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Liver International 2006: 26: 32–38

Liver International
DOI: 10.1111/j.1478-3231.2005.01198.x

Clinical Studies

Laparoscopic findings in patients with nonalcoholic steatohepatitis

Tanaka N, Ichijo T, Okiyama W, Mutou H, Misawa N, Matsumoto A, Yoshizawa K, Tanaka E, Kiyosawa K. Laparoscopic findings in patients with nonalcoholic steatohepatitis.
Liver International 2006: 26: 32–38. © Blackwell Munksgaard 2005

Abstract: *Background/Aims:* Laparoscopic observation of the liver is important to diagnose liver conditions accurately. However, the laparoscopic findings of nonalcoholic steatohepatitis (NASH) have not been characterized. The aim of this study was to clarify the laparoscopic characteristics of NASH. *Methods:* Twenty-four patients were enrolled. The degrees of hepatomegaly, color and irregularity of the liver surface, and the presence of depressions, patches, and vesicles were investigated. These laparoscopic findings were compared among NASH, alcoholic liver disease (ALD), and autoimmune hepatitis (AIH). *Results:* Mild hepatomegaly, dullness of the liver edge, increased fat accumulation of the round ligament, and whitish markings were found in most of the patients with NASH. Small depressions were observed in approximately 70% of the patients. As fibrosis developed, the liver surface became whiter and more uneven. Compared with patients with ALD and AIH, increased fat accumulation of the round ligament and dullness of the liver edge were observed more frequently in those with NASH. However, coarse and groove-like depressions were rare in NASH patients. *Conclusions:* Several findings, including mild hepatomegaly, increased fat accumulation of the round ligament, rounded liver edge, whitish markings, and small depressions were common in patients with NASH. However, coarse and groove-like depressions were rare. These findings may be helpful for confirming a diagnosis of NASH.

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Key words: hepatomegaly – laparoscopy – nonalcoholic steatohepatitis – small depressions – whitish markings

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Received 5 May 2005,
accepted 26 July 2005

In Western countries and in Japan, nonalcoholic steatohepatitis (NASH) is recognized as one of the major causes of chronic hepatitis. NASH is closely associated with metabolic syndrome characterized by visceral obesity, insulin resistance, hypertension, and hyperlipidemia (1). Because NASH has the potential to progress to cirrhosis (2), it is important to distinguish NASH from nonalcoholic fatty liver, which essentially has a benign course. At present, liver biopsy is indispensable for diagnosing NASH.

Laparoscopic liver biopsy is considered the most accurate method for diagnosing chronic liver diseases, especially cirrhosis (3). Compared with ultrasonography-guided biopsy, laparoscopy provides a great deal of additional information concerning not only the surface conditions and consistency of the liver but also the spleen, gallbladder, and peritoneum. It has been reported that characteristic laparoscopic findings are useful for the differential diagnosis of many kinds of chronic liver diseases, including viral hepatitis (4), alcoholic liver disease (ALD) (5), autoimmune hepatitis (AIH) (4, 6), primary biliary cirrhosis (PBC) (6, 7), and metabolic liver diseases. It has been reported that the laparoscopic findings of nonalcoholic fatty liver have a diffuse yellowish tone, yellowish round spots with fine red meshes (so-called leopard skin-like appearance), rounded margin of the right hepatic lobe, and mild hepatomegaly (8). Recently, Miyake et al. (9) reported a patient with liver cirrhosis because of NASH. A laparoscopy demonstrated diffuse small nodules on the liver surface, closely resembling the feature of alcoholic liver cirrhosis. However, the laparoscopic findings of NASH have not been summarized or characterized.

In the present study, we evaluated the laparoscopic findings of 24 patients with NASH and tried to correlate the appearance of the liver surface with the histological findings. We also

compared these findings among NASH, ALD, and AIH, and found some laparoscopic findings characteristic of NASH.

Patients and methods

Patients

Twenty-four patients with NASH (10 men, 14 women) were enrolled in this retrospective study. All patients underwent a laparoscopic liver biopsy between January 1995 and August 2004 at Shinshu University Hospital. The mean age was 60.2 years (range 37–77 years). The diagnosis of NASH was based on the following criteria: (1) no consumption of alcohol, (2) a liver biopsy specimen showing steatosis (lipid droplets in more than 10% of hepatocytes), ballooning degeneration, and pericellular/perivenular fibrosis, (3) the absence of past or current infection with the hepatitis B or hepatitis C virus, (4) the exclusion of other liver diseases such as drug-induced liver injury, AIH, PBC, primary sclerosing cholangitis, Wilson's disease, hereditary hemochromatosis, and α1-antitrypsin deficiency, and (5) the exclusion of secondary causes of NASH such as drugs (e.g., tamoxifen, amiodarone, and diltiazem), malnutrition, and surgical procedures (e.g., jejunoileal bypass and gastric bypass). Patients with positive antinuclear antibodies or with histological findings suggestive of overlapping AIH were excluded from the NASH group.

At the time of admission for laparoscopic examination, the body mass index (BMI) was calculated. Patients with more than 25 kg/m^2 of BMI were defined as obese according to the criteria of the Japanese Ministry of Health, Labour, and Welfare. The patients were considered hypertensive if they had a systolic pressure greater than 140 mmHg or a diastolic pressure greater than 90 mmHg, or if they were taking anti-hypertensive drugs. The patients were considered to meet the criteria for diabetes if they had a fasting glucose level equal to or higher than 126 mg/dl, or if they were taking insulin or oral hypoglycemic drugs. The patients were considered to have hyperlipidemia if their serum levels of cholesterol and triglycerides were at least 220 and 150 mg/dl, respectively, in a fasting state, or if they were taking lipid-lowering drugs.

Laboratory examination

The laboratory data were obtained in a fasting state. The homeostasis model assessment method index (HOMA-IR) was calculated by using the following equation: [fasting glucose (mg/dl) × fasting insulin (μU/ml)]/405. More than 2.0 of HOMA-IR was defined as the presence of insulin resistance. Two patients were excluded because their fasting glucose levels were higher than 150 mg/dl.

Laparoscopy

The standard technique of laparoscopic liver biopsy has been described in detail elsewhere (10). Briefly, after obtaining written informed consent from the patients, laparoscopy was performed in an endoscopy suite by a pair of hepatologists. The abdomen was cleaned and draped. The left paramedian site was anesthetized by 0.5% procaine hydrochloride, and a Veress needle was inserted into the abdomen to form a pneumoperitoneum with 2–3 l nitrous oxide. After an area 1 inch to the left of and cranial to the umbilicus was infiltrated with 0.5% procaine hydrochloride, an 11 mm Olympus trocar (Olympus, Tokyo, Japan) was inserted into the abdomen. Then, a 10 mm standard-type Olympus laparoscope (Olympus) was inserted into the abdomen to observe the liver, spleen, and peritoneum. The laparoscopic images were videotaped simultaneously. A palpation probe was introduced through the second puncture site in the right hypochondrium to examine the consistency of the liver. After replacing it with a 14-gauge Silverman needle, a liver biopsy was performed. To avoid excess bleeding, gelatin (Spongel^R, Yamanouchi, Tokyo, Japan) was filled into the biopsy site. After hemostasis was confirmed, the laparoscope was pulled out, the gas was allowed to escape, and the trocar and needles were removed. The abdominal wound was closed with a single silk suture. An overnight stay in the hospital was required for observation after the procedure.

For evaluation of laparoscopic findings, three experienced hepatologists blinded to the clinical, laboratory, radiological, and histological data reviewed all laparoscopic findings independently by video films. The gross laparoscopic findings were graded as follows: (1) hepatomegaly (classified into none, mild, moderate, and marked), (2) color of the liver surface (classified into reddish-brown, yellowish, yellowish-white, and whitish), and (3) irregularity of the liver surface (classified into smooth, uneven, granular, and nodular). The presence or absence of the following findings were also evaluated: (1) increased fat accumulation of the round ligament of the liver, (2) dullness of the liver edge, (3) perihepatic adhesion, (4) depressions, (5) nodules, (6) reddish patches, (7) whitish markings, and (8) lymphatic vesicles.

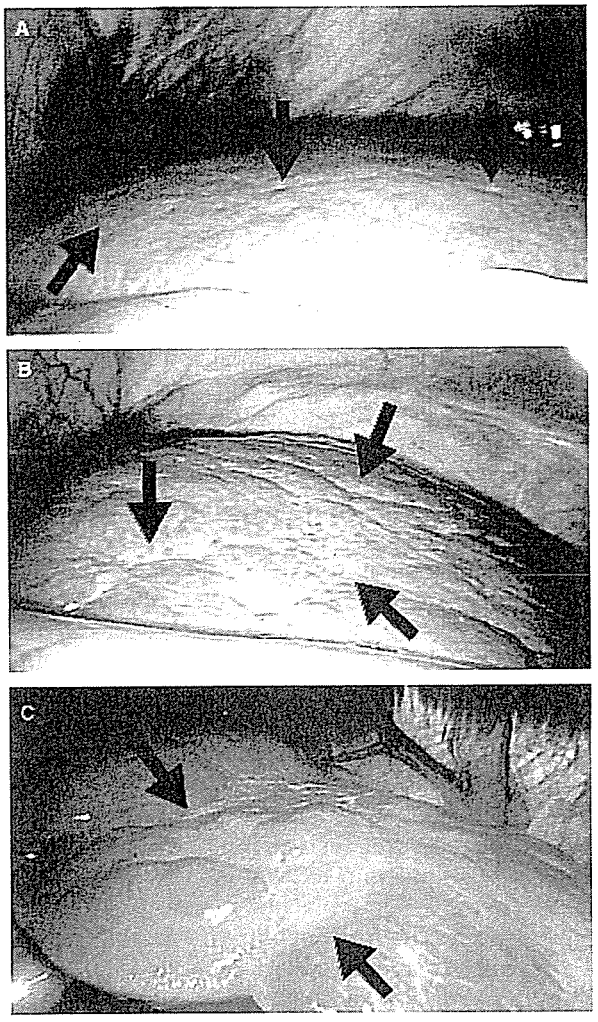

Fig. 1. Classification of depressions: (A) small depressions observed in a patient with NASH (arrows), (B) coarse depressions observed in a patient with autoimmune hepatitis (AIH) (arrows), (C) groove-like depression observed in a patient with AIH (arrows).

