Original Articles

NON-ALCOHOLIC STEATOHEPATITIS IN NARA MEDICAL UNIVERSITY HOSPITAL BETWEEN 2003 AND 2008; RETROSPECTIVE STUDY WITH CLINICOPATHOLOGIC ANALYSIS

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Abstract: Clinical and pathological features of 50 Japanese patients with biopsy proven non-alcoholic steatohepatitis (NASH) were reviewed. Incidence of NASH patients increased gradually after 2004, up to 8.06% in the series of liver biopsy specimens. Twenty-six were male and twenty-four were female with a mean age of 54.66 years (range 19-80 years old, male: 47.5, female: 62.4 years old). Forty-four percent of patients were preobese with a body mass index (BMI) between 25 and 30, while 28% of the patients were non-obese, and only 28% of the cases were morbidly obese, confirming that Japanese have a greater tendency to develop fatty liver disease than Western people. Dyslipidemia was found in 30%, diabetes mellitus in 34%, and hypertension in 26% of the patients. Abnormally elevated liver function tests were found in up to 90% of the patients and were characteristically mild with 2- to 3- fold elevation from the normal range in the majority of cases. Histological features of the liver were similar to those reported in Western literature; steatosis was found in every patient and most of them were score 1 (34%) and preferential macrovesicular in type. Lobular necroinflammation was typically mild (combined stage 1 and stage 2, 98%). Ballooned hepatocytes were frequently observed with characteristic small poorly formed Mallory bodies. Pericellular fibrosis, one of the key histologic features of NASH, was classified in stage 1 in the majority of the patients (54%), with characteristic acinar zone 3 involvement. As for the disease progression, portal fibrosis and/or bridging fibrosis could develop leading to liver cirrhosis (stage 4 NASH) in 10% of the patients.

Key words : steatohepatitis, metabolic syndrome, hepatocyte ballooning, Mallory bodies, perivenular/pericellular fibrosis, lipogranuloma

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) was recognized among the morphologic spectrum of non-alcoholic fatty liver disease (NAFLD) as a clinical entity comprised of hepatic steatosis, lobular inflammation, and pericellular fibrosis. It is currently considered as a hepatic manifestation of the metabolic syndrome[1]. Nonomura et al reported in 1992 that the incidence of NASH patients in a series of liver biopsies was 1.2% and also described in 2005

that clinical and pathological features of Japanese NASH patients were basically similar to those reported in Western people[2]. However, the majority of Japanese NASH patients were not morbidly obese but were preobese, indicating that Japanese have a greater tendency to develop insulin-resistance and fatty liver disease than Western people. Up to date, it was considered as a common liver disease in Japan[2], however, retrospective study about clinical relation and incidence of Japanese NASH was limited. Therefore, in the present study, we collected patients with NASH from the files of the pathology section of Nara Medical University Hospital between 2003 to 2008 and reviewed their clinical and pathological features to compare the results to those reported in previous Japanese literature.

MATERIALS AND METHODS

The definition of NASH implied both clinical and pathological findings in the liver, according to the criteria described by Brunt et al[3]. Only patients for whom clinical and laboratory evaluation had excluded significant alcohol abuse and a variety of liver diseases that may cause NASH-like histologic changes, such as chronic viral hepatitis C, Wilson disease, or autoimmune liver disease. After detailed evaluation, clinical and pathological features of 50 Japanese patients with biopsy proven non-alcoholic steatohepatitis (NASH) were collected from the files of Nara Medical University between 2003 and 2008.

Clinical and laboratory data were obtained and analyzed. Liver biopsy specimens were cut and stained with hematoxylin and eosin (H&E) and Azan-Mallory stain, and histologic features were examined under a microscope. Variable histologic changes were semi-quantitatively analyzed. NAFLD activity score (NAS) from scores 0-8 and histologic stages from stages 1-4 were assessed by the method described by Kleiner and Brunt et al[4, 5].

