A CLINICO−PATHOLOGICAL STUDY OF 163 UNTREATED CASES OF CHRONIC HEPATITIS C

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Abstract: We performed a clinico-pathological study of 163 untreated cases of chronic hepatitis C. Most of the patients were clinically asymptomatic and their physical examinations showed unremarkable or minimal changes at the time of the liver biopsy. Liver function tests tended to present slight abnormalities, involving mainly alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamil transferase (GGT) levels. In spite of these mild abnormalities, advanced chronic liver disease was histologically detected in the majority of the patients, mainly showing chronic active hepatitis. Most characteristic histological finding was an interlobular bile duct damage, which correlated with the presence of lymphoid aggregates in the portal tracts and with the development of fibrosis.

Key words: Chronic hepatitis C, Bile duct damage, Lymphoid aggregate, Histopathology

INTRODUCTION

Recently the genome of blood−borne non−A, non−B virus, designated hepatitis C virus (HCV), has been cloned and specific assays have been developed to detect anti−HCV antibodies (Choo et al., 1989; Kuo et al., 1989). Since the introduction of routine donor blood screening for hepatitis B surface antigen (HBs−Ag), the incidence of hepatitis B virus infection has decreased and hepatitis C has been the major cause of post−transfusional and sporadic hepatitis. The clinical presentation of chronic hepatitis C as reported in the literature suggests that the disease often runs a silent course, with few symptoms and mild biochemical abnormalities (Patel et al., 1991; Merican et al., 1993). In spite of the lack of symptoms and signs, severe liver damages have been reported to occur with high frequency (Patel et al., 1991; Scheuer et al., 1992; Merican et al., 1993).

The aim of this study is to report the results of the clinical, biochemical and histopathological examinations of 163 untreated cases of chronic hepatitis C, analyze the correlations or relationships among these findings and discuss the nature of chronic hepatitis C.

Received for Publication, October 12, 1994
Contribution No. 3026 from the Institute of Tropical Medicine, Nagasaki University

MATERIALS AND METHODS

We reviewed the clinical data and histological specimens from 1991 through 1994 pertaining to 163 untreated patients with chronic hepatitis C in the Department of Pathology, Institute of Tropical Medicine, Nagasaki University. The main reason for the biopsy procedure was to evaluate chronic alterations of serum aminotranferases and to have a histological diagnosis prior to interferon therapy. From 224 cases initially recorded, 61 were excluded on the basis of the presence of positive testing for HBs−Ag, presence of hepatocellular carcinoma, previous treatment with interferon, evidence of autoimmune hepatitis or alcoholic liver damage.

The sera from the patients were assayed for the presence of HBs−Ag and anti−C100−3 HCV using a radioimmunoassay kit (Dainabot, Japan and Ohtsuka assay Co. Ltd., Japan, respectively). Sera negative for both HBs−Ag and anti−C100−3 HCV were retested using a second generation assay which detects in addition to C100−3 antigen also the C22−3 and C 33−c antigen by radioimmunoassay (Ohtsuka assay Co. Ltd., Japan). Additional cases of HCV infection included those positive sera for HCV−RNA as detected by the polymerase chain reaction technique.

The liver biopsy specimens were fixed in buffered formalin, embedded in paraffin and stained with hematoxylin and eosin, Azan−Mallory and silver impregnation for reticulin fiber. All biopsy specimens were classified according to the type of chronic hepatitis; chronic persistent hepatitis (CPH), chronic active hepatitis (CAH) with mild or moderate degree and with or without cirrhosis. The specimens were graded with respect to the degree of piecemeal necrosis, portal and lobular inflammation and fibrosis according to the histological activity index (HAI) scoring system designed by Knodell et al (1981).

The presence or absence of the following histological findings were registered for each biopsy; bile duct damage and bile duct proliferation, plasma cell infiltration in portal tracts, acidophilic body, sinusoidal lining cell activation, fatty metamorphosis and dysplasia of hepatocytes. Bile duct damage was considered present when the following features were observed alone or in combination; multilayered or stratified epithelium with swollen epithelial cells, inflammatory cell infiltration in epithelium, vacuolization of epithelial cells, loss of polarity of epithelial cells and degeneration of epithelial cells.

The statistical significance of the results was evaluated by the Chi−square test. A P−value <0.05 was considered statistically significant.

