

ORIGINAL ARTICLE

LAPAROSCOPIC FEATURES AND INTEROBSERVER VARIATION OF HISTOLOGICAL DIAGNOSIS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background: There is a lack of information regarding the laparoscopic features and interobserver variation of histological diagnosis in patients with non-alcoholic fatty liver disease (NAFLD).

Methods: Thirty-five patients with NAFLD were studied for laparoscopic and histological findings. For the study of interobserver variation of histological diagnosis, two pathologists from different hospitals independently observed the 35 liver samples with patient names blinded to the pathologists. Assessment of laparoscopic findings on the diagnosis of non-alcoholic steatohepatitis (NASH) was also investigated.

Results: Histological diagnoses of the two pathologists were identical in 28 (five fatty liver, 23 NASH) patients, whereas they were not identical in seven patients (20%). The difference of diagnosis was mainly caused by the difference of judgment of minimal fibrosis and minimal necroinflammatory grade. Analysis of the laparoscopic findings revealed that small regular depressions were frequently found on the liver surface in patients with NASH. Scatter of dye on the liver surface facilitated the observation of this finding. Sensitivity and specificity of small depressions in the diagnosis of NASH was 73.9% and 80.0%, respectively.

Conclusion: Interobserver variation of the diagnosis was found in 20% of patients with NAFLD. Small regular depressions were characteristic findings of NASH. Laparoscopy is assessed to be useful for diagnosis of NAFLD, especially of early stage of NASH.

Key words: histological diagnosis, laparoscopy, liver biopsy, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis.

INTRODUCTION

Changes of lifestyle in recent years in many countries has resulted in a rapid increase of obesity, diabetes mellitus, and metabolic syndrome.^{1–3} Non-alcoholic fatty liver disease (NAFLD) is frequently associated with these conditions.^{4,5} Fatty liver (FL) and non-alcoholic steatohepatitis (NASH) are included in NAFLD.^{6–8} NASH was first reported by Ludwig *et al.* in 1980.⁹ They described a group of liver specimens with fatty liver accompanied by necroinflammatory change obtained from non-drinkers. NASH or NAFLD is reported to be an important cause of cryptogenic liver cirrhosis with/without hepatocellular carcinoma.^{10–12} Study of the prognosis of patients with NASH revealed that some proportion of the patients progress to liver cirrhosis, liver insufficiency, and hepatocellular carcinoma.^{4,13–17} In this context, it is important to correctly diagnose the patients with NASH.

The diagnosis of NAFLD is done by histological examination. Several guidelines or criteria have been proposed for

the histological diagnosis of NASH or NAFLD.^{4,7,18,19} However, precise criteria or consensus for each pathological findings has not been settled, therefore histological diagnosis of NASH sometimes differs among pathologists. This results in a difference of the frequency, clinical features, and the prognosis of NASH among hospitals. In the present study, laparoscopic features and the rate of interobserver variation of liver biopsy in patients with NAFLD were examined, and we tried to assess the role of laparoscopy in the diagnosis of NASH.

METHODS

Thirty-five patients (24 male and 11 female, age: 47.5 ± 17.5 years) diagnosed with NAFLD by liver biopsy under laparoscopy in Saiseikai Matsuyama Hospital and at the Department of Gastroenterology and Metabology of Ehime University Graduate School of Medicine were enrolled in this study. The method of laparoscopy, and its risks and advantages (accurate morphological diagnosis and lower risk of bleeding from biopsied site compared with biopsy under echogram by putting sponge gel into the biopsied site) were explained to the patients. Written informed consent was obtained from all patients. All of the patients consumed less than 20 g of alcohol per day, and all were

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Table 1. Histological diagnoses by the two pathologists