RESULTS

Clinical features

The incidence of NASH patients in the series of liver biopsies of Nara Medical University Hospital between 2003 and 2008 was shown in Table 1. Although not distinctly, a trend toward increased NASH patients incidence after 2004 was observed.

The mean age in relation to histologic stages in NASH patients is shown in Table 2. The

Table 1. Incidence of NASH patients of Nara medical university between 2003 and 2008

Year	No. of cases (%)
2003	6 / 115 (5.22%)
2004	6 / 84 (7.14%)
2005	14 / 133 (10.53%)
2006	9 / 103 (8.73%)
2007	5 / 127 (3.94%)
2008	10 / 124 (8.06%)

Table 2. Histologic stages in relation to the mean age of NASH patients

		No. of cases	Mean age (years)
Male	Total	26	47.5 −16.8
	Stage 1	16	46.3 −17.1
	Stage 2	4	37.0 −12.0
	Stage 3	4	53.2 −17.4
Female	Stage	2	67.0
	Total	24	62.4 −11.9
	Stage 1	11	59.8 −14.4
	Stage 2	4	63.2 −10.7
	Stage 3	6	63.3 −8.4
	Stage 4	3	68.7 −12.1
Total	Stage 1	27	54.2 −15.5
	Stage 2	8	50.1 −17.5
	Stage 3	10	59.3 −12.9
	Stage 4	5	68.0 −8.6

mean age increased in parallel with histologic stages in both male and female patients. The mean age difference between stage 1 and stage 4 in male patients was 20.7 years, and that in female patients was 8.9 years.

Among 50 patients, 26 were males and 24 were females. The mean age of all patients was 54.66 years old, with a range from 19 to 80 years old. The mean age of male patients was 47.5 years and that of females was 62.4. The mean age of male patients was about 15 years younger than that of female patients. In 68% of the patients, there were no signs or symptoms related to liver disease and the disease was found incidentally during a health check-up or during the course of treatment for other diseases, such as diabetes mellitus or hypertension. Only 32% of the patients had symptoms such as non-specific abdominal discomfort or general malaise. Dyslipidemia was found in 30%, diabetes mellitus in 34%, and hypertension in 26% of the patients. Twenty-eight percent of patients were morbidly obese with a BMI over 30, but 28% were non-obese with a BMI less than 25, and 44% of the patients had a BMI between 25 and 30. Aspartate aminotransferase (AST) levels were within the normal range in 10% of the patients, and were abnormally elevated in 90%. Most of the elevations, however, were characteristically mild with 2- to 3-fold elevation compared to the normal range, except the one patient who had 7-fold elevated AST level. Alanine aminotransferase (ALT) levels were within the normal range in 70% of the patients, and were abnormally elevated in 30%. The mean AST to ALT ratio was 0.89. Gamma glutamyl transpeptidase (γ-GTP) was abnormally elevated in 80% of the cases, but the elevation levels were within 3-fold of the normal range in 70% of the cases. Alkaline phosphatase (ALP) levels were abnormally elevated in 14% of the cases, and in all of the cases, the elevated levels were within 2-fold of the normal range. No correlation was found between different NASH stages and weight, BMI, mean ALP level, or AST/ALT ratio. Although not statistically significant, a trend toward increased AST and ALT level with increased lobular

inflammation was noted.

Pathologic features

Steatosis

The intensity of steatosis scores was score 0, 8% (n=4); score 1, 34% (n=17); score 2, 26% (n=13); and score 3, 32% (n=16). Steatosis in most of the specimens was predominantly in perivenular regions (acinar zone 3), whereas the distribution of steatosis was non-zonal (scattered or panacinar) in some stage 4 NASH patients (Fig. 1a). It was mainly macrovesicular in type admixed with a small number of microvesicular type. Unlike steatosis, glycogenated nuclei were typically observed in acinar zone 1 in NASH specimens.

