and the time of the liver biopsy. The demographic characteristics of the study population are shown in Table 1.

Liver Biopsies. Sections of paraffin-embedded needle-biopsy specimens were stained with hematoxylin and eosin, Masson's trichrome, and periodic acid-Schiff after diastase digestion. All liver biopsy sections underwent blind, independent evaluation by two pathologists. Morphologic features were classified as chronic persistent hepatitis, chronic active hepatitis, or cirrhosis. Any degree of piecemeal necrosis or evidence of previous piecemeal necrosis (portal expansion, hepatocyte trapping) was sufficient to rule out chronic persistent hepatitis and to categorize the biopsy as at least mild chronic active hepatitis. The histologic activity index (HAI) was calculated according to the scoring system of Knodell et al. (8).

Sera Reactivity Testing. Sera of all subjects were reactive for anti-HCV by both second-generation enzyme immunoassay (EIA-2) and second-generation recombinant immunoblot assay (RIBA-2) (Ortho Diagnostic, USA). Sera reactive for anti-HIV by EIA were confirmed by Western blot (Pasteur Institute, France). Tests for ALT, HBsAg, alpha-1-antitrypsin, iron, ceruloplasmin, anti-smooth muscle and/or antinuclear antibodies, and anti-liver kidney microsomal antibodies were negative or normal. All tests were performed and interpreted according to manufacturers' recommendations.

Quantitative HCV RNA Detection. Quantitation of serum HCV RNA levels was performed in all enrolled patients who met the following criteria: (1) availability of a sample collected no more than ± 2 weeks from performance of a liver biopsy and immediately frozen; and (2) sample storage time at -20°C for fewer than 2 years with no thawing. A total of 20 patients (11 HIV positive and 9 HIV negative) were selected for HCV load investigation. Demographic characteristics of these patients are shown in Table 2.

HCV RNA was tested by nested polymerase chain reaction (PCR). HPLC purified oligonucleotides I and II were used as outer primers and 33 and 48 as inner primers according to Genset Oligonucleotide Handbook sequences (9). Serum samples were diluted in 150 mM NaCl. One hundred μl of diluted sera were heated at 92°C for 30 sec and then cooled on ice.

Synthesis of complementary DNA was carried out in a volume of 20 μl containing the template (3 μl of the heated sample), 20 units of ribonuclease inhibitor (Boehringer Mannheim, Germany), 100 pmol of the antisense outer primer II, 20 units of AMV reverse transcriptase (Boehringer Mannheim), 1 mmol/l of each of the four dNTPs, PCR buffer (10 x PCR buffer contains 500 mmol/l KCl and 100 mmol/l Tris-HCl; pH 8.3), and 5 mmol/l of MgCl₂. The reaction was incubated at 42°C for 60 min, 99°C for 5 min, and 5°C for 5 min. The complementary DNA product was amplified by addition of 80 μl of a solution containing 100 pmol of sense outer primer I, 2.5 units of TAU polymerase (Boehringer Mannheim), PCR buffer, and 2 mmol/l of MgCl₂, and then overlaid with 100 μl of light mineral oil (Sigma, USA). The reaction was carried out in a DNA thermal cycler (Perkin-Elmer Cetus 480, USA) and consisted of an incubation at 95°C for 10 min followed by 35 cycles of 1 min each at 95°C, 1 min at 45°C, and 1 min at 72°C. One μl of the first amplification product was reamplified in a total volume of 50 μl containing 50 pmol of the inner primers, 0.2 mmol/l of each of the four dNTPs, PCR buffer, 0.1 mg/ml of gelatine, 1.5 mmol/l of MgCl₂, and 2.5 units of TAU polymerase. The reaction was incubated for 10 min at 95°C, 1 min at 46°C, and 1 min at 72°C. After the second amplification, 10 μl of PCR product was analyzed by electrophoresis on 4% multi-purpose agarose (Boehringer Mannheim).

Titers of HCV RNA were estimated in a semiquantitative fashion by means of serial tenfold dilutions. The highest dilution giving a positive PCR signal was taken as the viral titer. Special care was taken to reduce the risk of contamination, and all sample handling and mixture preparation was done in a different laboratory than that used for the amplifications. Negative controls were included during the cDNA synthesis and PCR assays. Assays were repeated at least twice and were considered valid if consistent results were obtained.

Recently, the 20 serum samples tested for HCV RNA titer by serial dilution were also analyzed by the HCV Monitor test procedure (Amplicor PCR Diagnostic, Switzerland). Results are given in Table 3.

Statistical Analysis. Results are expressed as means ± standard deviations. Study data were managed with the R-Sigma computer program (Horus, Spain). Data were tested for normality using the Kolmogorov-Smirnov test. Group means were compared by analysis of variance. Student's t test and the Mann-Whitney test were used to compare continuous variables between two groups. The chi-square test with Yates' correction and the Fisher exact test were used for analysis of frequency.

Results

As shown in Table 4, the distribution of the histological findings and HAI were similar in liver biopsies from both HIV-infected and noninfected patients. However, the mean interval from the estimated time of HCV infection to the performance of the liver biopsy was longer in HIV-negative patients than in coinfected patients (11.9 vs. 5.9 years; p < 0.001). In light of this finding, histological diagnoses made at different time intervals after HCV infection were analyzed (Table 5). In the first 15 years, 8 (6 drug abusers and 2 transfusion recipients) of 32 (25%) HIV-positive patients developed cirrhosis, in comparison with