Clinical, virological, and pathological significance of hepatic bile duct injuries in Chinese patients with chronic hepatitis C

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Purpose. Hepatic bile duct injuries are characteristic histological findings in patients with chronic hepatitis C virus (HCV) infection. However, the pathogenesis and clinical significance of this phenomenon remain unclear. The aims of this study were to evaluate the prevalence and clinical significance of hepatic bile duct injuries in Chinese patients with chronic hepatitis C.

Methods. One hundred and seventeen Chinese patients with chronic hepatitis C were enrolled. Clinical, biochemical, immunological (serum autoantibodies and cryoglobulinemia), histological, and virological data (serum HCV RNA titer and HCV genotype) were compared between patients with and without hepatic bile duct injuries.

Results. Eighty-three (71%) of the 117 patients with chronic hepatitis C had hepatic bile duct injuries. Patients with hepatic bile duct injuries had a significantly higher frequency of HCV genotype 1b; a higher mean serum globulin level; significantly higher mean scores for histological periportal necro-inflammation, portal inflammation, and fibrosis; and more severe portal lymphoid aggregation/follicles when compared with patients without hepatic bile duct injuries (P < 0.05, all). No significant differences in the presence of serum autoantibodies, cryoglobulinemia, mean serum HCV RNA titer, or response to interferon treatment were noted between the two groups. Multivariate logistic regression analysis showed that HCV genotype 1b infection, portal inflammation, and lymphoid aggregation/follicles were significant independent predictors associated with hepatic bile duct injuries.

Conclusions. The presence of hepatic bile duct injuries in Chinese patients with chronic hepatitis C was significantly correlated with HCV genotype 1b infection, and the patients with these injuries had more severe portal inflammation and formation of lymphoid aggregates/follicles.

Key words: autoantibodies, intrahepatic bile duct, chronic hepatitis C, cryoglobulinemia, viral genotype, histological activity index, lymphoid aggregates/follicles

Introduction

Hepatic bile duct injuries, lymphoid aggregates/follicles (lymphoid A/F), and steatosis are characteristic histological features often seen in patients with chronic hepatitis C.1–3 According to reports from Western countries, the prevalence of hepatic bile duct injuries (defined as portal biliary epithelial damage associated with lymphocyte infiltration) ranges from 15% to 91% in patients with chronic hepatitis C.1–11 These peculiar bile duct injuries have been called hepatitis duct injuries, hepatitis-associated bile duct lesions, and Poulsen-Christoffersen’s bile duct lesions, which are commonly seen in chronic aggressive viral hepatitis.1–3, 10

The pathogenesis and clinical significance of hepatic bile duct injuries in patients with chronic hepatitis C remain unclear. Previous reports have revealed that these hepatic bile duct injuries mimic the chronic nonsuppurative destructive cholangitis seen in patients with primary biliary cirrhosis.8,10 Patients with chronic hepatitis C frequently manifest immune disorders, such as positivity for serum autoantibodies, particularly antinuclear antibody (ANA) and anti-smooth muscle antibody (SMA); cryoglobulinemia; and sialadenitis.12–17 The relationship between hepatic bile duct injuries and these immunological manifestations in patients with chronic hepatitis C has not been clarified. Also, the associations between serum hepatitis C virus (HCV) RNA titer, HCV genotype, and hepatic bile duct injuries have not been reported previously. The aims of this study were to
assess the prevalence of hepatic bile duct injuries in Chinese patients with chronic hepatitis C and to compare the various clinical, biochemical, and immunological data of patients with and without hepatic bile duct injuries. In addition, liver histological changes in portal, periplural, and lobular necro-inflammation, as described by a modified histological activity index (HAI), and other characteristic findings of chronic hepatitis C, such as lymphoid A/F and steatosis, were compared between the two groups. Finally, serum HCV RNA titer, HCV genotype, and the response to interferon treatment were also compared between the two groups.

**Patients and methods**

During the period from January 1991 to August 1998, the clinical and pathological data of 117 Chinese patients with biopsy-proven chronic hepatitis C at the Division of Gastroenterology, Veterans General Hospital-Taipei, were reviewed. All these patients had elevated serum alanine transaminase (ALT) levels and were negative for serum hepatitis B surface antigen (HBsAg). None of these patients had a history of alcoholism, exposure to hepatotoxic drugs, or metabolic liver diseases.

