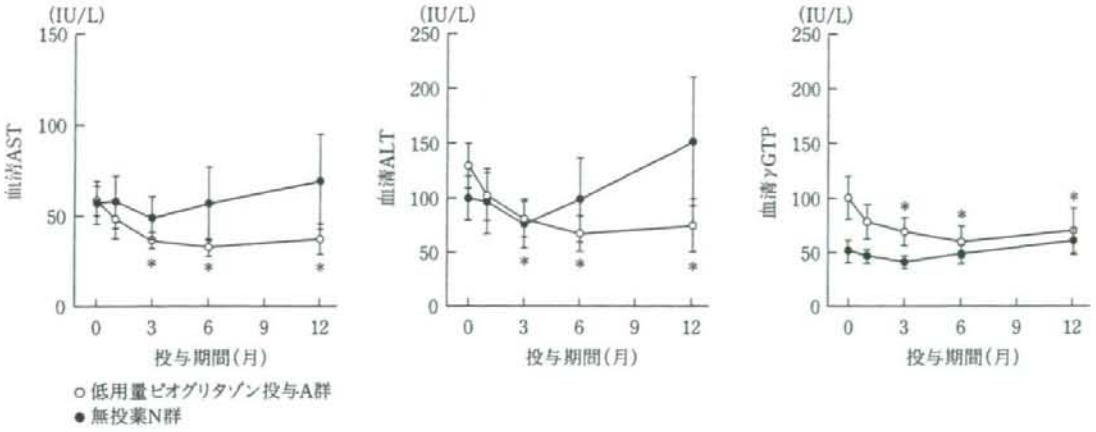

図 2　低用量ピオグリタゾン投与 A 群および無投薬 N 群の血清 AST・ALT・γGTP 値の推移
Mean±SEM
*$p<0.05$ vs. baseline levels by repeated-measure ANOVA on ranks

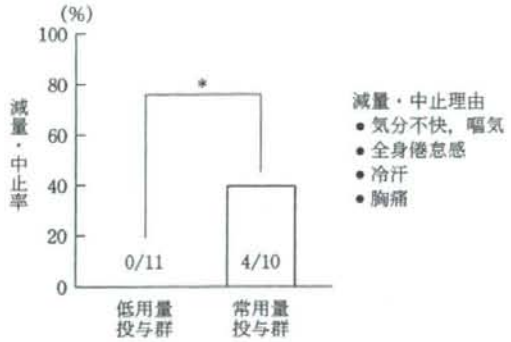

図 3　NAFLD 患者に対するピオグリタゾン投与の減量・中止率
*$p<0.05$ by Fisher's exact test

I　方　　法

インスリン抵抗性（HOMA-R：2.5 以上）を有する NAFLD 患者 18 例を無作為にピオグリタゾン低用量投与（7.5 mg/日）群（A 群：11 例），および無投薬群（N 群：7 例）に分け，3〜12 ヵ月継続して肝機能検査値の推移を観察した。

NAFLD の選定基準は，①画像診断あるいは肝組織診断にて脂肪肝を指摘され，②血清 AST 値＜血清 ALT 値であり，③アルコール摂取量が日本酒換算で平均 1 合/日以内（エタノール換算にて 20 g/日）であるものとした。

また，以前にピオグリタゾンの常用量（15〜30 mg/日）で投与開始した肝生検により診断された NASH 10 例（B 群）のデータをあわせて比較検討した。

II　結　　果

まず，低用量ピオグリタゾン投与の A 群と常用量投与の B 群の患者背景では，B 群において肝組織の grade や stage がやや高い傾向にあったが，有意な差は認めなかった。次に，BMI，HOMA-R，グリコアルブミン（GA）の推移を比較すると，いずれの群も治療開始前後において有意な変化はみられなかった。

さらに，A 群，N 群ともに投与開始 3 ヵ月後の時点では，血清 AST・ALT および γGTP 値の改善傾向をほぼ同様に認めた。ところが，6 ヵ月目以降では A 群でこれらの検査値のすべてがいっそう低下傾向を示したのに対し，N 群では投与前値とほぼ同様のレベルにまで再上昇した（**図 2**）。また，血清フェリチン値は A 群では，投与 3 ヵ月，6 ヵ月，12 ヵ月において投与前値より有意に低下した。さらに，血清Ⅳ型コラーゲン値は，N 群では投与 6 ヵ月，12 ヵ月において投与前値より有意な上昇を示した。