Lobular inflammation

Lobular inflammation was present in all biopsy specimens in varying amounts. The intensity of lobular inflammation scores was score 1, 28% (n=14); score 2, 68% (n=34); and score 3, 4% (n=2). Lipogranuloma and hepatocyte necrosis accompanying polymorphonuclear leukocytes (PMNs) were observed in the majority of the patients. Also, mononuclear inflammatory cells infiltration was occasionally found (Fig. 1c-d).

Hepatocyte ballooning

In the majority of patients this change was marked, score 2, 88% (n=44) with only 6 of the cases being mild, score 1, 12% (n=6). Ballooned hepatocytes were typically found in zone 3 together with macrovesicular steatosis, characterized by swollen cytoplasm containing eosinophilic granular or foggy aggregates, not sufficient to be identified as Mallory bodies. Poorly formed small Mallory bodies in NASH patients described by Nonomura et al were frequently observed[2] (Fig. 1b).

Fibrosis

Fibrosis was found in all cases, scored as stage 1 (zone 3 perisinusoidal fibrosis only), 54% (n=27); stage 2 (zone 3 perisinusoidal fibrosis with periportal or portal fibrosis), 16% (n=8); stage 3 (bridging fibrosis), 20% (n=10); and stage 4 (cirrhosis), 10% (n=5). Pericellular fibrosis can be detected simply by Azan-Mallory staining as delicate strands of collagen surrounding individual hepatocytes in acinar zone 3. Bridging fibrosis, comprised of central to central (C-C) and central to portal (C-P) pattern, was demonstrated with disease progression, accompanying portal mononuclear inflammatory cell infiltration (Fig. 2).

NASH and hepatocellular carcinoma versus cholangiocarcinoma

Hepatocellular carcinoma (HCC) was recognized among the patients with the steatohepatitis histologic background in 4 cases (8%), composed of 1 female and only 1 male. All of them were diabetes-associated, and interestingly, their ages were higher than the mean age of corresponding stage NASH patients. Moreover, the majority of the cases (n=3) exhibited non-cirrhotic histologic change but only periportal fibrosis with or without bridging fibrosis. Cholangiocarcinoma was noted in only 1 case without other underlying disease. On the contrary to hepatocellular carcinoma, overall activity was low (1 point for

hepatocyte ballooning and 1 point for lobular inflammation) and fibrosis was found only in the perivenular region.

Case report of HCC associated with NASH

The patient, a 73-year-old female, was admitted to the hospital with a lower abdominal pain. She had been obese since age 40 (height 142 cm, weight 60-63 kg) and was hospitalized two times for treatment of fatty liver at age 41 and 42. At age 36, she had been treated for chronic glomerulonephitis, and since age 50 she had been treated for systemic lupus erythematosus. Ultrasound, computed tomography and magnetic resonace imaging study revealed a hepatic mass sized about 20 mm in diameter in the right hepatic lobe (S7). Under a diagnosis of HCC, the tumor was surgically resected. The resected liver revealed a mass of 22 x 17 mm, white-yellowish, relatively well-defined tumor within a cirrhotic hepatic parenchyma (Fig. 3). Histological examination revealed well differentiated HCC (Fig. 4) in a background of steatohepatitis with precirroshis.

Other liver diseases with steatohepatitis-like features

Between 2003 and 2008, 14 liver biopsy specimens of other diseases with steatohepatitis-like histologic changes were identified; 9 were chronic viral hepatitis C, 2 were chronic viral hepatitis B, 2 were autoimmune hepatitis, and 1 was primary biliary cirrhosis. Typical histologic features of NASH can be observed in this group, such as pericellular fibrosis (acinar zone 3), ballooned hepatocytes, and macrovesicular steatosis. However, the majority of chronic viral hepatitis specimens, both B and C type (n=10), revealed a moderate to marked degree of portal inflammation (score 3 and score 4 respectively) and intralobular degeneration score was moderate to marked in almost all cases (n=9) according to Knodell histology activity index (HAI) scoring. Autoimmune hepatitis of both cases showed lobular inflammation, steatosis, and pericellular fibrosis resembling those of NASH cases but the key point was predominant plasma cell infiltration in the portal area. One case of primary biliary cirrhosis contained small epithelioid granulomas, a characteristic feature of this entity. All of them were offirmed by clinical manifestations and laboratory chemical tests.

