

HEPATOLOGY

Comparison of liver histopathology between chronic hepatitis C patients and chronic hepatitis B and C-coinfected patients

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Key words

chronic hepatitis, coinfection, HBV, HCV, histology.

Accepted for publication 22 March 2006.

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Abstract

Background: The aim of the present study was to compare the histological characteristics of livers between chronic hepatitis C (CHC) patients with and without hepatitis B virus (HBV) coinfection.

Methods: A total of 336 CHC patients (male/female: 204/132, mean age: 46.1 ± 11.7 years) were enrolled in the study; 32 patients (9.8%) were positive for hepatitis B surface antigen (HBsAg). The histological characteristics of livers were described according to the Knodell and Scheuer scoring system.

Results: The proportion of non-intralobular necrosis (score 0) was significantly lower and the mean intralobular necrosis score was higher among CHC patients with HBV coinfection than those without coinfection (43.8% vs 64.5%; 0.84 ± 1.05 vs 0.53 ± 0.89). The epidemiological and virological parameters, and other histological scores (periportal necrosis, portal inflammation, total necroinflammation and fibrosis) were not significantly different between these two groups.

Conclusion: Chronic hepatitis C patients with HBV coinfection tend to have more severe intralobular necrosis than those with isolated HCV infection.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common causes of chronic liver disease in the world. Both viruses induce chronic hepatitis, which may progress to cirrhosis and eventually to hepatocellular carcinoma.^{1–3} Previous reports have shown that dual infection with HBV and HCV is not uncommon in patients in both Asian^{4,5} and Western countries.^{6,7} The prevalence is approximately 10–15% in patients with chronic HBV infection, although it may vary from one country to another. Some clinical reports have suggested that patients with dual HBV and HCV infection might have more severe liver disease.^{5–9} The seroprevalence of hepatitis B surface antigen (HBsAg) in chronic C patients has been rarely investigated. Indeed, no study has compared the replication patterns of the viruses in anti-HCV-positive patients who were chronically infected with both hepatitis B and C. The purpose of the present study was to compare the histological characteristics of livers in patients with chronic hepatitis who had dual HBV and HCV infection with those suffering from HCV infection alone.

Methods

Between 1995 and 1999, 336 anti-HCV-positive patients with consistently high levels (more than twofold the upper limit of the normal range) of alanine aminotransferase for more than 6 months were enrolled in the study. Liver biopsies from all patients showed chronic hepatitis lesions. None of the patients had any other obvious cause of hepatitis (other viruses, autoimmune disease, drug hypersensitivity, hemochromatosis, Wilson's disease, α -antitrypsin deficiency). All patients had an alcohol intake <40 g/day. No patient had received previous treatment with immunosuppressants or antiviral agents such as interferon. Of the 336 patients, 32 (9.8%) were HBsAg positive.

Serum HBsAg, antihepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg), and anti-hepatitis B e antigen (anti-HBe) were assayed using commercially available kits (General Biological HBsAg radio-immunoassay [RIA] and HBeAg/Anti-HBe RIA; General Biological, Taiwan). Anti-HCV was studied using a second-generation ELISA (Abbott Diagnostics, North Chicago, IL, USA).

Detection of serum HCV-RNA was performed using a standardized automated qualitative reverse transcriptase–polymerase chain reaction (RT-PCR) assay (Cobas Amplicor Hepatitis C Virus Test, version 2.0; Roche, Branchburg, NJ, USA). The detection limit was 50 IU/mL. The HCV genotypes 1a, 1b, 2a, 2b, and 3a were determined by amplification of the core region using genotype-specific primers as described by Okamoto *et al.*¹⁰ Serum HCV-RNA levels were measured using the branched DNA assay (Quantiplex HCV-RNA 2.0, Bayer, Emeryville, CA, USA), performed strictly in accordance with the manufacturer's instructions. The quantification range was 0.2–120 Meq HCV-RNA/mL.

The HBV-DNA level was measured using the Cobas Amplicor HBV monitor test (COBAS-AM test; Roche Molecular Systems, Pleasanton, CA, USA) according to the manufacturer's instructions.¹¹ The dynamic range of the test was 2×10^2 – 2×10^5 HBV-DNA copies/mL. Serial dilutions were performed using the specimen dilution protocol provided by the manufacturer.

Liver biopsy specimens were obtained from all patients included in the study. The biopsy was performed using a Menghini needle. Liver biopsy specimens were fixed in 10% formalin. Hepatic histopathological findings were interpreted independently of clinical and biochemical data by two pathologists, according to the scoring system described by Knodell and Scheuer.^{12–14}

The χ^2 test was used for analysis of categorical variables. The Student's *t*-test was used for analysis of continuous variables. $P < 0.05$ was considered statistically significant; 95% confidence intervals were also calculated when appropriate.

Results

The two groups of patients were similar in terms of demographic and virological parameters (Table 1). The level of HCV-RNA did not differ between the two groups. The distribution of HCV genotypes was also not different between the two groups.

Except for six patients, all of the patients with chronic hepatitis B and C infections were negative for HBV-DNA as determined by a DNA assay. Among the six patients, two of them had serum HBV-DNA levels $>10\,000$ copies/mL. Their mean total necroinflammation score was 6 and the stage of fibrosis score was 2.