最後に，A 群の全 11 例では明らかな副作用を認めず服薬継続可能であったが，B 群では 40%の症例が投与開始 3 ヵ月以内に浮腫，気分不快などの副作用発現のため服薬中止ないし減量しており，副作用発現率が有意に高いことが判明した（$p<0.05$）（**図 3**）。

なお，B群で服薬継続可能であった症例では，12ヵ月後の時点でもトランスアミナーゼ値の有意な低下傾向が維持されており，長期投与可能例では，肝組織像における線維化の著明な改善も確認された。

欧米の報告ではNASH症例に対しチアゾリジン系薬剤の中ないし高用量投与での有用性が報告されているが[2,3]，日本人のNAFLD症例に対しては低用量のピオグリタゾン投与でも十分な治療効果が得られる可能性が示唆された。とくに副作用発現の観点からは常用量での導入は望ましくなく，低用量からの導入が副作用回避とそれに伴うコンプライアンス向上の観点から有用であると考えられた。

*　　*　　*

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Familial Aggregation in Patients with Non-Alcoholic Steatohepatitis

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□ CASE REPORT □

Familial Aggregation in Patients with Non-Alcoholic Steatohepatitis

Katsutoshi Tokushige, Satoru Yatsuji, Etsuko Hashimoto, Ayae Kabutake, Maki Tobari, Makiko Taniai and Keiko Shiratori

Abstract

We encountered three families that showed NASH accumulation. In family #1, a 21-year-old son and 10-year-old daughter were diagnosed with nonalcoholic steatohepatitis (NASH). They shared two adiponectin-gene single nucleotide polymorphisms (SNP). In family #2, a 51-year-old mother and 27-year-old son were diagnosed with NASH and shared the SNPs of other genes. In family #3, a 66-year-old mother and 34-year-old son were diagnosed with NASH and shared the SNPs of other genes. SNP sites differed among the three families, suggesting that the genes associated with the occurrence of NASH might be different in each patient.

Key words: NASH, SNP, familial aggregation

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Introduction

In lifestyle-related diseases such as type 2 diabetes mellitus (DM/) hypertension, accumulation in a family is often observed (1, 2). Hara et al reported that two single nucleotide polymorphism (SNP) sites, Intron 2 and Exon 2 of the adiponectin gene, are associated with DM onset and insulin resistance (3). In addition, SNPs of the PPARγ2 gene, calpains 10 (intron polymorphism) gene and adrenergic receptor (Trp64Ayg polymorphism) gene have been reported to be associated with the onset of DM, suggesting that genetic background might be associated with DM (4-6). In hypertension, a polymorphism of the angiotensinogen gene (M235 T) is reported to be correlated with serum renin levels and to related to hypertension (7). It is reported that insertion/deficiency (I/D) polymorphisms of the angiotensin-converting enzyme (ACE) gene are correlated with the serum ACE level, which is related to cardiovascular disease and essential hypertension (8). These results suggested that the occurrence and progression of lifestyle-related diseases are associated with genetic background as well.

Non-alcoholic fatty liver disease (NAFLD) has recently been recognized as a leading cause of abnormal liver function tests. Its spectrum ranges from simple fatty liver, which is usually a benign and non-progressive condition, to non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis (9, 10). Patients with NASH usually have insulin resistance syndrome as well. In addition, NASH is increasingly being recognized as a major cause of cryptogenic cirrhosis and as an indication for liver transplantation. Its etiology remains unclear, but most investigators agree that development of NASH requires underlying steatosis followed by a "second hit" that induces inflammation, fibrosis, or necrosis (10). As NASH is included in metabolic syndrome, familial aggregation is suggested (11-13).

Genetic SNPs of the β3 adrenergic receptor gene, microsomal triglyceride transfer protein (MTP) gene, MnSOD gene, and interleukin 1β gene are reported to be associated with NASH (14, 15). We also demonstrated that SNPs of the TNF promoter region might be associated with the "second hit" of NASH (16). Here, we report three families in which NASH accumulation was observed, and analyze the disease course and gene polymorphisms of these patients.