RESULTS

Clinical characteristics

Table 1 describes demographic data of 163 patients with chronic hepatitis C. The mean age of the patients was 54 years old and the male to female ratio was 2:1. Most of the patients (70%) were between 50 and 70 years old. Thirty nine out of 105 male patients (38%) and 24 out of 58 (41%) female patients had a history of blood transfusion.

Table 2 shows the symptoms and physical signs of the patients, when they were submitted for liver biopsy. One hundred thirty nine out of 163 patients (85%) were asymptomatic and the most frequent symptom was general fatigue (11%), followed by vague abdominal discomfort (3%) and itching (1%). Ninety eight out of 163 patients (60%) presented no signs of physical examination and 57 cases (36%) presented hepatomegaly, followed by splenomegaly (2%) and hepatosplenomegaly (2%). Among the patients with hepatomegaly, there was a male predominance (43/105;41%), as compared with the female group (15/58; 25%) and this trend occurred more frequently in the age groups older than 50 years.

Biochemical Findings

The mean levels, ranges and distribution of AST, ALT and GGT of the patients are shown in Table 3 and 4. Biochemical alterations of AST, ALT and GGT tended to be mild and no correlation could be established among any biochemical determination and distinct groups of age or symptoms and physical signs.

Table 1. Demographic data of patients with chronic hepatitis C

	Number	Mean age (range)
Male	105	54 (24−72)
Female	58	55 (27−75)
Total	163	54 (24−75)

Table 2. Clinical symptoms and signs in chronic hepatitis C

Symptoms	
No complaint	85%
Fatigue	11%
Abdominal discomfort	3%
Itching	1%
Signs	
No change	60%
Hepatomegaly	36%
Splenomegaly	2%
Hepatosplenomegaly	2%

Table 3. AST. ALT and GGT levels in chronic hepatitis C

	Mean level (range)
AST	61 (13−421)
ALT	77 (15−348)
GGT	63 (11−241)

AST (aspartate aminotransferase): normal level=40IU/L
ALT (alanine aminotransferase): normal level=40IU/L
GGT (gamma−glutamil transferase): normal level=50IU/L

Table 4. AST. ALT and GGT distributions in chronic hepatitis C

	No. of patients (%)
AST (IU/L)	
<40	55 (34%)
40−80	66 (40%)
>80	42 (26%)
ALT (IU/L)	
<40	30 (18%)
40−80	79 (49%)
>80	54 (33%)
GGT (IU/L)	
<50	88 (54%)
50−100	46 (28%)
>100	29 (18%)

Histological Findings

The general histological findings in chronic hepatitis C are indicated in Table 5. The Christoffersen−Paulsen type bile duct damage (Photo. 1) and bile ductular proliferation were seen in 57% and 47% of the patients, respectively. Lymphoid follicle formations with or without germinal center in portal tracts (Photo. 2) were seen in 42% of the patients. The majority of the cases (85%) showed mild degree of piecemeal necrosis. Fatty metamorphosis of the hepatocytes was detected in 29% of all the cases. Most cases presented more advanced fibrosis, as in the cases of fibrous portal expansion and bridging fibrosis.

Table 6 shows a correlation between bile duct damage and portal inflammation in chronic hepatitis C. Bile duct damage was significantly correlated with the degree of portal inflammation, and especially all cases of lymphoid follicle formation with germinal center in portal tracts showed bile duct damage ($p<0.05$).

A correlation between bile duct damage and degree of fibrosis is indicated in Table 7. Bile duct damage was significantly correlated with advanced fibrosis ($p<0.05$), except cirrhosis.

Table 5. Histological findings of chronic hepatitis C

	n=163
Portal tract lesions	
Lymphoid infiltrate	
None	3 (2%)
Loose condensation	92 (56%)
Lymphoid follicle without germinal center	62 (38%)
Lymphoid follicle with germinal center	6 (4%)
Piecemeal necrosis	
None	11 (7%)
Mild	139 (85%)
Moderate	12 (8%)
Marked	1 (1%)
Bile duct damage	93 (57%)
Bile ductular proliferation	63 (47%)
Plasma cell infiltration	48 (29%)
Parenchymal lesions	
Sinusoidal lining cell hyperplasia	149 (91%)
Spotty necrosis	158 (97%)
Fatty metamorphosis	47 (29%)
Dysplasia of hepatocytes	16 (10%)
Fibrosis	
Limited in portal tracts	9 (6%)
Portal fibrous expansion	60 (37%)
Bridging fibrosis	74 (45%)
Cirrhosis	20 (12%)