A percutaneous liver biopsy was performed in each patient, using a Menghini needle. All biopsy specimens were larger than 1.5 cm in length, and contained more than five portal areas for examination. Specimens were fixed in buffered formalin, processed into a paraffin block, and routinely stained with hematoxylin/eosin and Masson’s trichrome. The liver histology was evaluated by two pathologists who had no knowledge of the patient’s clinical and laboratory findings. Hepatic bile duct damage, lymphoid A/F, and steatosis were determined using the scoring system described by Scheuer et al., with modification. The severity of the hepatic bile duct injuries was scored based on the sum of grades 0–2 (absent, mild, or severe) for the following criteria: inflammatory cell infiltration, stratification, cytoplasmic vacuolization, and degeneration of interlobular biliary epithelial cells. Lymphoid A/F was scored as 0 (no lymphoid infiltrates), 1 (ill defined condensations of lymphoid infiltrates), 2 (definite aggregations or follicles without identifiable germinal centers), and 3 (lymphoid follicles with germinal centers). Steatosis was scored as 0 (none), 1 (mild to moderate), and 2 (severe) according to the degree of severity. Liver inflammatory grade and fibrotic stage were determined with an HAI scoring system according to the classifications of Knodell and Scheuer, with minor modification. If the scores for the same patient differed between the two pathologists, the slides were reviewed and a consensus was reached.

In order to compare the prevalence of hepatic bile duct injuries in chronic hepatitis C patients with that in patients with chronic hepatitis other than type C, hepatic bile duct injuries were also evaluated in 44 patients with chronic hepatitis B whose age, sex, and serum ALT were matched with those of the chronic hepatitis C patients.

Serum samples, for measuring liver biochemistry, cryoglobulin, autoantibodies, HCV RNA titer, and HCV genotype, were collected and the tests performed within 3 days of the liver biopsy in 99 (85%) patients, within 1 week in 10 patients, and within 2 weeks in the remaining 8 patients.

Liver biochemical tests, including ALT, aspartate transaminase (AST), gamma-glutamyl transpeptidase (γ-GT), alkaline phosphatase (Alk-P), albumin, globulin, and total bilirubin, were measured by an autoanalyzer (model 736 automatic analyzer; Hitachi, Tokyo, Japan). Serum anti-HCV was measured by a second-generation enzyme immunoassay kit (Abbott Laboratories, Chicago, IL, USA). HBSAg was measured using a radioimmunoassay kit (Abbott Laboratories). Serum ANA, SMA, and anti-mitochondrial antibody (AMA) were measured by an indirect immunofluorescence assay (Fluoro-Kit; Inestar, Stillwater, MN, USA). An ANA titer above 1:80, an SMA titer above 1:20, and an AMA titer above 1:10 were recorded as positive. Cryoglobulinemia was determined using a standard method, as described in our previous report.

Quantitative measurements of serum HCV RNA were performed by the branched DNA signal amplification (bDNA) assay (Quantiplex 2.0; Chiron, Emeryville, CA, USA) following the manufacturer’s instructions. The assay produced results in terms of numbers of HCV genome equivalents per ml (Eq/ml). The detection limit of this assay was 200,000 Eq/ml. Samples below the detection limit of the bDNA assay were further tested by home-brew reverse transcription-nested polymerase chain reaction (RT-nested PCR), as described previously. Serum HCV RNA titers were transformed logarithmically for statistical analysis. HCV genotyping was performed using RT-nested PCR with type-specific primers (method of Okamoto et al.,) and results were reported as types 1a, 1b, 2a, 2b, and 3a according to the classification of Simmonds et al.

Fifty-seven patients underwent interferon therapy after liver biopsy. Twenty-three of the 57 patients received interferon α-2b, 3 million units, three times a week for 24 weeks; six patients received consensus interferon (CIFN), 3 μg; 12 patients received CIFN, 9 μg; and 16 patients received CIFN, 15 μg, three times a week for 24 weeks. Sustained response was defined as persistent normalization of serum ALT and undetect-