Depressions on the liver surface were classified into small, coarse, or groove-like ones according to the following criteria (Fig. 1): (1) *small depression*: a depressed area on the liver surface less than 5 mm in diameter, (2) *coarse depression*: a coarsely depressed area on the liver surface more than 5 mm, and (3) *groove-like depression*: a groove-shaped long depression on the liver surface extending for more than 20 mm. Coarse and groove-like depressions indicated massive or zonal necrosis.

The nodules were classified into either micronodules, which were defined as small nodules of up to a few millimeters in diameter, or, when the nodules were over 10 mm in diameter, classified as either mound-like or hemispherical nodules according to shape.

For the comparison of surface irregularities or nodules among patients with NASH, ALD, and AIH, the stage of fibrosis was matched to the advanced stage showing bridging fibrosis or cirrhosis. The frequency of each representative laparoscopic finding was calculated in patients with NASH, ALD, and AIH, respectively, and expressed as a percentage.

Histological examination

The liver biopsy specimens were immediately fixed in 10% neutral formalin. The sections were cut at 4-μm thickness and stained with hematoxylin and eosin, or using the Azan–Mallory method. Fibrosis was assessed using a staging system according to the classification published by Brunt et al. (11). Stage 1 denotes the presence of perivenular/pericellular fibrosis in zone 3, stage 2 represents the changes of stage 1 with focal or extensive periportal fibrosis, stage 3 denotes the changes of stage 2 plus bridging fibrosis, and stage 4 indicates cirrhosis.

Statistical analysis

All statistical analyses were performed using SPSS software 11.0J for Windows (SPSS Japan Inc., Tokyo, Japan). Qualitative variables were expressed as percentages and were compared using Fisher's exact probability test. Quantitative data were expressed as means ± standard deviations, and were compared using Student's *t*-test. Probability values less than 0.05 were considered statistically significant.

Results

Laparoscopic findings of NASH

Fourteen patients were obese, and 10 were diabetic. Laboratory data revealed mild elevation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γGT), glucose, and ferritin levels. HOMA-IR was more than 2.0 in all patients. The histological stages of the 24 patients were as follows: stage 1 in three patients, stage 2 in 11, stage 3 in three, and stage 4 in seven.

The laparoscopic findings of the 24 patients with NASH are summarized in Table 1, and typical laparoscopic findings are shown in Figs 2 and 3. The common gross findings were mild hepatomegaly (83.3%), dullness of the liver edge (95.8%), and increased fat accumulation in the round ligament of the liver (100%). No special findings were found in the gallbladder. The spleen seemed to be enlarged in some patients, but it was

Table 1. Laparoscopic findings in patients with NASH ($n = 24$)

Hepatomegaly	
None	3 (12.5%)
Mild	20 (83.3%)
Moderate	1 (4.2%)
Marked	0 (0%)
Color of the liver surface	
Reddish-Brown	0 (0%)
Yellowish	2 (8.3%)
Yellowish-White	7 (29.2%)
Whitish	15 (62.5%)
Irregularity of the liver surface	
Smooth	6 (25.0%)
Uneven	12 (50.0%)
Granular	0 (0%)
Nodular	6 (25.0%)
Other findings	
Dullness of the liver edge	23 (95.8%)
Increased fat accumulation of round ligament	24 (100%)
Increased vessel markings	24 (100%)
Perihepatic peritoneal adhesion	0 (0%)
Whitish markings	22 (91.7%)
Reddish patch/markings	2 (8.3%)
Lymphatic vesicles	1 (4.2%)
Small depressions	17 (70.8%)
Coarse depressions	0 (0%)
Groove-like depressions	1 (4.2%)

NASH, nonalcoholic steatohepatitis.

difficult to evaluate the size of the spleen accurately because it was often covered with a thick fatty omentum. No peritoneal adhesion was detected around the liver.

In more than 90% of the patients, the liver surface was diffusely yellowish-white or whitish, but not yellowish. A leopard skin-like appearance was not found. Whitish markings and increased vessel markings, which are suggestive of chronic liver injury and hepatic fibrosis, were observed in 91.7% and 100% of the patients, respectively. However, reddish patches, reddish markings, and lymphatic vesicles, which suggest strong lobular inflammation and lymphatic stasis, were rare in livers with NASH.

Next, the irregularity of the liver surface was investigated. The liver surface was smooth in 25% of the patients and uneven in 50%. Nodules were observed in 25% of the patients. In approximately 70% of the patients, small depressions were scattered on the liver surface. However, coarse depressions and groove-like depressions were extremely rare.

In the 24 NASH patients we investigated, 10 patients were not obese (BMI < 25). The frequencies of these laparoscopic findings did not differ between obese and nonobese patients with NASH.

Relationship between macroscopic and microscopic findings

The relationship between the color of the liver surface and histological stage is shown in Table 2.

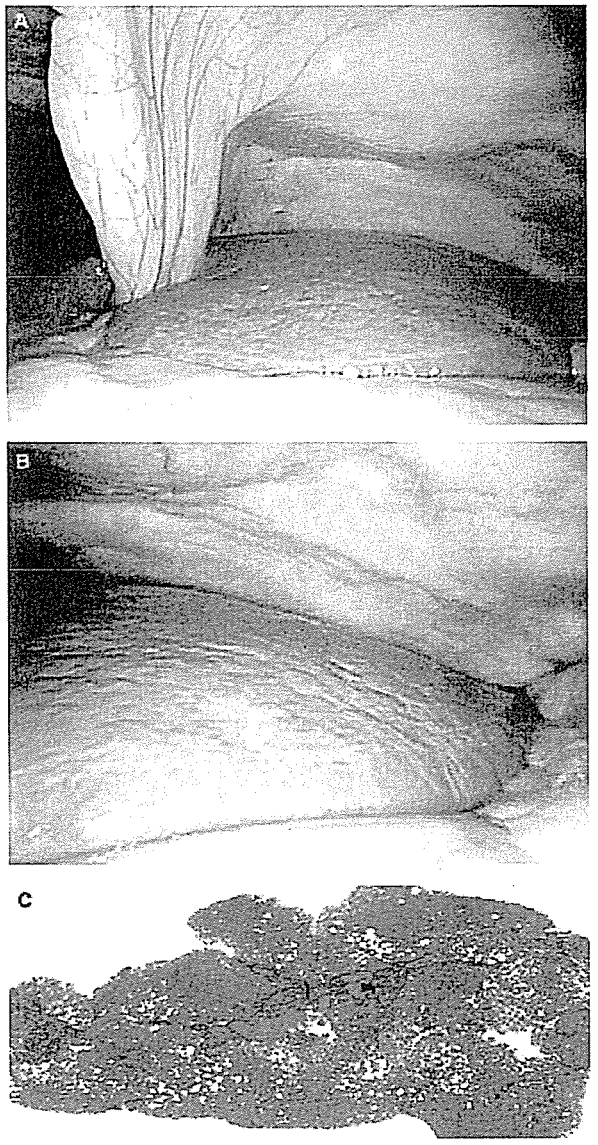

Fig. 2. Laparoscopic findings of NASH (stage 3): (A) fat accumulation of the hepatic round ligament is markedly increased, (B) whitish liver surface, mild hepatomegaly, dullness of the liver edge, and scattered small depressions are recognized, (C) Azan–Mallory staining. Moderate fatty deposition and stage 3 fibrosis are seen (× 100).

Livers representing a yellowish surface, which reflects severe hepatic steatosis, were only in stages 1 and 2. With advances in the stage of fibrosis, the liver surface became more whitish than yellowish.