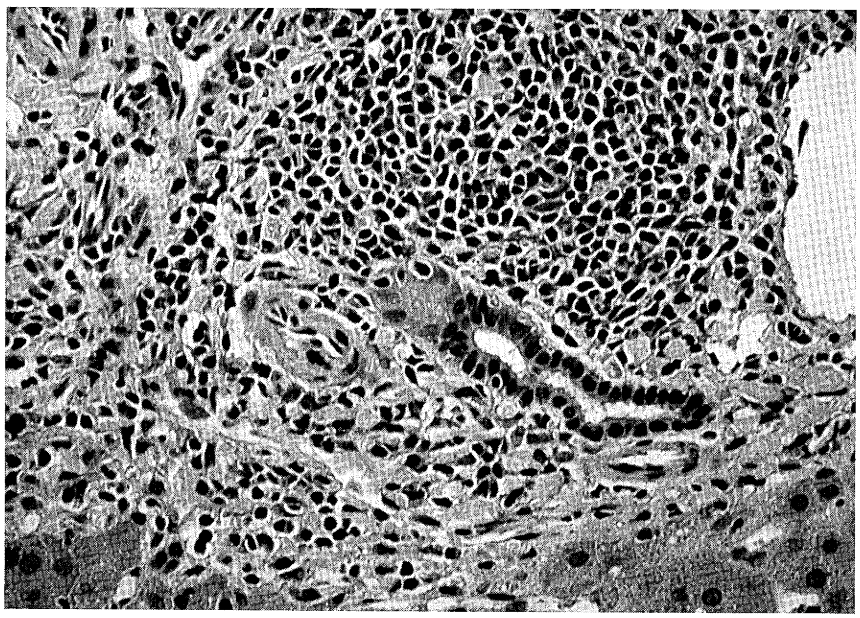

Photo. 1. Christoffersen-Paulsen type bile duct damage (HE, ×100)

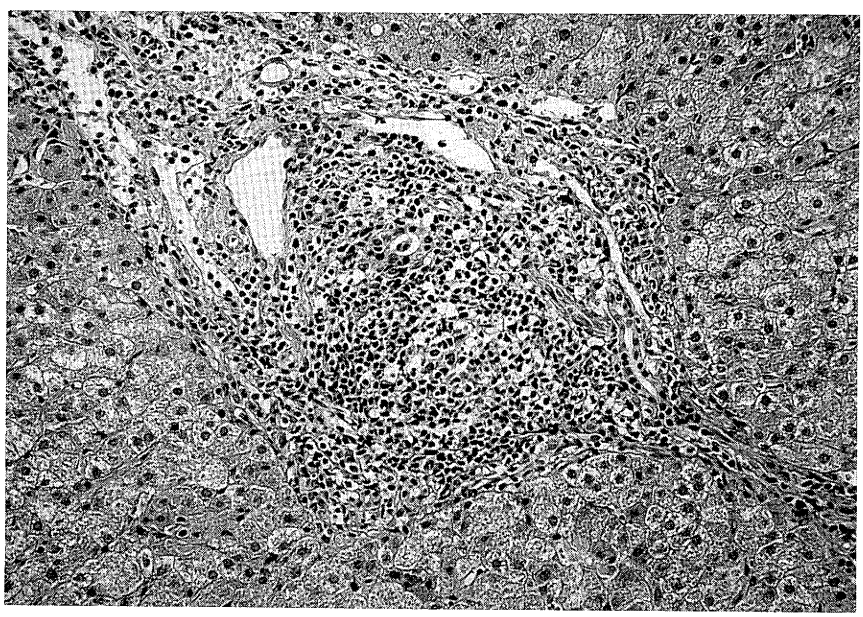

Photo. 2. Lymphoid follicle formation in portal tract (HE. ×40)

Table 6. Bile duct damage (BDD) and lymphoid infiltrate in portal tract

Lymphoid infiltrate	BDD	
	present	absent
None (n=3)	1 (33%)	2 (67%)
Loose condensation (n=92)	42 (46%)	50 (54%)
Follicle without germinal center (n=62)	44 (71%)	18 (29%) *
Follicle with germinal center (n=6)	6 (100%)	0 (0%) *

* Statistically significant, $p<0.05$

Table 7. Bile duct damage (BDD) and fibrosis

Fibrosis	BDD	
	present	absent
Limited in portal tracts (n=9)	3 (33%)	6 (67%) *
Portal fibrous expansion (n=60)	36 (60%)	24 (40%) *
Bridging fibrosis (n=74)	50 (68%)	24 (32%) *
Cirrhosis (n=20)	15 (75%)	5 (25%)