Scoring or grouping system [†]	Category		Pathologist 1	Pathologist 2	
Brunt <i>et al.</i>	Stage	0	10	6	Identical: 21 Non-identical: 14
		1	13	13	
		2	7	6	
		3	2	7	
		4	3	3	
Brunt <i>et al.</i>	Grade	0	7	8	Identical: 18 Non-identical: 17
		1	14	18	
		2	14	8	
		3	0	1	
Matteoni <i>et al.</i>	Type	1	7	7	Identical: 28 Non-identical: 7
		2	3	2	
		3	0	0	
		4	25	26	
Kleiner <i>et al.</i> [‡]	Score	0	0	1	Identical: 7 Non-identical: 28
		1	0	3	
		2	1	3	
		3	6	8	
		4	14	7	
		5	6	10	
		6	5	1	
		7	3	2	

[†]Histological scoring or grouping system proposed by Brunt *et al.*,¹⁸ Matteoni *et al.*⁷ and Kleiner *et al.*¹⁹

[‡]Non-alcoholic fatty liver disease (NAFLD) activity score.

negative for both hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus antibody (anti-HCV). Liver diseases with other known causes such as autoimmune hepatitis, primary biliary cirrhosis, Wilson's disease, and congestive liver diseases were excluded.

The liver surface was observed under laparoscopy (laparoscope 5 mm in diameter; Olympus Medical Systems, Tokyo, Japan). Liver biopsy samples were obtained under laparoscopy using a 14-gauge Silverman-needle. The sizes of the biopsied liver specimens were longer than 15 mm. Two pathologists, one from Matsuyama-Saiseikai Hospital who had 21 years experience in the field of pathology and the other from Ehime University School of Medicine with 22 years experience, independently observed the 35 liver specimens with patient names blinded to the pathologists. Histological diagnosis was done according to the criteria proposed by Brunt *et al.*¹⁸ In the present study, NASH was defined when specimens had findings of more than grade 2 necroinflammatory grade, or both more than grade 1 necroinflammatory grade and more than stage 1 fibrosis score. Other samples that did not fulfill the above criteria were diagnosed as fatty liver (FL). Besides the staging and grading of Brunt *et al.*, histological findings were also judged by the grouping (typing) and scoring system proposed by Matteoni *et al.* and Kleiner *et al.*, respectively.^{7,19}

Usefulness of laparoscopic findings on the diagnosis of NASH was analyzed. Assessment of laparoscopic findings was investigated, by studying sensitivity, specificity, positive predictive value, negative predictive value, and accuracy on the diagnosis of NASH. In addition, the interobserver variation of laparoscopic findings between the two endoscopists was studied by judging the laparoscopic findings of the photographs independently, with the names of the 35 patients

blinded to the endoscopists. Laparoscopic findings of the left lobe and those of the right lobe of the liver were also compared.

Statistical analyses were done by Student's *t*-test and chi-squared test. *P*-value less than 0.05 was considered significant.

RESULTS

Table 1 shows the histological diagnosis of stage and grade determined by the two pathologists. Stage diagnosed by the two pathologists was identical in 21 specimens, whereas it was not identical in 14 (40.0%) specimens (1-stage difference in 13 and 2-stage difference in one). Grade was identical in 18 of 35 specimens but not in 17 (48.6%) specimens (1-grade difference in 15 and 2-grade in two). Diagnosis of NASH and FL was identical in 23 and five specimens, respectively, whereas diagnoses of seven specimens (20%) were not identical. These seven specimens were classified as discrepancy cases in the present study. As for the grouping proposed by Matteoni *et al.*, 28 specimens were identical between the two pathologists, whereas only seven specimens were identical in NAFLD activity score (NAS). In the 28 non-identical specimens by NAS, difference of score was 1 in 12 specimens, 2 in 12 specimens, and 3 in four specimens.