The relationship between the irregularity of the liver surface and histological stage was also examined. All patients with a smooth liver surface were histologically diagnosed as being in stage 1 or 2. As expected, all patients with nodules were in stage 4. However, in livers bearing small depressions, 65% were diagnosed as being in stage 1 or 2, and 17.6% were in stages 3

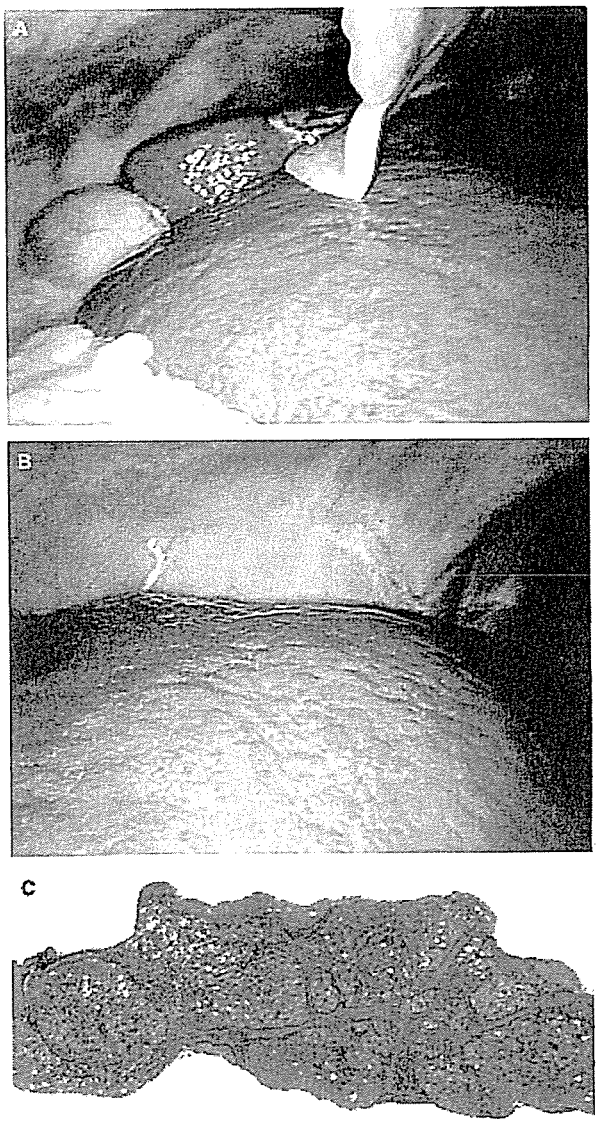

Fig. 3. Laparoscopic findings of NASH (stage 4): (A) long-distance view, an enlarged left lobe with a rounded edge is observed; (B) close-up view of the left lobe, the liver surface is uneven, and whitish markings are prominent. Nodular formation is still partial and incomplete; (C) Azan–Mallory staining. Mild fatty deposition and stage 4 fibrosis are seen (× 100).

Table 2. Relationship between laparoscopic findings and histological stage

	Stage 1–2 ($n=14$)	Stage 3 ($n=3$)	Stage 4 ($n=7$)
Color of the liver surface			
Yellowish ($n=2$)	2	0	0
Yellowish-White ($n=7$)	7	0	0
Whitish ($n=15$)	5	3	7
Irregularity of the liver surface			
Smooth ($n=6$)	6	0	0
Uneven ($n=12$)	8	2	2
Nodular ($n=6$)	0	1	5
Small depressions ($n=17$)	11	3	3

Table 3. Comparison of clinical backgrounds and laparoscopic findings between NASH, alcoholic liver disease (ALD), and autoimmune hepatitis (AIH) in stage 3–4

	NASH ($n=10$)	ALD ($n=9$)	AIH ($n=11$)
Patient backgrounds			
Gender (female/male)	7/3	0/9	9/2
Age	67 ± 5	53 ± 13[a]	64 ± 15
Obesity (BMI > 25)	7 (70%)	1 (11.1%)[a]	3 (27.3%)[a]
Hypertension	5 (50%)	1 (11.1%)[a]	3 (27.3%)[a]
Diabetes	7 (70%)	0 (0%)[a]	1 (9.1%)[a]
Hyperlipidemia	4 (40%)	0 (0%)[a]	1 (9.1%)[a]
BMI (kg/m^2)	28.1 ± 3.1	22.9 ± 2.3[a]	24.5 ± 3.6[a]
AST (IU/l)	53 ± 18	70 ± 50	83 ± 44[a]
ALT (IU/l)	51 ± 27	52 ± 29	78 ± 63
γGT (IU/l)	76 ± 42	187 ± 158[a]	121 ± 65
Total cholesterol (mg/dl)	185 ± 31	177 ± 70	201 ± 65
Triglycerides (mg/dl)	124 ± 43	154 ± 98	125 ± 52
Immunoglobulin G (mg/dl)	1551 ± 327	1931 ± 434	2038 ± 573[a]
Patients in stage 4	7 (70%)	7 (77.8%)	6 (54.5%)
Laparoscopic findings			
Hepatomegaly	9 (90%)	8 (88.9%)	5 (45.5%)
Dullness of the liver edge	10 (100%)	5 (55.6%)[a]	6 (54.5%)[a]
Increased fat accumulation of round ligament	9 (90%)	2 (22.2%)[a]	2 (18.1%)[a]
Perihepatic adhesion	0 (0 %)	1 (11.1%)	4 (36.4%)
Reddish patch/markings	2 (20 %)	2 (22.2%)	6 (54.5%)
Lymphatic vesicles	1 (10 %)	3 (33.3%)	3 (27.3%)
Small depressions	6 (60 %)	3 (33.3%)	0 (0%)[a]
Coarse depressions	0 (0 %)	7 (77.8%)[a]	10 (90.9%)[a]
Groove-like depressions	1 (10 %)	1 (11.1%)	8 (72.7%)[a]
Nodular formation in stage 4	6/7	5/7	6/6
Micronodules	2	3	0
Mound-like nodules	4	2	2
Hemispherical nodules	0	0	4

NASH, nonalcoholic steatohepatitis; BMI, body mass index. Values are expressed as means ± standard deviations. [a]$P<0.05$ compared with the NASH group.

and 4, respectively. Small depressions were generally found, regardless of the histological stage (Table 2).

Comparison of laparoscopic findings among NASH, ALD, and AIH

To differentiate NASH from ALD or AIH using laparoscopic findings, we compared representative laparoscopic findings between NASH and ALD and between NASH and AIH, in the advanced stages (Table 3). Patients with NASH in stages 3 and 4 ($n = 10$) had complicated such as obesity, hypertension, diabetes mellitus, and hyperlipidemia at a high frequency relative to patients with ALD ($n = 9$) and AIH ($n = 11$) in the same fibrotic stage. Patients with AIH showed higher levels of serum aminotransferases and immunoglobulin G than those of NASH or ALD. Compared with ALD, the dullness of the liver edge and increased fat accumulation of the round ligament were more common in NASH (100% vs. 55.6%, $P = 0.033$, and 90% vs. 22.2%,

$P = 0.005$, respectively). Coarse depressions were significantly less common in NASH than in ALD (0% vs. 77.8%, $P = 0.001$). Lymphatic vesicles seemed to be uncommon in NASH, but were not statistically significant. When laparoscopic findings were compared between NASH and AIH, dullness of the liver edge and increased fat accumulation were more common in NASH (100% vs. 54.5%, $P = 0.023$, and 90% vs. 18.1%, $P = 0.002$, respectively). Small depressions were also more common in NASH (60% vs. 0%, $P = 0.004$), but coarse depressions and groove-like depressions were extremely rare in NASH compared with AIH (0% vs. 90.9%, $P < 0.001$, and 10% vs. 72.7%, $P = 0.006$, respectively). The appearance rates of reddish patches and markings did not differ significantly among the three groups, and neither did the shape of the nodules. Whitish markings and increased vessel markings were observed in all patients in the respective groups.

Discussion

As far as we know, this is the first study concerning the laparoscopic findings of NASH. Iwamura previously described the laparoscopic features of fatty liver with inflammation; that is, steatosis hepatis according to Kalk's classification (12), as an irregular spotty thickening of the hepatic capsule and a shallow depression in the hepatic parenchyma (8). He also showed laparoscopic pictures of the nodular liver of steatosis, histologically corresponding to fatty cirrhosis of the liver, in his review (8). Because these conditions are now defined as NASH, these previous observations agree with the results of this study.

We defined the criteria of NASH as strictly as possible in this study. To differentiate NASH from ALD and AIH completely, we excluded NASH patients with minimal alcohol consumption or positive autoantibodies. We also tried to exclude patients with cryptogenic cirrhosis, who are unable to be histologically diagnosed with NASH because of the reduction of lipid droplets, that is, burned-out NASH. Although the number of patients analyzed was limited, these results are considered valid when the laparoscopic characteristics of NASH are discussed.

Because NASH is strongly associated with visceral obesity, it was not surprising to find that an increase in fat accumulation of the hepatic round ligament was very common in NASH. Chronic alcohol abuse results in energy wastage and in the inhibition of adipose tissue accumulation, so alcoholics are sometimes not obese despite a high total energy intake (13). Indeed, in this study, about 90% of patients with advanced ALD had a BMI of less than 25 kg/m^2 (Table 3). Moreover, the association between visceral fat accumulation and chronic liver diseases, except in NASH, is not known. Therefore, increased fat accumulation of the hepatic round ligament may be a useful finding to differentiate NASH from other chronic liver diseases, especially ALD.

According to the results of this study, the laparoscopic appearance of NASH is classified as a whitish liver, as well as chronic inactive hepatitis. As shown in Table 2, the liver surface became less yellowish and more whitish as hepatic fibrosis developed. Inui et al. (14) demonstrated that the coexistence of hepatic steatosis and fibrosis tended to make it difficult to observe a spotty yellow-colored surface. The color change of the liver surface observed in patients with NASH may be associated not only with the thickening of the hepatic capsule because of persistent hepatocyte injury and fibrosis but also attenuation of steatosis. In fact, the histological evaluation in this study demonstrated that the degree of steatosis tended to be milder as hepatic fibrosis progressed (data not shown). Therefore, a whitish change of the liver surface is one of the important findings that strongly suggests NASH rather than a nonalcoholic fatty liver or simple steatosis.