Case Report

Between January 1991 and December 2005, 249 patients

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Table 1. Laboratory Data

	Case1	Case2	Case3	Case4	Case5	Case 6	Unit
T-bil	0.7	1.5	0.3	0.8	0.6	0.8	(mg/dl)
AST	280	61	43	134	66	30	(IU/l)
ALT	504	154	98	292	69	65	(IU/l)
γ-GTP	54	23	41	184	74	36	(IU/l)
T-chol	205	139	256	211	249	186	(mg/dl)
TG	85	79	83	368	246	224	(mg/dl)
Plt	33.7	24.5	27.2	19.4	20.3	20.9	(10^4/mm^3)
PT%	85.3	82.5	100	89.4	93.8	100	(%)
FBS	99	95	115	91	140	137	(mg/dl)
HbA1c	4.9	4.9	6.2	5.6	8.0	7.3	(%)
IRI	10.5	3.3	9.1	16.2	16.6	11.2	(μU/ml)
HOMA-R	2.57	0.77	2.58	3.64	5.74	3.79	
HBsAg	(-)	(-)	(-)	(-)	(-)	(-)	
HCV Ab	(-)	(-)	(-)	(-)	(-)	(-)	
DM	(-)	(-)	(+)	(-)	(+)	(+)	
HT	(-)	(-)	(-)	(-)	(-)	(+)	
Hyperlipidemia(-)		(-)	(+)	(+)	(-)	(+)	

were diagnosed as having biopsy-proven NASH at Tokyo Women's Medical University Hospital. Three families were found to have NASH between siblings or between mother and child. The diagnosis of NASH was based on the following criteria: 1) the presence of steatosis (>10%), lobular inflammation, and ballooning, with or without Mallory bodies and perivenular or pericellular fibrosis; 2) intake of less than 40 g of ethanol per week, as confirmed by physicians and family members in close contact with the patient; and 3) appropriate exclusion of other liver diseases such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, and metabolic liver diseases.

Family #1 (Case 1, 10-year-old girl; Case 2, 21-year-old man)

In the summer of 1999, Case 1, a 10-year-old girl had eaten ice cream every day and had gained 3 kg of body weight in one month. Because her mother had type 2 DM, a parent-and-child medical examination for DM was performed, revealing hepatic dysfunction. Laboratory and physical data show BMI 21.8 kg/m^2, AST 280, ALT 504 (Table 1). Liver biopsy showed steatohepatitis (Stage 1, Grade 2-3) according to Brunt's NASH classification (17). Thus, she was diagnosed with NASH. Diet and exercise therapy was started, and her serum AST/ALT levels normalized. Her older brother (Case 2) had had mild liver dysfunction since 1999. His body weight had increased by 5 kg from 2002 to 2003, and his serum AST/ALT levels were markedly increased. Laboratory and physical data showed BMI 23.3 kg/m^2, AST 61, and ALT 154 (Table 1). Liver biopsy showed steatohepatitis (Stage 1, Grade 2), and he was diagnosed with NASH. After diet and exercise therapy, his serum AST/ALT levels improved to the normal range. We investigated the lifestyle and food preference by questionnaires. In cases 1 and 2, both liked to eat snacks and ice cream. Figure 1 shows the family tree of these cases. Their mother had DM. Both her children (cases 1 and 2) were diagnosed with NASH at relatively young ages. In addition, we investigated 10 SNP sites reported to be associated with NASH or DM (3, 14-16), and found that both cases shared two adiponectin-gene SNP sites that have been suggested to be related to DM (Table 2).