Comparison of histological difference in the livers from patients with chronic hepatitis B and C and the livers from chronic hepatitis C patients is presented in Table 2. The mean intralobular necrosis score was higher in livers from patients with dual HBV and HCV infection than in livers from patients infected with hepatitis C alone (0.84 ± 1.05 vs 0.53 ± 0.89 , $P = 0.06$). No difference of histological lesions was observed between the two groups, including interface hepatitis, portal inflammation, total necroinflammation score and fibrosis. However, the proportion of patients with no intralobular necrosis (score 0) was significantly lower among the patients with chronic hepatitis B and C infections than in the patients chronically infected with hepatitis C only (48.3% vs 64.5%, $P < 0.05$).

Discussion

Previous studies have suggested that patients coinfecting with both HBV and HCV suffer from a more serious form of liver disease,^{4–9,15,16} particularly, when patients are infected with HBV and superinfected with HCV. Two independent studies from

Table 1 Subject characteristics

	HCV + HBV (<i>n</i> = 32) <i>n</i> (%)	HCV (<i>n</i> = 304) <i>n</i> (%)
Age (years) (mean \pm SD)	44.8 \pm 10.8	46.3 \pm 11.8
Sex (M/F)	20/12	184/120
Route of infection		
Blood transfusion	8 (25)	76 (25)
Operation	5 (16)	39 (13)
Dental procedure	2 (6)	15 (5)
Tattoo, acupuncture	2 (6)	12 (4)
Injection with recycle needle	1 (3)	9 (3)
Other percutaneous procedure	6 (19)	52 (17)
Unknown	8 (25)	101 (33)
ALT (IU/L) (mean \pm SD)	97.6 \pm 77.6	136.7 \pm 147.3
Genotype 1b	17 (53)	140 (46)
2a	7 (22)	93 (30)
2b	5 (16)	38 (13)
Other	3 (9)	33 (11)
HCV-RNA mean level (log /mL) (mean \pm SD)	6.05 \pm 0.90	6.26 \pm 0.79
HBV-DNA positivity	6 (18.7)	—
HBV-DNA level ($>10\,000$ copies/mL)	2 (6.2)	—

ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 2 Histological subject characteristics (mean \pm SD)

	HCV + HBV (<i>n</i> = 32)	HCV (<i>n</i> = 304)
Necroinflammation		
Interface hepatitis	0.88 \pm 1.07	1.25 \pm 1.39
Intralobular necrosis*	0.84 \pm 1.05	0.52 \pm 0.59
Portal inflammation	1.71 \pm 1.19	1.92 \pm 1.21
Total score	3.37 \pm 2.32	3.69 \pm 2.48
Fibrosis	1.09 \pm 1.11	1.37 \pm 1.32
Cirrhosis (F4)	1 (3.2%)	17 (5.6%)

* $P = 0.06$.

Taiwan have shown that 10–20% of fulminant/subfulminant hepatitis in chronic HBsAg carriers could be attributed to HCV superinfection.^{17,18} Indeed, liver disease appears to be more severe in terms of histology in patients seropositive for both HBsAg and anti-HCV than in patients seropositive for HBsAg alone.^{7,9,19} Furthermore, case–control studies have indicated that dual infection with HCV and HBV results in a much higher relative risk for the development of hepatocellular carcinoma.^{1–3}

In the present study we compared the histological characteristics of livers from anti-HCV- and HBsAg-positive patients with chronic hepatitis to those from patients positive only for anti-HCV. All patients were positive for HCV-RNA as shown by PCR. The two groups of patients were similar in terms of demographic and virological parameters. This study confirms the severity of intralobular necrosis in patients chronically infected with hepatitis B and C. In addition, the total necroinflammation score was more severe in the patients who had a HBV-DNA level $>10\,000$ copies/mL.²⁰ These findings indicate that HBV may play a dominant role

in contributing to the severity of liver disease in the majority of patients.^{21,22}

Earlier studies in animal models have shown that HCV superinfection exerts an inhibitory effects on the replication of the pre-existing HBV.²³ Clinical studies have confirmed that anti-HCV-positive patients have a low HBV-DNA polymerase activity⁴ or weak HBV-DNA positivity.^{7,9,24} In the present study all patients except six were negative for HBV-DNA. The suppression of HBV expression by HCV replication has been reported in an *in vitro* cultured cell system.²⁵ This is the first hypothesis proposed to explain the interference phenomenon. The interaction between HBV and HCV may also be related to other factors, such as interferon or cytokines.²

It is possible that the timing of infection by each virus in a dual infection influences the evolution and severity of liver disease. Some work suggests that the more recent virus infection tends to suppress the preexisting virus.¹⁸ The HCV superinfection was reported to cause much more severe liver disease in patients with chronic HBV infection with a risk of fulminant/subfulminant hepatitis than HBV superinfection in patients with chronic HCV infection.^{19,26}

In conclusion, the present study indicates that (i) chronic infection with both hepatitis B and C is associated with a more severe intralobular liver necrosis than that of patients chronically infected with hepatitis C alone; and (ii) there is viral interference between HBV and HCV replicative patterns. The mechanism underlying liver injury and the interaction between the two viruses remain to be elucidated.

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