DISCUSSION

NASH was formally described as a clinical entity by Ludwig et al in 1980 and is currently recognized as component of the metabolic syndrome, particularly type 2 diabetes mellitus and dyslipidemia. It is one of the most common causes of abnormally elevated hepatic enzymes in both Western and Japanese patients. Many studies reported a potential for progression to end-stage liver disease, probably accounting for many cases of so called "cryptogenic cirrhosis". Nowadays, no laboratory test can discriminate steatohepatitis from other hepatic diseases. Histologic examination is the gold standard for diagnosis, for evaluation, and for exclusion of other liver diseases[5-7]. Whereas Japanese alcoholic liver disease shows some clinical and pathological features different from those in Western people, Nonomura et al reported that those of NASH patients of both Japanese and Western patients are basically similar[2]. Compared to the previous study about NASH cases in liver biopsy

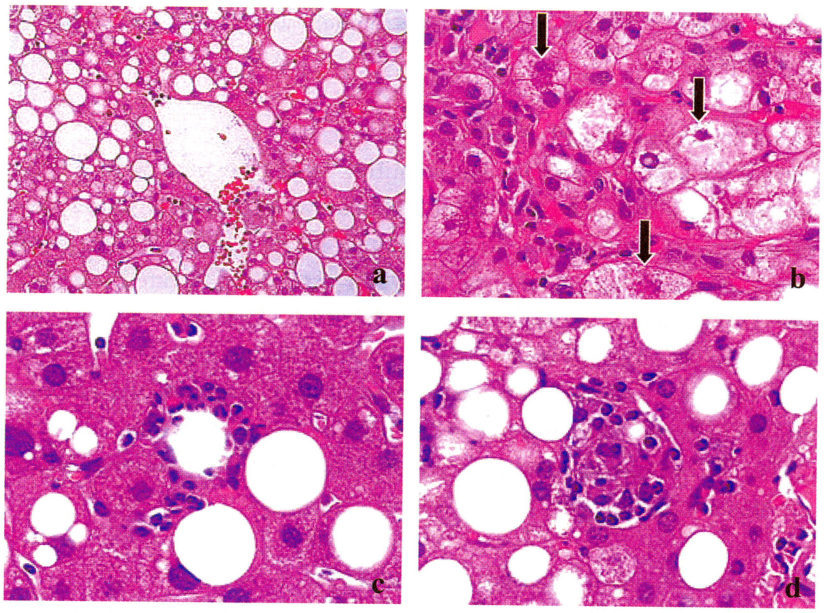

Fig. 1. Hepatocellular abnormalities of NASH; steatosis (a), hepatocyte ballooning (b), lipogranuloma (c), and hepatocyte necrosis (d). Steatosis was predominantly located in acinar zone 3 and typically macrovesicular in type. Ballooned hepatocytes show dilated clear cytoplasm with small poorly formed Mallory bodies (b, arrows). Lipogranuloma was noted accompanied by neutrophils and histiscytes, and frequently around an area of steatosis. Necrotic hepatocyte was occasionally infiltrated by mononuclear inflammatory cells. These characteristic histopathologic changes of NASH were typically located in acinar zone 3. H&E stain (all).