In NASH, small depressions were frequently observed in most patients regardless of the fibrotic stage, and were more common laparoscopic findings compared with ALD or AIH. On the other hand, coarse and groove-like depressions were rarely observed in patients with NASH. Depressions are generally caused by the necrosis of hepatocytes, collapse, and the resultant fibrosis. Thus, these differences in the shape of the depressions indicate that the necroinflammatory change is milder in NASH than in ALD or AIH. Although it is supposed that, even in NASH, the depressions may be deeper and larger with disease progression, we failed to prove this hypothesis in the present study.

Accurate diagnosis of NASH can be difficult, because no specific diagnostic markers of NASH have been found (15). We have occasionally found that it is difficult to make a diagnosis of either NASH or AIH because of the high positive rate of nonspecific autoantibodies in NASH patients (16). Although the number of patients was small, this study demonstrated that dullness of the liver edge and small depressions were common, whereas coarse and groove-like depressions were very rare in patients with NASH; these were entirely distinct from patients with AIH. Therefore, these laparoscopic differences may be help-

ful for the accurate diagnosis of either NASH or AIH. Laparoscopy may be valuable for patients with nonalcoholic fatty liver disease with positive autoantibodies and/or elevated immunoglobulin G levels.

There were some limitations to this study. First, a number of NASH patients analyzed were limited. To confirm our results, a large-scale analysis with enrollment of many patients with NASH is needed. Second, the presence or absence of typical laparoscopic findings alone is insufficient to lead to a detailed evaluation of the extent of activity or the progression of NASH. The establishment of a laparoscopic scoring system may be useful not only for accurately diagnosing NASH but also for evaluating the relationship between the laparoscopic findings and the clinical, biochemical, and histological ones. Finally, it was not possible to demonstrate the time course of the laparoscopic findings in the same patients. It would be interesting to observe how the laparoscopic findings changed in accordance with disease progression or with the treatment of NASH. Although laparoscopy is, to some extent, an invasive examination, the information obtained by serial laparoscopy will be of great value when the natural history of NASH is reviewed.

In conclusion, this study demonstrates that mild hepatomegaly, dullness of the liver edge, increased fat accumulation of the hepatic round ligament, whitish markings, and small depressions were common laparoscopic findings in NASH. However, compared with stage-matched patients with ALD or AIH, coarse and groove-like depressions were rare in those with NASH. Laparoscopic examination may be useful for providing additional information to make an accurate diagnosis of NASH.

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Journal of Medical Virology 78:68–73 (2006)

Patients With and Without Loss of Hepatitis B Virus DNA After Hepatitis B e Antigen Seroconversion Have Different Virological Characteristics

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The characteristic differences between patients with and without loss of hepatitis B virus (HBV) DNA after achieving hepatitis B e antigen seroconversion were analyzed by comparing changes in HBV DNA and HBV core-related antigen levels during a period from 3 years before to 3 years after the seroconversion. Of the 24 seroconverters, 6 (inactive replication group) showed continuous loss of HBV DNA in serum after the seroconversion and the remaining 18 did not lose HBV DNA (active replication group). The HBV DNA level was similar between the two groups, while the HBV core-related antigen level was significantly lower in the active replication group than in the inactive replication group before the seroconversion. The levels of both HBV DNA and HBV core-related antigen decreased remarkably around the time of seroconversion in the inactive replication group, while these levels did not change or decreased slightly in the active replication group. After the seroconversion, the HBV DNA level was significantly higher in the active replication group than in the inactive replication group, while the HBV core-related antigen level was similarly low between the two groups. Because the serum level of HBV core-related antigen mainly reflects that of HBe antigen, the low level of HBV core-related antigen seen after seroconversion in both groups might have contributed to the occurrence of seroconversion. The precore and core promoter mutations which cause diminished excretion of hepatitis B e antigen were significantly more frequent in the active replication group than in the inactive replication group. It was therefore considered that the seroconversion was caused mainly by a decrease in viral replication in the inactive replication group, and mainly by a decrease in HBe antigen production in the active replication group. ***J. Med. Virol. 78:68–73, 2006.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: HBV DNA; seroconversion; HBV core-related antigen; precore mutation; core promoter mutation

INTRODUCTION

A total of 350 million people worldwide are estimated to be carriers of hepatitis B virus (HBV) [Maynard, 1990; Maddrey, 2000]. HBV is important as a causative agent for liver diseases such as chronic hepatitis and hepatocellular carcinoma, especially in Asian countries [Lee, 1997]. In the natural history of chronic HBV infection, seroconversion from hepatitis B e (HBe) antigen to its antibody (anti-HBe) is usually accompanied by a decrease in HBV replication and remission of hepatitis [Realdi et al., 1980; Hoofnagle et al., 1981; Liaw et al., 1983]. Thus, HBe antigen seroconversion is a favorable sign for patients with chronic hepatitis B. However, there are some patients who continue to have elevated HBV DNA levels in the serum and active liver disease after the seroconversion [Bonino et al., 1986; Hsu et al., 2002].

Although the detailed mechanisms of HBe antigen seroconversion have not been fully clarified, several mutations in the HBV genome have been reported to be associated with the phenomenon. When the precore (pre-C) and core genes in the HBV genome are

Grant sponsor: Ministry of Health, Labour and Welfare of Japan; Grant number: 1640013-41.

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Accepted 29 September 2005
DOI 10.1002/jmv.20505
Published online in Wiley InterScience (www.interscience.wiley.com)

transcribed and translated in tandem, HBe antigen is produced and secreted into circulation [Bruss and Gerlich, 1988; Garcia et al., 1988]. The G to A mutation at nucleotide (nt) 1896 in the pre-C region (G1896A), which converts codon 28 for tryptophan to a stop codon, is associated with the loss of HBe antigen [Carman et al., 1989; Okamoto et al., 1990]. The double mutation (A1762T and G1764A) in the core promoter (CP) has been shown to reduce the synthesis of HBe antigen by suppressing the transcription of precore mRNA [Okamoto et al., 1994; Takahashi et al., 1995; Buckword et al., 1996]. Convincing lines of evidence have indicated a close association of HBe antigen seroconversion with the appearance of precore and core promoter mutations [Okamoto et al., 1994; Takahashi et al., 1995; Buckword et al., 1996; Yamaura et al., 2003] as well as the severity of liver disease [Kosaka et al., 1991; Aritomi et al., 1998; Lindh et al., 1998].

A chemiluminescence enzyme immunoassay (CLEIA) was developed previously for the detection of HBV core-related antigen [Kimura et al., 2002; Rokuhara et al., 2003]. The HBV core-related antigen is expressed on HBe and core (HBc) antigens; both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical. The HBVcrAg CLEIA measures the serum levels of HBe and HBc antigens simultaneously, using monoclonal antibodies, which recognize common epitopes of these two denatured antigens. However, the amount of HBV core-related antigen mainly reflects that of HBe antigen, because the concentration of HBe antigen in serum is much higher than that of HBc antigen [Kimura et al., 2002]. In the present study, the characteristic differences that may exist between patients with and without HBV DNA in serum after HBe antigen seroconversion were examined by comparing chronological changes of HBV DNA and HBV core-related antigen as well as by testing HBV genome mutations associated with the seroconversion.

MATERIALS AND METHODS

Patients

The present study is a retrospective one using stored sera from Japanese patients with chronic hepatitis B seen in Shinshu University Hospital. The clinical database was reviewed to identify all patients who had been followed from January 1985 to June 2001 and also showed seroconversion from HBe antigen to anti-HBe during the follow-up period. A total of 24 patients were recruited in the present study. The 24 patients consisted of 17 men and 7 women with a median age of 39 years. Seroconversion of HBe antigen was defined as disappearance of HBe antigen accompanied by the development of anti-HBe on at least two consecutive visits. All 24 patients met the following three criteria: (1) follow-up was performed for at least 3 years before and after the seroconversion; (2) chronic hepatitis without liver cirrhosis was confirmed by histological examination; and (3) serum samples were available for testing every 6 months during the follow-up period. Of the 24 patients, 12 patients received interferon administration of at most 4 weeks and none received nucleotide analogs such as lamivudine, adefovir, or entecavir during the follow-up period.

Serum concentrations of HBV DNA and HBV core-related antigen were determined every 6 months during the follow-up period, which ran from 3 years before to 3 years after the seroconversion. The presence or absence of the pre-C mutation of A1896 and the double mutation in the CP (T1762/A1764) was determined every year during the follow-up period. The serum samples had been stored at −20°C or below until tested. Written informed consent was obtained from each patient.

Serological Markers for HBV

Conventional HBV markers, including HBe antigen and anti-HBe, were tested using CLEIA kits (Fuji Rebio, Tokyo, Japan). Six major genotypes (A–F) of HBV were determined using the method reported by Mizokami et al. [1999], in which the surface gene sequence amplified by PCR was analyzed by restriction fragment length polymorphism.