Laparoscopic features and histological findings of liver specimens from patients with FL, with NASH, and a discrepancy case are shown in Fig. 1. Figure 1a–c was from a 24-year-old male patient diagnosed with fatty liver. The surface of the liver was smooth and no depressions were observed. Both pathologists diagnosed the biopsied specimen with FL (stage 0, grade 0). Another patient (Fig. 1d–f) was a 67-year-old woman diagnosed with NASH. Her liver surface showed

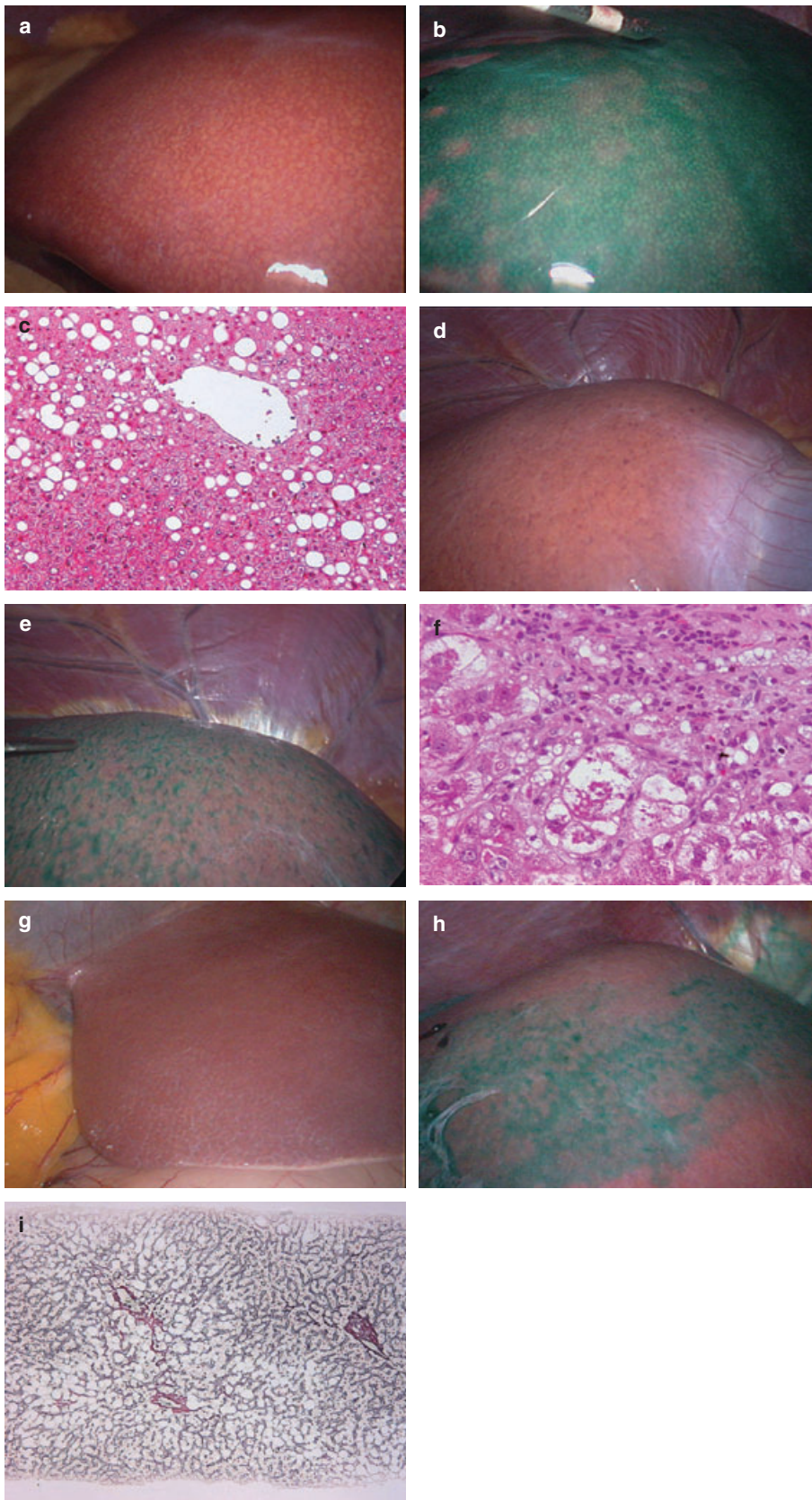


Fig. 1. Laparoscopic and histological features. Photographs taken under laparoscopy without a dye, after scattering indocyanine green solution (ICG) on the liver surface, and microphotographs of liver specimens stained with hematoxylin and eosin or silver staining of three patients are shown. (a–c) Patient who was diagnosed with fatty liver by both pathologists. (a,b) Liver surface is smooth and no depressions are observed. (c) Liver biopsy specimen shows simple steatosis without inflammatory change nor fibrosis. (d–f) Patient who was diagnosed with non-alcoholic steatohepatitis (NASH) by both pathologists. (d) Liver surface is not smooth, and (e) small regular depressions are obvious, especially in the photograph of liver under scattering ICG solution. (f) Liver biopsy specimen shows ballooning of hepatocytes, infiltration of inflammatory cells, including neutrophils and Mallory bodies. (g–i) Discrepancy case. (g) Small regular depressions are not obvious in the photograph without ICG, but (h) it has become clear that there are some small depressions on the liver surface. (i) One pathologist judged the liver biopsied specimen as fatty liver, whereas the other judged it as NASH with mild fibrosis and mild necroinflammatory grade.

Table 2. Histological diagnoses of the discrepancy cases

Case	Grade	Pathologist 1 Stage	Diagnosis	Grade	Pathologist 2 Stage	Diagnosis
1	0	0	FL	1	1	NASH
2	0	0	FL	1	1	NASH
3	1	1	NASH	0	0	FL