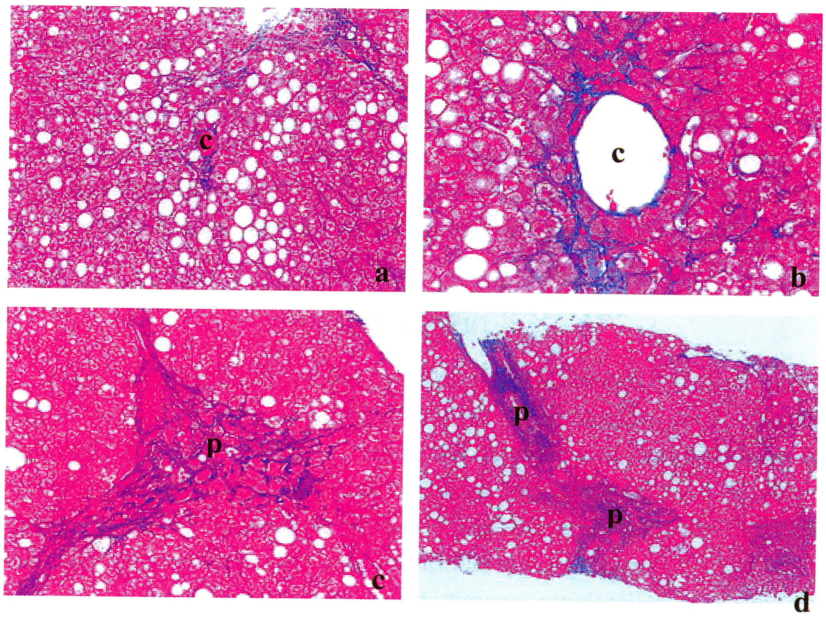

Fig. 2. Histologic features of perivenular and pericellular fibrosis of NASH. In the early stage, pericellular fibrosis was predominantly located in acinar zone 3 (a). Delicate strands of collagen fibers surrounded individual hepatocytes revealing a chicken-wire appearance (b). Portal fibrosis was observed in stage 2 NASH (c). As disease progression, bridging fibrosis was developed (d). C: centrilobular area; P: portal area. Azan-Mallory method (all).

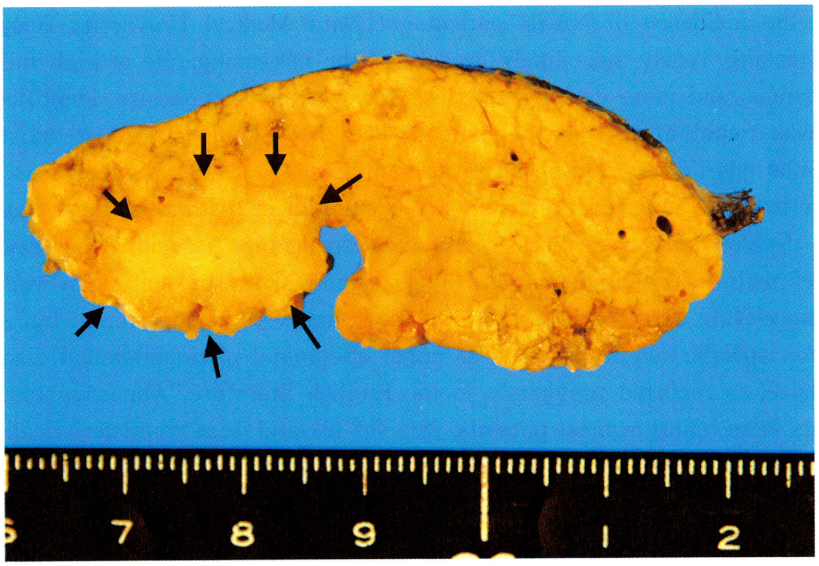

Fig. 3. Cut surface of the liver tumor, showing a relatively well-defined, 20 x 17 mm mass (arrows) within a precirrhotic yellowish liver.