The Pre-C and CP mutations were determined on nucleic acids extracted from 100 μl of serum with a DNA/RNA extraction kit (Smitest EX-R and D; Genome Science Laboratories Co., Ltd., Tokyo, Japan). The stop codon mutation in the Pre-C region (A1896) was detected with an enzyme-linked mini-sequence assay kit (Smitest; Genome Science Laboratories). In principle, G1896 in the wild-type HBV and A1896 in the mutants were determined by mini-sequence reactions using labeled nucleotides that are complementary to either the wild-type or mutant. The results were expressed as a percent mutation rate according to the definition by Aritomi et al. [1998]. The sample was judged positive for the pre-C mutation when the mutation rate exceeded 50% in the present study, because the mutation rate steadily increase to 100% afterward once it exceed the rate of 50% [Yamaura et al., 2003]. The double mutation in the CP was detected using an HBV core promoter detection kit (Smitest; Genome Science Laboratories) [Aritomi et al., 1998]. This kit detects T1762/G1764 or A1762/T1764 by a polymerase chain reaction (PCR) with primers specific for either the wild-type or mutant. The results were recorded in three categories, that is, wild, mixed, and mutant types. In the present study, the sample was considered positive for the CP mutation when the results were in the mutant type category. The detection limits of the pre-C and the CP mutation kits are both 1,000 copies/ml according to the manufacturer. The pre-C mutation could be determined in 136 (99%) of 137 samples, which had HBV DNA levels higher than 1,000 copies/ml and in 30 (97%) of 31 samples which had levels lower than 1,000 copies/ml. Similarly, the CP mutation could be determined in 136 (99%) of 137 samples and in 28 (90%) of 31 samples.

The serum concentration of HBV DNA was determined using an Amplicor HBV monitor kit (Roche,

Tokyo, Japan) which had a quantitative range of 2.6–7.6 log copies/ml [Kessler et al., 1998]. Sera containing over 7.0 log copies/ml HBV DNA were diluted 10- or 100-fold in normal human serum and measured again to obtain the end titer.

The serum concentration of HBV core-related antigen was measured using the CLEIA reported previously [Kimura et al., 2002; Rokuhara et al., 2003]. In summary, 100 μl serum was mixed with 50 μl pretreatment solution containing 15% sodium dodecylsulfate and 2% Tween 60. After incubation at 70°C for 30 min, 50 μl pretreated serum was added to a well coated with monoclonal antibodies against denatured HBc and HBe antigens (HB44, HB61, and HB114) and filled with 100 μl assay buffer. The mixture was incubated for 2 hr at room temperature and the wells were washed with buffer. Alkaline phosphatase-labeled monoclonal antibodies against denatured HBc and HBe antigens (HB91 and HB110) were added to the well, and incubated for 1 hr at room temperature. After washing, CDP-Star with Emerald II (Applied Biosystems, Bedford, MA) was added and the plate was incubated for 20 min at room temperature. The relative chemiluminescence intensity was measured, and the HBV core-related antigen concentration was read by comparison to a standard curve generated using recombinant pro-HBe antigen (amino acids, 10–183 of the precore/core gene product). The HBV core-related antigen concentration was expressed as units/ml (U/ml) and the immunoreactivity of recombinant pro-HBe antigen at 10 fg/ml was defined as 1 U/ml. In the present study, the cut-off value was set tentatively at 3.0 log U/ml. Sera containing over 7.0 log U/ml HBV core-related antigen were diluted 10- or 100-fold in normal human serum and measured again to obtain the end titer.

Statistical Analyses

The Mann–Whitney U test was used to analyze continuous variables. The Fisher's exact test was used in the analysis of categorical data. The Manzel Haentel chi-square test was used to evaluate positive rates for the pre-C and CP mutations. The Wilcoxon test was used to analyze the change in the level of HBV DNA and HBV core-related antigen. *P*-values less than 0.05 were considered significant. Statistical analyses were performed using an SPSS 11.5 J statistical software package (SPSS, Inc., Chicago, IL).

RESULTS

Grouping of Seroconverters According to HBV DNA Outcome

The 24 seroconverters enrolled in the present study were classified into two groups according to changes in serum levels of HBV DNA. The HBV DNA level decreased substantially around the time of the seroconversion and then became continuously undetectable in one group (inactive replication group), and the level decreased slightly and did not become continuously undetectable even after the seroconversion in another group (active replication group). In the present study, the former group of patients were defined as those whose HBV DNA levels were lower than 2.6 log copies/ml at each of the time points of 1.5, 2, 2.5, and 3 years after the seroconversion, and the latter group of patients were defined as those whose HBV DNA levels were not. Of the 24 seroconverters, 6 belonged to the inactive replication group and the remaining 18 belonged to the active replication group.

The clinical backgrounds of the active and inactive replication groups are compared in Table I. The median age, gender ratio, and history of interferon therapy did not differ between the two groups. All patients were infected with genotype C HBV. Normalization of serum alanine aminotransferase (ALT) after seroconversion was considered to have occurred in cases in which ALT was normal at each of the time points of 2, 2.5, and 3 years after the seroconversion in the present study. The normalization of ALT was more frequent in the inactive replication group than in the active replication group, but the difference was not statistically significant.

Changes in HBV DNA and HBV Core-Related Antigen Concentration

Changes in the serum level of HBV DNA are compared between the active and inactive replication groups in Figure 1A. At the start-point of the follow-up, the level was distributed within a similarly high range in both groups. In the inactive replication group, the median

TABLE I. Comparison of Clinical Backgrounds Between the Inactive and Active Replication Groups

Characteristics	Inactive replication group n = 6	Active replication group n = 18	*P*
Age at seroconversion (yr)[a]	37 (23-65)	39 (17-64)	>0.2*
Gender (M:F)	4:2	13:5	>0.2**
Genotype C[b]	6 (100%)	18 (100%)	>0.2**
History of interferon therapy[b]	3 (50%)	9 (50%)	>0.2**
ALT normalization[c]	4 (67%)	5 (28%)	0.150**

*Mann–Whitney U test.
**Fisher's exact test.
[a]Data are expressed as the median (range).
[b]Data are expressed as a positive number (percent).
[c]Normalization of serum ALT level after seroconversion (the ALT value was within the normal range at each of the time points of 2, 2.5, and 3 years after the seroconversion).

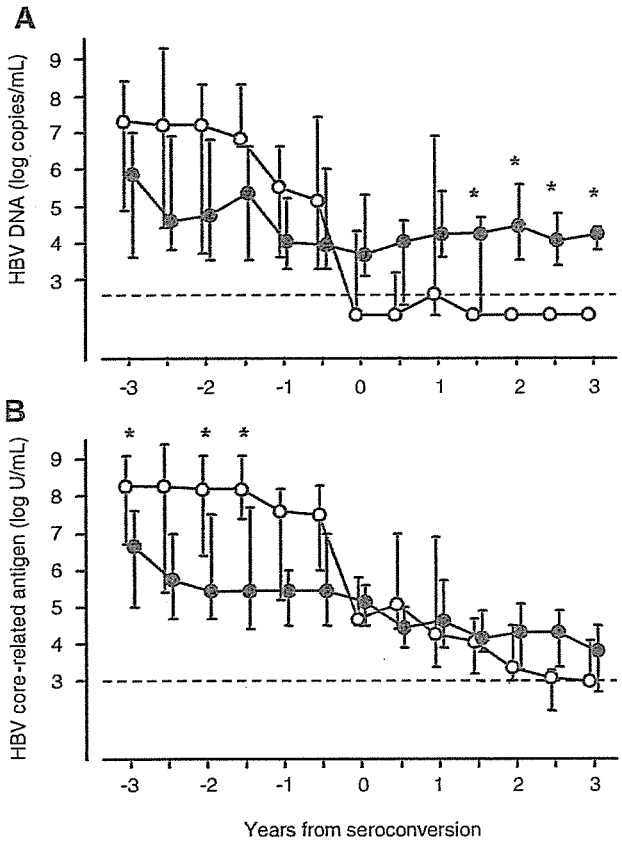

Fig. 1. Comparison of changes in HBV DNA (A) and HBV core-related antigen (B) levels between the inactive and active replication groups. Data are shown as the median ±25% ranges. The broken lines indicate the detection limits of the HBV DNA and HBV core-related antigen assays, respectively. Open circles indicate inactive replication group and closed circles indicate active replication group. *$P < 0.05$ between the inactive and active replication groups.

concentration decreased around the time of seroconversion and became continuously undetectable thereafter. In the active replication group, on the other hand, the median concentration tended to decrease around the time of seroconversion, but was not undetectable even at 3 years after seroconversion. The median HBV DNA level in the active replication group was significantly higher than that in the inactive replication group at 1.5 years after the seroconversion and each of the subsequent time points.

Changes in the serum concentration of HBV core-related antigen are compared between the active and inactive replication groups in Figure 1B. The concentration of HBV core-related antigen was significantly higher in the inactive replication group than in the active replication group at the start of the follow-up and at 1.5 and 2 years before the seroconversion point. The median concentration of HBV core-related antigen in the inactive replication group appeared to decrease around the time of seroconversion and reached a level comparable to that in the active replication group. The median HBV core-related antigen level was similar between the inactive and active replication groups at all time points after the seroconversion, and it decreased slowly with time in both groups.

Changes in the log ratio of HBV core-related antigen/HBV DNA concentrations are compared between the inactive and active replication groups in Figure 2. The values of HBV core-related antigen and HBV DNA were substituted by their corresponding detection limit values when they were under the detection limit. The log ratio was similar between the two groups at points before the seroconversion. The log ratio decreased after the seroconversion in the active replication group, but did not change in the inactive replication group. The log ratio of HBV core-related antigen/HBV DNA was significantly lower in the active replication group than in the inactive replication group at all post-seroconversion time points except 1 year.

Comparison of Pre-C and CP Mutations

The positive rates for the pre-C and CP mutations at the time points before and after the seroconversion are compared between the inactive and active replication groups in Figure 3. The pre-C mutation did not appear during the follow-up period in the inactive replication group. On the other hand, the positive rate for the pre-C mutation was around 30% before the seroconversion, and then increased to around 60% after the seroconversion in the active replication group. The difference in the positive rate was significant at the time points of 2 and 3 years after the seroconversion. The positive rate for the CP mutation was less than 40% in the inactive replication group during the follow-up period except at the last time point, while it was over 60% in the active replication group throughout the follow-up period. The difference in the positive rate was statistically significant at the time points of 2 and 3 years before the seroconversion and at 1 and 2 years after it.