4	0	0	FL	1	1	NASH
5	1	1	NASH	0	0	FL
6	1	0	FL	1	1	NASH
7	1	0	FL	2	1	NASH

FL, fatty liver; NASH, non-alcoholic steatohepatitis.

Table 3. Clinical data of the subjects

<i>n</i>	NASH 23	Discrepancy case 7	Fatty liver 5
BMI (kg/m ²)	26.3 ± 3.7	26.1 ± 2.9	24.2 ± 3.0
AST (IU/L)	68.0 ± 41.4	61.3 ± 41.1	35.2 ± 3.6
ALT (IU/L)	117.7 ± 85.1	135.6 ± 112.9	83.6 ± 29.8
TG (mg/dL)	178.2 ± 104.0	135.7 ± 63.2	143.6 ± 115.0
CRP (mg/dL)	0.35 ± 0.45	0.11 ± 0.05	0.16 ± 0.12
Ferritin (mg/dL)	228.4 ± 269.4	114.0 ± 96.2	127.0 ± 74.3
Platelets (10 ⁴ /μL)	21.3 ± 7.8	22.8 ± 4.3	24.6 ± 4.1
K-ICG	0.17 ± 0.05	0.19 ± 0.02	0.18 ± 0.07

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; K-ICG, disappearance rate of indocyanine green; TG, triglycerides.

small regular depressions. This finding became more obvious after the scatter of indocyanine green (ICG) dye (25 mg ICG dissolved in 10 mL saline) on the liver surface. She was diagnosed with NASH (stage 3, grade 2) by both pathologists. The third patient was a 59-year-old woman, who was a discrepancy case. Her liver surface showed white markings that were similar to those observed in patients with alcoholic liver disease. Small regular depressions were not clear by regular laparoscopic observation; however, when ICG dye was scattered on the liver surface, the finding of small depressions became obvious (Fig. 1g,h). The liver specimen showed very slight perisinusoidal fibrosis in zone 3 of the liver lobule (Fig. 1i). One pathologist judged this finding as pathological fibrosis and diagnosed NASH, whereas the other judged it as within the normal range and diagnosed FL.

Histological diagnosis of staging and grading by the two pathologists of the seven specimens from the discrepancy cases is shown in Table 2. Diagnosis of staging was different in all seven specimens; one pathologist diagnosed them as stage 0 and the other as stage 1. Diagnosis of grading was also different in six of the seven specimens.