* Statistically significant, $p<0.05$

DISCUSSION

The results of our study confirm the general impression that chronic hepatitis C runs a protracted course with few nonspecific symptoms and signs, along with mild biochemical abnormalities, despite the presence of, or evolution to, advanced chronic liver disease. Most of the patients (85%) were asymptomatic at the time of diagnosis, and were referred for liver biopsy to elucidate chronic serum elevation of aminotransferases or to provide histological information for further interferon therapy. Physical examination was unremarkable in the majority of the patients (60%). The most common physical abnormality was hepatomegaly in 35 % of the patients, more frequently in males and specially among older patients. It has been reported that older patients present more severe manifestations of the disease (Merican et al., 1993). Among the biochemical tests, abnormalities involved mainly serum levels of AST, ALT and GGT, but no correlation could be established with the degree of piecemeal necrosis and portal and intralobular inflammation. This lack of correlation with hepatic histology has already been pointed out (Patel et al., 1991; Merican et al., 1993), and could be a result of sampling error due to the fluctuanting course of aminotransferases and advanced fibrosis. In this regard, the clinical severity cannot be assessed by the serum aminotransferase level alone, and liver biopsy is essential in assessing the extent of liver damage (Patel et al., 1991; Healy et al., 1994). The typical fluctuation in aminotransferase level may represent alteration

in viral replication, host immunity, or both (Patel et al., 1991). These fluctuant levels correlate to episodes of lobular necrosis, thought to be responsible for the progression to chronic disease (Scheuer, 1992).

The most conspicuous histological findings in our study was bile duct damage (BDD), which was observed in 57% of our cases, while the incidence rates in the literature differ from 22% (Scheuer et al., 1992), 25% (Robberts et al., 1993), 30% (Lefkowitch and Apfelbaum, 1989), 31% (Lefkowitch et al., 1993), 90% (Danque et al., 1993) to 91% (Bach et al., 1992). The affected bile ducts are small or medium sized interlobular ducts. The BDD appears to involve immunological mediated reaction to antigens on bile duct epithelium, possibly histocompatibility (HLA) antigens or HLA antigens displayed with virus-related antigens (Lefkowitch et al., 1993; Danque et al., 1993). An association between BDD and a better response to interferon therapy in patients with chronic hepatitis C has been documented, and may be of prognostic significance (Lefkowitch et al., 1993).

The occurrence of BDD has a significant correlation with the degree of portal/periportal inflammation in our cases. The affected bile ducts were usually surrounded by a dense lymphoid infiltrate, sometimes with a germinal center, close to the damaged ducts. Lymphoid follicles have been reported to occur in frequencies varying from 49% (Bach et al., 1992; Lefkowitch et al., 1993), 52% (Lefkowitch and Apfelbaum, 1989), 63% (Robberts et al., 1993) to 78% (Vyberg, 1993). In our study, 42% of the patients displayed lymphoid follicles and 73 % of them had BDD. Lymphoid follicle is a feature of a variety of chronic inflammatory diseases, often of autoimmune nature and their presence in hepatitis C reflects an ongoing immunologic reaction. Lymphoid follicles may be formed early in the acute stages of hepatitis C (Schmid et al., 1982). The appearance or disappearance of lymphoid follicles during the course of the illness does not seem to correlate with either improvement or deterioration of the histological activity index (Poulsen and Christoffersen, 1969).

A significant correlation between the occurrence of BDD and the degree of hepatic fibrosis in non-cirrhotic livers, which has been described in several reports (Vyberg, 1993; Elloway et al., 1992), was also detected in our cases. While 37 out of 60 (62%) in the cases involving fibrous portal expansion and 45 out of 74 (61%) in the cases involving bridging fibrosis presented BDD, only 3 out of 9 (33%) in the cases involving limited fibrosis in the portal tract showed BDD.

In conclusion, chronic hepatitis C is a relentlessly chronic progressive disease, frequently running a silent course, with mild or unremarkable biochemical abnormalities, which do not correlate with the histological activity of this disease. Liver biopsy is the only method to assess the degree of liver damage. The most single characteristic histological feature of chronic hepatitis C is a BDD, surrounded by a lymphoid follicle.

ACKNOWLEDGMENTS

The first author was sponsored by Japan International Cooperation Agency (JICA) under the programme of Training Course in Research for Tropical Medicine.

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