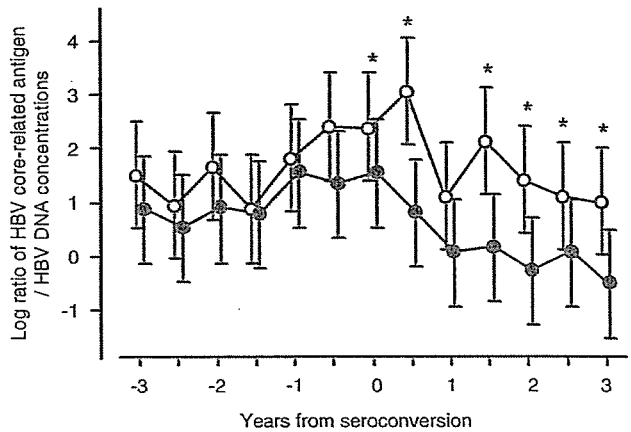

Fig. 2. Comparison of changes in the log ratio of HBV core-related antigen/HBV DNA levels between the inactive and active replication groups. Data are shown as the median ±25% ranges. Open circles indicate inactive replication group and closed circles indicate active replication group. *$P < 0.05$ between the inactive and active replication groups.

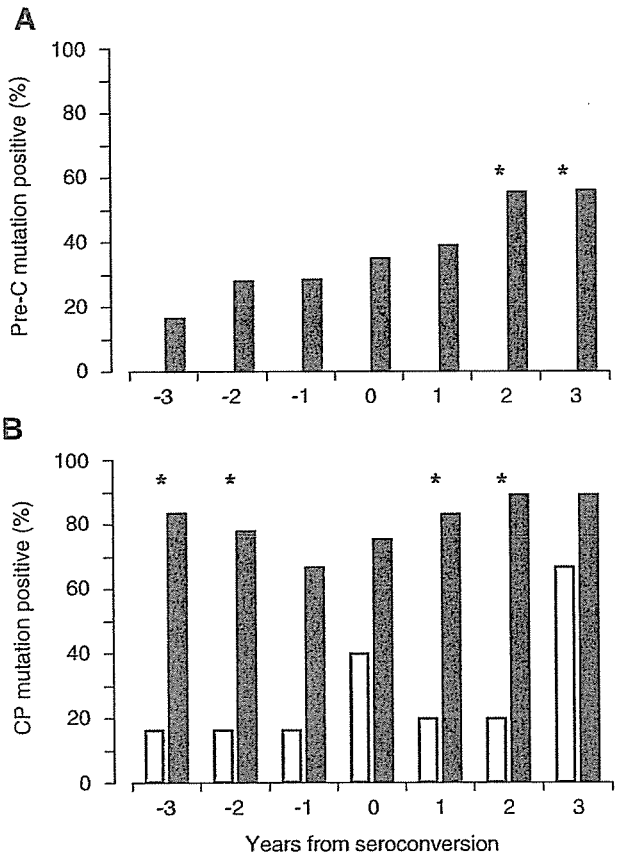

Fig. 3. Comparison of positive rates for the pre-C (A) and CP (B) mutations between the inactive and active replication groups. Open bars indicate inactive replication group and closed bars indicate active replication group. Number of patients in the inactive replication group is six at each time point except the followings: point 0 year (n = 5) in A, and points 0 year (n = 5), 1 year (n = 5), and 2 years (n = 5) in B. Number of patients in the active replication group is 18 at each time point except the followings: point 0 year (n = 17) in A and point 0 year (n = 17) in B. *$P < 0.05$ between the inactive and active replication groups.

DISCUSSION

Seroconverters were divided tentatively into two groups according to their levels of serum HBV DNA in the present study. It has been reported that older age and female gender are factors predicting occurrence of HBe antigen seroconversion in patients with chronic hepatitis B [Alward et al., 1985; Lok et al., 1987; McMahon et al., 2001]. On the other hand, in the present study, median age and gender distribution were similar between the inactive and active replication groups. A history of interferon treatment was recorded in half of the patients enrolled. The treatment history did not seem to be associated with the loss of HBV DNA after seroconversion, because the history was similarly distributed between the two groups and the duration of interferon therapy was as short as 4 weeks at most. Although the difference was not statistically significant, patients in the inactive replication group tended to show continuous normalization of ALT. Further, none of the six patients in the inactive replication group developed end stage liver diseases such as cirrhosis and hepatocellular carcinoma after the follow-up period, while 4 of the 18 patients in the active replication group developed them (data not shown). High viral load, which is usually associated with active hepatitis, has been reported to be a risk factor for development of hepatocellular carcinoma even in patients with chronic hepatitis B who achieved HBe antigen seroconversion [Ikeda et al., 2003; Ohata et al., 2004]. We could not compare long-term prognosis between patients in the inactive and active replication groups in the present study. However, patients in the active replication group tended to show active hepatitis after the seroconversion and to develop end stage liver diseases. Thus, further analysis of patients whose active viral replication continues after the seroconversion would be of clinical significance.

Analysis of the changes in HBV DNA and HBV core-related antigen revealed a clear contrast between the two. Namely, the HBV DNA level was similar between the two groups, while HBV core-related antigen was significantly lower in the active replication group than in the inactive replication group before seroconversion. The levels of both HBV DNA and HBV core-related antigen decreased remarkably around the time of seroconversion in the inactive replication group, while these levels did not change or decreased slightly in the active replication group. After seroconversion, the HBV DNA level was significantly higher in the active replication group than in the inactive replication group, while the HBV core-related antigen level was similar between the two groups. Because the discrepancy in the log ratio of HBV core-related antigen/ HBV DNA between the two groups first appeared at the time of seroconversion and continued thereafter, the difference between the HBV DNA and HBV core-related antigen changes was suggested to be closely associated with the seroconversion. The results obtained in the present study indicate that the mechanism of seroconversion was different between the two groups.

Because the serum level of HBV core-related antigen mainly reflects that of HBe antigen [Kimura et al., 2002], the low level of HBV core-related antigen seen after seroconversion in both the inactive and active replication groups might have contributed to the occurrence of seroconversion. The pre-C and CP mutations, which were associated with the seroconversion, were frequent in the active replication group and rare in the inactive replication group, at least at around the time of seroconversion. The decrease of HBV core-related antigen excretion seen after seroconversion was thought to have been caused mainly by the decrease of viral replication in the inactive replication group, because viral replication did not resume in this group. On the other hand, the decrease of HBV core-related antigen was thought to have been caused mainly by the appearance of pre-C and/or CP mutations, because active viral replication continued in this group. These results suggested that the two groups had different mechanisms of seroconversion.

It has been reported that the frequency of the pre-C and the CP mutations differs among HBV genotypes. Orito et al. reported that the CP mutation was significantly associated with genotype C [Orito et al., 2001]. Yamaura et al. [2003] reported that the CP mutation was already commonly seen several years before the seroconversion in patients with genotype C. These results are consistent with the present finding that the majority of patients in the active replication group had the CP mutation from the start of follow-up. The fact that patients in the active replication group had a lower level of HBV core-related antigen before the seroconversion may be attributable to the frequent CP mutation seen in this group.

In conclusion, the present study showed that there were different mechanisms of HBe antigen seroconversion between patients in whom HBV viraemia continued after the seroconversion and those in whom it did not. Measurement of HBV core-related antigen in addition to HBV DNA was suggested to be useful in examining specific conditions of chronic hepatitis B.

ACKNOWLEDGMENTS

We thank Ms. Toyo Amaki and Ms. Asami Yamazaki for their technical assistance. This research was supported in part by a Grant-in Aid from the Ministry of Health, Labour and Welfare of Japan (no. 1640013-41).

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Hepatology Research 34 (2006) 35–40
HEPATOLOGY RESEARCH
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Timing of interferon therapy and sources of infection in patients with acute hepatitis C

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Received 13 June 2005; received in revised form 22 August 2005; accepted 31 August 2005
Available online 15 December 2005

Abstract

Background/Aims: Controversy over the selection of patients and optimum therapeutic method for acute hepatitis C has continued. The aims of this study were to investigate the source of infection, and to evaluate the timing of interferon (IFN) therapy in patients with acute hepatitis C in Japan.
Methods: The records of 102 patients from 12 facilities in Japan who developed acute hepatitis C after 1990 were investigated. In the patients treated with IFN, we performed multivariate analysis to investigate factors related to sustained virological response (SVR).
Results: Medical procedure was the most common source of infection, accounting for 32.4% in the 102 patients (33/102). Of 81 patients treated with IFN, 71 patients were followed after IFN therapy, and 57/71 (80.3%) had SVR. The SVR rate was significantly higher in patients treated with IFN within 24 weeks from onset of symptoms than the SVR rate in those treated after 25 weeks ($P = 0.0016$). Multivariate analysis revealed that only the duration between onset of symptoms and initiation of IFN therapy (within 24 weeks) was related to SVR.