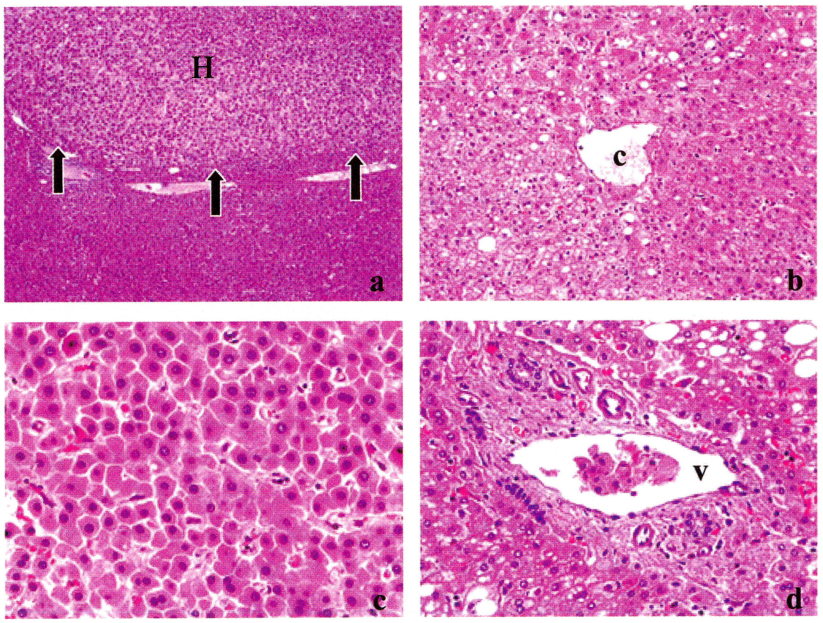

Fig. 4. NASH with HCC (resection specimen). Hepatocellular carcinoma was developed in a background of steatohepatitis. Arrows represented the junction between the cancer and non-neoplastic hepatic tissue (a). Surrounding hepatic tissue exhibited mild degree of steatosis and lobular inflammation (b). Well-differentiated HCC displayed solid or sheet pattern of malignant hepatocytes revealing enlarged nuclei with pleomorphism, dense eosinophilic cytoplasm, and increased N: C ratio. Portal triad was absent (c). Cancer emboli bearing dysplastic hepatocytes are observed in some portal tracts (d). H: hepatocellular carcinoma; C: centrilobular area; V: hepatic venules in the portal area. H&E stain (all)

specimens[8], the incidence of NASH patients of Nara Medical University Hospital in our study is increasing, yearly most likely because of the increasing rate of high-fat diet due to life-style changes and more detectable cases. However, morbid obesity, identified as a BMI over 30%, was found in 28% of the patients with NASH, in contrast to most studies in English populations that morbid obesity was found in 69% to 100% of the cases. Our results were similar to those reported previously by Nonomura et al that Japanese have a greater tendency to develop fatty liver disease than Western people. Associated incidence of NASH with diabetes mellitus, dyslipidemia, and hypertension in the present study was 34%, 30%, and 26%, respectively. Most patients (68%) of the series were asymptomatic, but some have a variety of non-specific symptoms, such as upper abdominal pain, abdominal discomfort, and general malaise, as reported previously in the English literature. Abnormal results of liver function tests were found in most patients, 98% (n=49), and were characteristically mild with a 2- to 3-fold elevation above the normal range. Brunt et al corroborated a lack of statistically significant association between clinical laboratory tests and individual histopathologic findings[9]. However, it is reported that a mean AST/ALT ratio greater than 1 was one of an advanced disease predictor[1]. In the present study, no correlation was found between assessed features (steatosis, necroinflammatory grade, and fibrosis) and weight, BMI, and mean ALP level. Although not statistically significant, a trend toward increased mean AST/ALT ratio with increased fibrotic stage was observed.

