Laparoscopic findings in patients with nonalcoholic steatohepatitis

Naoki Tanaka, Tetsuya Ichijo, Wataru Okiyama, Hidetomo Muto, Noriko Misawa, Akihiro Matsumoto, Kaname Yoshizawa, Eiji Tanaka, and Kendo Kiyosawa

Division of Gastroenterology, Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

Reprint requests and correspondence:

Naoki Tanaka, M.D., Ph.D., Division of Gastroenterology, Department of Internal Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, 390-8621, Japan.

E-mail: naopi@hsp.md.shinshu-u.ac.jp

Tel: (81) 263-37-2634

Fax: (81) 263-32-9412

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Abstract
Background/Aims: Laparoscopic observation of the liver is important to accurately diagnose liver conditions. 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 presence of depressions, patches, and vesicles were investigated. These laparoscopic findings were compared among NASH, alcoholic liver disease, and autoimmune hepatitis.
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 alcoholic liver disease and autoimmune hepatitis, 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.

Key Words: nonalcoholic steatohepatitis, laparoscopy, hepatomegaly, whitish markings, small depressions

Introduction

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 the present time, 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) [5, 7], and metabolic liver diseases. It has been reported that the laparoscopic findings of nonalcoholic fatty liver are 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. reported a patient with liver cirrhosis due to NASH. A laparoscopy demonstrated diffuse small nodules on the liver surface, closely resembling the feature of alcoholic liver cirrhosis [9]. 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 those 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 systolic pressure greater than 140 mmHg or 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 mg/dL 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) x fasting insulin (μU/mL)]/405. More than 2.0 of HOMA-IR were 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 allowed to escape, and the trocar and needles 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.

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 millimeters 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, and 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 $\pm$ 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 had obesity, and 10 had diabetes mellitus. 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 3 patients, stage 2 in 11, stage 3 in 3, and stage 4 in 7.

The laparoscopic findings of the 24 patients with NASH are summarized in Table 1, and typical laparoscopic findings are shown in Figures 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 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 non-obese 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. Livers representing a yellowish surface, which reflects severe hepatic steatosis, were only in stages 1-2. 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 stage 1 or 2. As expected, all patients with nodules were in stage 4. However, in livers bearing small depressions, 65% were diagnosed as stage 1 or 2, and 17.6% were stages 3 and 4, respectively. Small depressions were generally found, regardless of the histological stage (Table 2).

Comparison of laparoscopic findings among NASH, ALD and AIH

~~It can be difficult to accurately diagnose NASH, because no specific diagnostic markers to NASH have been found [11]. When a past history of alcohol consumption is unclear, distinguishing between NASH and ALD is impossible [11]. In addition, we have occasionally found that it is difficult to diagnose patients with either NASH or AIH, because of a high positive rate of nonspecific autoantibodies in NASH patients [12]. In order to use laparoscopic findings~~ 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 3-4 (n = 10) were complicated with obesity, hypertension, diabetes mellitus, and hyperlipidemia in 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. 50%, $p = 0.033$, and 90% vs. 12.5%, $p = 0.005$, respectively). Coarse depressions were significantly less common in NASH than in ALD (0% vs. 75%, $p = 0.001$). 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 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 completely differentiate NASH from ALD and AIH, 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 due to 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 wasting 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, has not been 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. demonstrated that the coexistence of hepatic steatosis and fibrosis tended to make it difficult to observe a spotty yellow-colored surface [14]. The color change of the liver surface observed in patients with NASH may be associated not only with thickening of the hepatic capsule due to persistent hepatocyte injury and fibrosis, but also with 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 suggest 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 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 helpful 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 that enrolls 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 ~~and characteristic~~ laparoscopic findings in NASH. However, compared with stage-matched patients with ALD or AIH, coarse and groove-like depressions were ~~uncommon~~ rare in those with NASH. Laparoscopic examination may be useful for providing additional information to make the accurate diagnosis of NASH.

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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 Liver Surface		
Reddish-Brown	0	(0%)
Yellowish	2	(8.3%)
Yellowish-White	7	(29.2%)
Whitish	15	(62.5%)
Irregularity of Liver Surface		
Smooth	6	(25.0%)
Uneven	12	(50.0%)
Granular	0	(0%)
Nodular	6	(25.0%)
Other Findings		
Dullness of 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%)

Table 2. Relationship between Laparoscopic Findings and Histological Stage

	Stage 1-2 (n = 14)	Stage 3 (n = 3)	Stage 4 (n = 7)
Color of Liver Surface			
Yellowish (n = 2)	2	0	0
Yellowish-White (n = 7)	7	0	0
Whitish (n = 15)	5	3	7
Irregularity of 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 Stages 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%) [b]
Hypertension	5 (50%)	1 (11.1%) [a]	3 (27.3%) [b]
Diabetes	7 (70%)	0 (0%) [a]	1 (9.1%) [b]
Hyperlipidemia	4 (40%)	0 (0%) [a]	1 (9.1%) [b]
BMI (kg/m^2)	28.1 ± 3.1	22.9 ± 2.3 [a]	24.5 ± 3.6 [b]
AST (IU/L)	53 ± 18	70 ± 50	83 ± 44 [b]
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 [b]
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 liver edge	10 (100%)	5 (55.6%) [a]	6 (54.5%) [b]
Increased fat accumulation of round ligament	9 (90%)	2 (22.2%) [a]	2 (18.1%) [b]
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%) [b]
Coarse depressions	0 (0 %)	7 (77.8%) [a]	10 (90.9%) [b]
Groove-like depressions	1 (10 %)	1 (11.1%)	8 (72.7%) [b]
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

Values are expressed as means ± standard deviations. a, $p < 0.05$ compared with NASH group; b, $p < 0.05$ compared with NASH group.

Figure Legends

Fig. 1. Classification of depressions.

(A) Small depressions observed in patient with NASH (arrows).

(B) Coarse depressions observed in patient with autoimmune hepatitis (arrows).

(C) Groove-like depression observed in patient with autoimmune hepatitis (arrows).

Fig. 2. Laparoscopic findings of NASH (stage 3).

(A) Fat accumulation of hepatic round ligament is markedly increased.

(B) Whitish liver surface, mild hepatomegaly, dullness of liver edge and scattered small depressions are recognized.

(C) Azan-Mallory staining. Moderate fatty deposition and stage 3 fibrosis are seen (x 100).

Fig. 3. Laparoscopic findings of NASH (stage 4).

(A) Long-distance view. Enlarged left lobe with rounded edge is observed.

(B) Close-up view of left lobe. 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 (x 100).