DOI of related article:10.1016/j.hepres.2005.10.002.
Abbreviations: HCV, hepatitis C virus; IFN, interferon; ALT, alanine aminotransferase; SVR, sustained virological response; Peg-IFN, pegylated interferon
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1386-6346/$ – see front matter
doi:10.1016/j.hepres.2005.08.012

Conclusions: Our multicenter cooperative survey revealed that medical procedure was the most frequent source of infection in acute hepatitis C. As concerns the therapy, interferon treatment should be initiated within 24 weeks after onset of symptoms.

Keywords: Hepatitis C virus (HCV); Acute hepatitis; Medical procedure; Interferon

1. Introduction

There are about 170 million people infected with the hepatitis C virus (HCV) worldwide [1], and the infection progresses to hepatic cirrhosis in 10–30% [1,2]. Since patients often lack subjective symptoms even in acute hepatitis C [3], infection is often realized by patients when the pathology progresses to hepatic cirrhosis and hepatocellular carcinoma. There are a variety of sources of infection, such as medical procedure, intravenous drug use, and sexual behavior [4,5]. In addition, vertical transmission of HCV has been reported, and it seems that maternal viral load is significant for infection to fetus [6]. On the other hand, as a therapy for acute hepatitis C, interferon (IFN) administration has been established to be effective [4,5,7–13].

Although the initial prevention of hepatitis C virus (HCV) infection is ideal, the most effective method of preventing progression to the chronic hepatitis C is still controversial in the acute phase. In Japan, the development of acute hepatitis C due to blood transfusion has markedly decreased after introduction of the HCV antibody test for screening of blood donors [14]. However, infection from intravenous (i.v.) drug use and incidences due to accidental contamination of medical staff are still important problems [15,16]. Investigation for the sources of infection in acute hepatitis C is very important for the prevention. In this study, we investigated a national survey on the route of infection of acute hepatitis C and the therapeutic effectiveness according to the timing of IFN therapy. This survey consists of the largest number of case reports and may reflect the current situation of acute hepatitis C in Japan.

2. Patients and methods

2.1. Patients

A retrospective study was performed in patients of 12 facilities nationwide who developed acute hepatitis C after 1990. The total number of patients at the facilities was 102. Informed written consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Age, gender, source of infection, HCV serotype or genotype, HCV-RNA level, histology of liver biopsy, fluctuation in alanine aminotransferase (ALT) level, presence or absence of IFN therapy, course when not treated with IFN, duration between onset of symptoms and IFN therapy, type of IFN, total dose of IFN, administration method, total duration of administration, and therapeutic results were investigated in each patient.

2.2. Diagnosis of acute hepatitis C

The diagnostic criteria of acute hepatitis C were HCV-RNA detectable at the time of an elevated ALT level, followed by development conversion of HCV antibody. Patients in whom HCV antibody was already positive at the onset were excluded.

2.3. Natural course

In patients who followed the natural course without any treatments, the chronic hepatitis was defined as persistence of HCV-RNA positivity for 6 months or longer, and resolution was defined as a disappearance of serum HCV-RNA within 6 months followed by persistent negativity for 6 months or longer.

2.4. Definition of fluctuation of ALT

In patients diagnosed with acute hepatitis C, when one peak of the serum ALT level was observed, the fluctuation was designated as monophasic, and when two or more peaks were observed, the fluctuation was designated as bi- or multiphasic.

2.5. Serologic tests

Anti-HCV antibody was determined using a second-generation or third-generation enzyme-linked immunosorbent assay (Ortho Diagnostics Systems, Tokyo, Japan). Hepatitis C virus RNA was quantified by using the bDNA signal amplification assay (Chiron Corp.) or the Cobas Amplicor HCV Monitor test ver1.0 or 2.0 (Roche Diagnostic Systems, Tokyo, Japan). The data were represented as Meq/ml, K copies/ml, and KIU/ml, respectively. Detection of HCV-RNA to determine the response of IFN treatment was used by Amplicor HCV (Roche Diagnostics K.K., Japan). Hepatitis C virus serotype was determined using the genotyping enzyme-linked immunosorbent assay (International Reagents Corporation, Tokyo, Japan) to be type 1 or 2 [17].

2.6. IFN therapy

For IFN, IFN-α (natural form, gene recombinant, or consensus IFN), or IFN-β was used (Table 4). No concurrent treatment with IFN and ribavirin was administered to any patient. Among patients treated with IFN, the sustained

virological response (SVR) was defined undetectable HCV-RNA in serum at least 6 months after cessation of therapy. Non-response was defined as detectable HCV-RNA for 6 months after cessation of therapy.

2.7. Statistical analysis

Data were expressed as the mean ± standard deviation for continuous variables and as counts for categorical variables. The results were compared using the Chi-square test, Fisher's exact probability test, or Mann–Whitney *U*-test, depending upon the type of data analysed. Logistic regression was used to analyse the factors contributing to SVR with IFN therapy. *P* values <0.05 were considered significant. Statistical analyses were performed by using Stat View software (version 5.0; SAS Institute Inc., Cary, NC).

3. Results

3.1. Patient characteristics

The baseline characteristics of the 102 patients in this study are shown in Table 1. The distribution of patients by gender and age is shown in Table 2.

3.2. Natural course

The natural course of the disease was followed in 21 patients, and the course could be followed to the outcome in 18 patients (the prognosis was unknown in three patients) (Table 3). The disease progressed to chronic hepatitis C in 61.1% of the patients and resolved spontaneously in 38.9% of the patients. The age and the fluctuation pattern of the ALT level were significantly different between the two groups. As for gender, serum HCV-RNA level, and serogroup, no correlation with spontaneous resolution or chronic hepatitis C was observed.

Table 1
Base-line characteristics of 102 patients

Age	38.6 ± 16.2 (16–84)
Male/female (mean age)	46 (39.2 ± 16.0)/56 (38.2 ± 16.5)
Source of infection (%)	
Medical procedure	33 (32.4)
Accidental needle stick	21 (20.6)
Sexual behavior	8 (7.8)
Drug abuse	6 (5.9)
Tattoo	3 (2.9)
Unknown	31(30.4)
Viral load (high[a]/low/N.D.)	46/45/11
HCVserotype(1/2/N.D.)	54/23/25
IFN/without IFN	81/21

N.D., not determined; IFN, interferon. Details of the routes in medical procedure: surgery 14, blood transfusion 5, endoscopy 3, intravenous injection 4, invasive procedure 3, dental therapy 3, dialysis 1.
[a] Viral load (high): more than 100 KIU/ml or 1 Meq/ml.

3.3. IFN therapy

Table 4 shows the backgrounds of the 81 patients treated with IFN. Of 71 patients in whom the effect was clarified,

Table 2
Distribution of patients according to gender and age

Age (years)	Number of patients					
	Medical procedure (M/F)	Accidental needlestick (M/F)	Sexual behavior (M/F)	Drug abuse (M/F)	Tattoo (M/F)	Unknown (M/F)
<19	0/1	0/0	0/0	0/1	0/0	0/1
20–29	5/1	3/8	1/3	2/1	3/0	2/6
30–39	4/3	3/3	2/1	0/1	0/0	3/3
40–49	2/4	0/4	1/0	0/1	0/0	2/3
50–59	4/3	0/0	0/0	0/0	0/0	2/3
60–69	4/1	0/0	0/0	0/0	0/0	2/0
70–79	0/0	0/0	0/0	0/0	0/0	1/1
>80	0/1	0/0	0/0	0/0	0/0	0/2
Total	19/14	6/15	4/4	2/4	3/0	12/19

M, male, F, female.

Table 3
Base-line characteristics of 18 untreated patients

	Resolved group (seven cases)	Chronic group (11 cases)	*P* value
Age	64.4 ± 15.2	45.6 ± 14.3	0.0331[a]
Gender (male/female)	2/5	4/7	>0.9999
HCV RNA level (high[b]/low/N.D.)	2/4/1	6/4/1	0.6084
Serogroup (1/2/N.D.)	4/0/3	4/2/5	0.4667
Fluctuation of ALT level (monophasic/bi- or multiphasic/N.D.)	5/0/2	0/8/3	0.0008[a]

N.D., not determined; ALT, alanine aminotransferase. Fluctuation of ALT level: monophasic; one peak of the serum ALT was observed, bi- or multiphasic; two or more peaks of the serum ALT were observed (N.D. was excluded from statistical comparisons).
[a] Statistically significant.
[b] Viral load (high): more than 100 KIU/ml or 1 Meq/ml.

Table 4
Base-line characteristics of 81 patients treated with interferon

Age	38.6 ± 16.2
Gender (male/female)	43/38
HCV RNA level (high[a]/low/N.D.)	38/36/7
HCV serogroup (1/2/N.D.)	46/21/14
Fluctuation of ALT level (monophasic/bi- or multiphasic/N.D.)	21/53/7
Type of IFN (α/β)	63/18
Total IFN dose (MU)	470 ± 228.1 (52–972)
Duration of IFN administration (w)	17.6 ± 8.9 (4.0–42.0)
Outcome (SVR[b]/NR/N.D.)	57/14/10

N.D., not determined; ALT, alanine aminotransferase; IFN, interferon; MU, million units; SVR, sustained virological response; NR, non-response: detectable HCV RNA in serum for 6 months after cessation of therapy.
[a] HCV RNA level (high): more than 100 KIU/ml or 1 Meq/ml.
[b] Sustained virological response: undetectable HCV RNA in serum at least 6 months after cessation of therapy.