Histologic changes of the liver in NASH patients were a combination of various features composed of hepatocellular steatosis, lobular necroinflammation, hepatocellular degeneration associated with Mallory body formation, and pericellular/ perisinusoidal fibrosis. Characteristically, these findings were predominantly located in acinar zone 3. The most important clue for distinguishing steatohepatitis from simple steatosis is the presence of ballooning hepatocyte characterized by partly cleared cytoplasm in which residual cytoplasmic fragments condense to form the Mallory body[5]. In the present study, ballooning hepatocytes were observed in all cases, in which occasionally found were the Mallory bodies that were typically poorly formed and smaller than those of alcoholic steatohepatitis. Eosinophilic granular aggregates were often demonstrated and also characteristic cytoplasmic expressions of liver cells in NASH, as described by Nonomura et al[2]. Liver cell necrosis accompanying polymorphonuclear leukocytes was a typical finding for NASH but mononuclear inflammatory cell infiltrates can be found. In the present study, only 4% of the patients (n=2) were score 3, indicating that lobular inflammation in NASH was usually mild[2]. Pericellular fibrosis was one of the key histologic features of NASH in adults and was preferentially located in acinar zone 3. However, in severe cases, central to central (C-C) and portal to central (P-C) bridging fibrosis can occur, eventually leading to cirrhosis. Similarly, steatosis can extend to a panacinar distribution as progression of the disease [2,5].

Hubscher et al described that hepatocellular carcinoma is a rare complication of precirrhotic NASH, especially in diabetes patients[5]. All 4 NASH patients with HCC in our study have an underlying diabetes mellitus with negative hepatitis viral markers. The interesting point was that their ages were higher than the mean age of corresponding stage NASH patients. Moreover, most of them (6%, n=3) were in non-cirrhotic stage (stage 2 or stage 3) whereas only one of them disclosed stage 4 full blown cirrhosis, indicating that

hepatocellular carcinoma can occur as a late complication of NASH without cirrhotic background. Staging of the histological lesions of NASH patients from stage 1 to 4 has been proposed by Brunt et al. In the present series, 27 were in stage 1, 8 in stage 2, 10 in stage 3, and 5 in stage 4, giving a mean age of 54.2, 50.1, 59.3, and 68 years old, respectively, indicating that it takes about 14 years from the initial stage 1 to develop to the final stage 4 of cirrhosis, as reported by Nonomura et al[2].

Some liver diseases can produce histologic changes resembling those seen in steatohepatitis. They were excluded from the present study and should be mentioned here because typical histologic features of NASH can be observed in this group. Chronic viral hepatitis, both B and C type, was the most common liver disease that mimics NASH histologically. However, the majority of chronic viral hepatitis specimens revealed a moderate to marked the degree of portal inflammation or intralobular inflammation with predominantly mononuclear inflammatory cells infiltrate. These can be discriminated from NASH because the degree of portal inflammation and lobular inflammation are usually mild in steatohepatitis with characteristic neutrophils. Autoimmune hepatitis of both cases generated all typical features of NASH but the key histologic point was predominant plasma cell infiltration in the portal area. Primary biliary cirrhosis showed steatosis and lobular inflammation similar to those of NASH but can be morphologically recognized by the presence of epithelioid granuloma. These results suggest that the pathologist should consider that typical histologic features can be observed in other liver diseases. Patient's clinical data and careful microscopic examination coupled with clinical chemistry tests are essential in distinguishing NASH and other liver diseases.

It is reported that mild portal inflammation associated with interface hepatitis and periportal fibrosis, characteristic features of various forms of hepatitis, are commonly present in NASH. However, another concurrent disease should be considered if the severity of the portal inflammation is disproportionate to that of lobular inflammation[5]. In the present study, mildly non-significant portal mononuclear cell infiltration was occasionally observed in some specimens.

In conclusion, we collected 50 patients of Nara Medical University Hospital with non-alcoholic steatohepatitis to summarize and compare the findings with those reported in previous Japanese literature. The interesting finding in this study was a continuously increased incidence of NASH patients in Japan since 2004, strongly consistent with more modified lifestyle changes. However, the majority of our NASH patients were preobese. Other clinical manifestations and histologic features as well were considered similar to those reported in Western people.

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