Table 3 shows clinical data of patients with FL, NASH, and the discrepancy cases. The average data of body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), C-reactive protein (CRP), and ferritin were high in the NASH group and the disappearance rate of indocyanine green (K-ICG) was low in the NASH group; however, significant differences were not found. Table 4 shows the laparoscopic findings of the three groups. Findings of small depressions (small regular depres-

sions) were frequently found in NASH whereas not in FL. No difference was found in yellowish color, and yellow regular leopard-like spots. Assessment of the laparoscopic findings on the diagnosis of NASH is shown in Table 5. Sensitivity and specificity of the finding of small depressions were 73.9% and 80.0%, respectively. Specificity was also 80% in findings of whitish clouded liver surface, increase of vessels, and white markings; however, sensitivity was less than 50% in these findings. Table 6 shows the laparoscopic findings of the seven patients within the discrepancy group. Two of the seven had the finding of small depressions, whereas the other five patients did not have this finding. Both of the two patients with small depressions were obese (BMI 28.5, 25.6, respectively), and had diabetes mellitus or glucose intolerance, whereas the number of platelets and K-ICG were within the normal range.

Interobserver variation and variation between left and right hepatic lobes are shown in Table 7. Laparoscopic findings judged by the two endoscopists were identical in more than 27 of 35 patients (≥77.1%) in all findings except the finding of an increase of vessels (62.9%). Regarding the variation between the two hepatic lobes, the percentage of identical cases was 77.1–94.2%.

DISCUSSION

Interobserver variation is one of the major problems of the histological diagnosis of NASH, and efforts are being made to eliminate the variation.^{19,20} The present study revealed that

Table 4. Laparoscopic findings

	NASH	Discrepancy case	Fatty liver
Whitish clouded liver surface (%)	7/23 (30.4)	5/7 (71.4)	1/5 (20)
Small depressions (%)	17/23 (73.9)	2/7 (28.6)	1/5 (20.0)
Yellowish color (%)	20/23 (87.0)	6/7 (85.7)	5/5 (100)
Regular yellow leopard-like spots (%)	12/23 (52.2)	5/7 (71.4)	2/5 (40.0)
Increase of vessels (%)	9/23 (39.1)	5/7 (71.4)	1/5 (20.0)
White markings (%)	11/23 (47.8)	2/7 (28.6)	1/5 (20.0)

Number of patients showing the laparoscopic findings are shown.
NASH, non-alcoholic steatohepatitis.

Table 5. Assessment of laparoscopic findings

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Whitish clouded liver surface (%)	7/23 (30.4)	4/5 (80.0)	7/8 (87.5)	4/20 (20.0)	11/28 (39.3)
Small depressions (%)	17/23 (73.9)	4/5 (80.0)	17/18 (94.4)	4/10 (40.0)	21/28 (75.0)
Yellowish color (%)	20/23 (87.0)	0/5 (0)	20/25 (80.0)	0/3 (0)	20/28 (71.4)
Regular yellow leopard-like spots (%)	11/23 (47.8)	3/5 (60.0)	12/13 (92.3)	3/14 (21.4)	13/28 (46.4)
Increase of vessels (%)	9/23 (39.1)	4/5 (80.0)	9/10 (90.0)	4/18 (22.2)	13/28 (46.4)
White markings (%)	11/23 (47.8)	4/5 (80.0)	11/12 (91.7)	4/16 (25.0)	15/28 (53.6)

Table 6. Laparoscopic features of the discrepancy cases

Case	Color	White markings	Small depressions	Increase of vessels
1	Dark red-brown	+	+	+
2	Yellow-red	-	-	-
3	Yellow-red	-	-	-
4	Yellow-red	-	-	-
5	Yellow-red	-	-	+
6	Yellow-red	+	+	+
7	Yellow-red	-	-	+

Table 7. Comparison of laparoscopic findings between the two endoscopists and between the left and right lobes