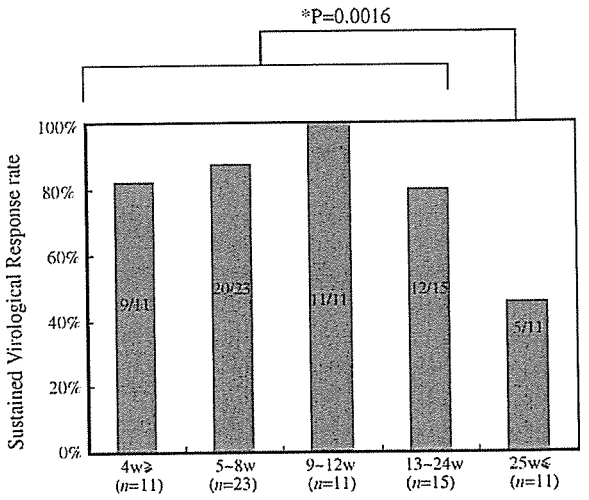

Fig. 1. Sustained virological response rate according to duration between onset of symptoms and initiation of IFN therapy. The groups treated with IFN 0–24 weeks after onset of symptoms and treated after 25 weeks were compared. Comparison by the Chi-square test. (*) Statistically significant; w, week.

57 patients (80.3%) had SVR. Table 5 shows the results of the logistic regression analysis of SVR-related factors. Age, gender, serogroup, HCV-RNA level, fluctuation of ALT, duration between onset and initiation of IFN, type of IFN, total IFN dose, and duration of IFN administration were evaluated statistically by univariate and multivariate analysis. On multivariate analysis as well as univariate analysis, the duration between onset of symptoms and initiation of IFN therapy was the only factor related to SVR.

The SVR rate according to the duration before initiation of IFN therapy was investigated (Fig. 1), and the SVR rate was found to be significantly higher in patients treated before 24 weeks than in patients treated after 25 weeks. However, immediate administration has not been associated with higher SVR rate (0–8 weeks versus 9–24 weeks).

On comparison of the SVR rate by the source of infection, the SVR rate was 100% in the patients infected by accidental needlestick (19/19) (the prognosis was unknown in two of 21 patients infected by needlestick). This was significantly higher than that in patients infected via other routes (19/19 versus 38/52, $P < 0.05$). The duration between onset of symptoms and initiation of IFN therapy was investigated according to the source of infection, and the duration was shortest in the needlestick group (9.7 ± 5.3 weeks).

4. Discussion

We examined the source of infection and optimal timing of therapy in patients with acute hepatitis C at 12 facilities in Japan. Since there has been no study performed in more than 100 patients with acute hepatitis C in Japan, this study may reflect the current situation in Japan. HCV serogroup of 25 patients were not determined (Table 1). Several reasons are considered. Firstly, the study is retrospective. Secondly,

Table 5
Logistic regression analysis of odds ratio for sustained virological response

Variable	Odds ratio	95% CI	*P* value
Univanate			
Age(40>/40≤)	2.48	0.73–8.46	0.147
Gender (female/male)	2.48	0.74–8.33	0.143
Serogroup (1/2)	1.03	0.23–4.54	0.969
HCV RNA level (high[a]/low)	1.75	0.46–6.68	0.413
Fluctuation of ALT (monophasic/bi-or multiphasic)	1.57	0.38–6.45	0.531
Duration between onset and initiation of IFN (≤24w/≥25w)	7.50	1.85–30.48	0.005[b]
Type of IFN (alpha/beta)	4.33	0.52–36.18	0.176
Total IFN dose (>300MU/≤300MU)	2.27	0.63–8.15	0.208
Duration of IFN administration (≥24w/<24w)	1.43	0.44–4.67	0.551
Multivariate			
Duration between onset and initiation of IFN (≤24w/≥25w)	15.78	1.37–181.61	0.027[b]

ALT, alanine aminotransferase; IFN, interferon; MU, million units; 95% CI, 95% confidence interval.
[a] HCV RNA level high: More than 100 KIU/ml or 1 Meq/ml.
[b] Statistically significant.

titer of anti-HCV is often low in early phase of acute hepatitis C. Many patients were considered to be infected during a medical procedure. Studies on risk of surgery for the development of acute hepatitis C have been reported previously [18]. Alfonso et al. performed a large-scale surveillance in Italy and found that 25.5% of patients (261/1023) with acute hepatitis C had undergone an invasive procedure. Therefore, medical care should be recognized as an important source of infection in the sporadic incidence of acute hepatitis C. On the other hand, in blood donors of Western Mexico, the most frequent risk factors for HCV transmission were transfusion (42%) and household exposure (14.8%) [19]. Therefore, the main risk factors for infection may differ with countries.

Since IFN therapy for acute hepatitis C is not covered by the health care insurance, the therapy could not be administered to all patients. The progression to the chronic hepatitis C in the 18 patients with natural courses without IFN therapy was almost consistent with previous reports [20,21]. As shown in Table 3, a significant difference was observed in age, but this may have been due to the two patients in their 80s in the spontaneous resolution group (data not shown). The important point is that the ALT fluctuation was monophasic in all patients in the spontaneous resolution group. In contrast, the fluctuation was bi- or multiphasic in patients who progressed to chronic hepatitis C. As a characteristic of acute hepatitis C in which spontaneous elimination of the virus is likely to occur, it has been reported that many cases are accompanied by subjective symptoms, such as jaundice and influenza-like symptoms [22,23]. Subjective symptoms are sometimes influenced by the patient's subjective sense. In contrast, the fluctuation of the ALT level may be a more objective index. Hofer et al. observed the natural course for at least 30 days after onset, and when serum HCV-RNA became negative during this period, the disease was resolved at a high rate, suggesting that IFN therapy should be administered to patients in whom negative conversion of HCV-RNA did not occur within 30 days [22]. Combined with our results, it might be likely that the disease resolves spontaneously in patients in whom the ALT level followed the monophasic course, as well as in those in whom the disease is symptomatic and negative conversion of HCV-RNA occurs in the early stage.

As the results of IFN therapy, the SVR rate was 80.3% (57/71) as shown in Table 4. Our present study, albeit retrospective analysis, revealed that therapy initiated within 24 weeks was the only factor related to the SVR in both univariate and multivariate analysis (Table 5). In the randomized controlled study by Hwang et al., the factor related to SVR was the HCV-RNA level before initiation of therapy [9]. However, there were only 33 patients, which may have led to a result different from our results. On the other hand, Nomura et al. recently performed a randomized controlled trial in patients with acute hepatitis C, and their results demonstrate that the SVR rate was significantly higher in the early-intervention group (IFN therapy was initiated 8 weeks after the onset) than in the late-intervention group (IFN therapy was initiated after 1 year observation from the onset) (87% versus 40%) [24]. Otherwise, Gruner et al. prospectively investigated the T-cell dynamics in patients with acute hepatitis C, and found that activity of HCV-specific IFN-γ-producing T cells started to decrease 24 weeks after onset [25]. In addition, T cell actions have been reported to be important for elimination of HCV in the early stage of infection [26–30], and the defective functions of HCV-specific T cells might contribute to viral persistence in chronically infected patients [31]. It is interesting that our results support their reports.

Next, we evaluated the optimal timing of initiation of therapy within 24 weeks. In our previous study, we administered therapy after observation of the course for about 4 weeks when signs of the chronic hepatitis began to appear, not immediately after the onset, and obtained good results [32,33]. Licata et al. investigated the optimum timing of IFN therapy by meta-analysis [34]. Their analysis shows that delaying therapy 2 months after the onset of the disease does not affect the efficacy of treatment, therefore, they suggest that patients should be treated within 60 days from the onset to avoid the unnecessary treatment of affected patients who would spontaneously recover. In our study, the highest SVR rate was obtained in the group treated 9–12 weeks after onset of symptoms as shown in Fig. 1, which was consistent with their analytical results.

The SVR rate obtained by combination therapy with Pegylated-IFN (Peg-IFN) and ribavirin for chronic hepatitis C was 30–54% [35–37], but for acute hepatitis C, the therapeutic result was good even when IFN was administered alone. To elucidate this difference, it may be important to investigate not only the T-cell dynamics but also viral genome in various aspects [7]. In our present study, no patients were treated with Peg-IFN. Recently, the efficacy of Peg-IFN monotherapy with acute hepatitis C has been reported. Santantonio et al. evaluated the delaying Peg-IFN therapy, targeting sixteen patients who failed to spontaneously clear the virus within 12 weeks from the onset. They reported that 15/16 patients (94%) showed SVR [38]. Since the highest SVR was obtained in the group treated 9–12 weeks after onset in our study, it is important to start the IFN therapy in optimal timing regardless of the kind of IFN. The high SVR has been obtained by IFN monotherapy, so that, it is necessary to investigate whether ribavirin should be administered concurrently with IFN.

In conclusion, the major sources of infection of acute hepatitis C in Japan were the medical procedure and accidental needlestick. The disease may be likely to resolve spontaneously in patients in whom fluctuation of the ALT level follows the monophasic course. The SVR rate was significantly higher in the group treated with IFN within 24 weeks after the onset of symptoms than in the group treated after 25 weeks. In cases of acute hepatitis C, it is desirable to administer IFN at least within 24 weeks when the ALT level starts to follow a multiphasic course.

Acknowledgments

This study was supported, in part, by a grant-in-aid from the Ministry of Health, Welfare, and Labour of Japan. The authors thank Kendo Kiyosawa, M.D., Keisuke Hino, M.D., Kyosuke Kaji, M.D., Akihiro Iwamitsu, M.D., Tomoo Naito, M.D., Yasuhito Tanaka, M.D., for assistance with data collection.

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