	No. and percentage of identical cases	
	Between two endoscopists	Between left and right lobes
Whitish clouded liver surface (%)	27/35 (77.1)	33/35 (94.2)
Small depressions (%)	30/35 (85.7)	31/35 (88.6)
Yellowish color (%)	27/35 (77.1)	33/35 (94.2)
Regular yellow leopard-like spots (%)	30/35 (85.7)	33/35 (94.2)
Increase of vessels (%)	22/35 (62.9)	27/35 (77.1)
White markings (9%)	31/35 (88.6)	31/35 (88.6)

the discrepancy in the diagnoses of stage and grade in specimens with NAFLD was found in 40.0% and 48.6% of cases, respectively, between two pathologists. Diagnosis of NASH or FL was also different in 20% of cases. It is suspected that the lack of consensus for each pathological finding is the cause of the discordance. The results in the present study support that interobserver variation is a great problem in the diagnosis of NASH.

Besides interobserver variation, sampling variation in NAFLD has also been reported.²¹ From these results and reports, there was a limitation of exact diagnosis by percutaneous liver biopsy with one sample. In this context, we tried to clarify the laparoscopic findings of NASH and FL, and tried to assess the diagnostic significance of laparoscopy. Only a few reports have been described regarding the laparoscopic feature of NASH.²²⁻²⁴ The present study revealed

that small depressions were found in NASH but were rare in patients with FL. Both the sensitivity and the specificity of this finding on the diagnosis of NASH were high, therefore this finding is useful for the diagnosis of NASH. Scatter of dye facilitated the observation of this finding. Specificity of the findings of whitish clouded liver surface, increased number of vessels and white marking on the diagnosis of NASH was also high, but sensitivity was low in these findings. The finding of increased number of vessels is suspected to reflect the disturbance of local circulation caused by fibrosis and/or necroinflammatory change. White markings may reflect fibrosis. Further study is needed to clarify whether these findings have relation to NASH. Yellow color and regular yellow leopard-like spots were not useful for distinguishing NASH and FL. These findings reflect the fat deposition and fatty change in zone 3 of the hepatic lobule, respectively. Pattern of fatty change may be similar in NASH and FL, therefore these findings were not useful for the diagnosis of NASH. However, the pattern of fatty change might be varied in advanced stages of NASH, therefore this issue should be studied further.

In the present study, diagnostic discrepancy was seen in seven of 35 patients. Histological findings of all of these patients showed minimal or mild fibrosis and necroinflammatory change, therefore these cases were 'borderline cases'. It is not possible to conclude the final diagnosis of these patients because diagnosis of NASH is done histologically. However, two of the seven patients showed findings of small regular depressions. Existence of small regular depressions may indicate that NASH might be the correct diagnosis in these two patients. Theoretically, a third pathologist's opinion or histological confirmation by an outside independent committee is required in the study design of this work; however, such analyses are very difficult and complicated, so in the present study, a third pathologist was not included. Further study is needed to achieve the final and correct diagnosis of patients with NAFLD; however, laparoscopy may be useful for diagnosing patients with NAFLD, especially with minimal or mild fibrosis and necroinflammatory change.

Interobserver variation of laparoscopic findings must also be studied. In the present study, findings judged by the two endoscopists were identical in approximately 80% of the investigated findings except for increase of vessels (62.9%). There are no definite criteria for the judgment of laparoscopic findings, and this may be the reason for the discrepancy in the present study. This issue also needs further discussion. Sampling variability of liver biopsy is another problem in the histological diagnosis of NASH.²¹ In the present study, 77–94% of the subjects showed similar laparoscopic findings (except for increase of vessels) between the left and right hepatic lobes. This result indicates that approximately 10–20% of patients may show different histological findings between the left and right lobes. Laparoscopy is a great tool to avoid sampling error because the entire liver surface can be observed under the laparoscope, and information from the biopsied area of the liver surface is available.

In conclusion, a considerable rate of interobserver variation in histological diagnosis of NAFLD was found, and laparoscopy gives us additional information that may be useful for the diagnosis of NAFLD.

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