Family #2 (Case 3, 51-year-old woman; Case 4, 27-year-old man)

Case 3 had been treated for DM from 1990. Around 2000, liver dysfunction was discovered, and she was referred to our hospital in 2001. Laboratory and physical data showed BMI 26.4 kg/m^2, AST 43, ALT 98 (Table 1). Liver biopsy showed steatohepatitis (Stage 0-1, Grade 2), and she was diagnosed with NASH. Figure 2 shows her clinical course. We recommended diet and exercise therapy, but her body weight did not decrease. Serum AST/ALT levels increased and decreased in direct proportion to the increase and decrease in her body weight. In 2004, her serum AST/ALT levels were markedly increased. A second biopsy was performed, showing steatohepatitis (Stage 2, Grade 2-3), and the fibrosis was observed to have progressed during the four years since diagnosis. Her son (Case 4) had had hepatic dysfunction since his late teens. Serum AST/ALT levels, which at one time were normalized, had increased again in 2004, prompting a medical examination at that time. In 2005, his serum AST/ALT levels were increased to 134/292 when his body weight increased from 78 kg to 82 kg, as shown in Fig. 2. Liver biopsy showed steatohepatitis (Stage 1, Grade 3). With his weight loss, liver function improved. In cases 3

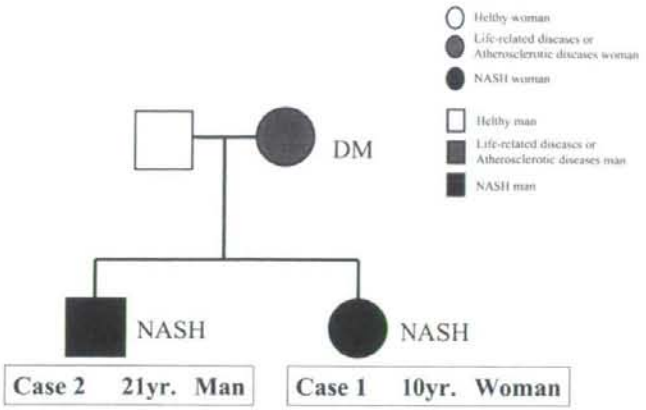

Figure 1. The family tree of family #1. Cases 1 and 2 were diagnosed with NASH. Their mother had type 2 diabetes mellitus (DM).

Table 2. SNP Analysis

	TNF-α -238	TNF-α -308	TNF-α -863	TNF-α -1031	Adipo +45	Adipo +276	IL-1β +511	β3-Ad	MTP	MnSOD
Case1	G/G	G/G	C/C	T/T	G/T	G/G	T/T	Trp/Arg	T/G	T/T
Case2	G/G	G/G	C/A	C/T	G/T	G/G	C/T	Trp/Trp	G/G	C/T
Case3	G/G	G/G	C/A	C/T	T/T	G/G	T/T	Trp/Arg	G/G	C/T
Case4	G/G	G/G	A/A	C/C	G/T	G/G	C/T	Arg/Arg	G/G	T/T
Case5	G/G	G/G	C/C	T/T	G/T	T/G	C/T	Trg/Trg	G/G	T/T
Case6	G/G	G/G	C/C	T/T	T/T	T/T	T/T	Trp/Arg	G/G	T/T

Shaded genotypes indicate those thought to contribute to the occurrence or progression of NASH or DM.

TNF, tumor necrosis factor

Adipo, adiponectin

IL-1, interleukin-1

β3-Ad, β3-adrenergic receptor

MTP, microsomal triglyceride transfer protein

and 4, there was no common tendency of lifestyle or food preference. Figure 3 shows the family tree of Cases 3 and 4. DM and hypertension were found in 3 other relatives. In the SNP analysis, both cases shared the SNPs of TNF-α promoter regions -1,031 and -863, which have been suggested to be related to TNF-α production, and the SNPs of adiponectin gene, the β3-adrenergic receptor and MTP genes, reportedly related to NASH (Table 2).

Family #3 (Case 5, 34-year-old man; Case 6, 66-year-old woman)

Case 5 had been obese since primary school age. At age 24, his serum AST/ALT levels were slightly increased and his body weight had increased to 90 kg (BMI 28.7 kg/m^2). He was diagnosed with DM at age 29. As his control of DM was poor, medical treatment was started. In 2004, he was referred for hepatic dysfunction to our hospital. Laboratory and physical data showed BMI 33.5 kg/m^2, AST 66, ALT 69 (Table 1). Liver biopsy showed steatohepatitis (Stage 3, Grade 3). We recommended diet and exercise therapy, after which his serum AST/ALT levels decreased. His mother (Case 6) had had hypertension from about age 50. At about age 60, hyperlipemia, hyperuricemia, and fatty liver were discovered. At age 63, therapy against DM was started. She was referred to our hospital for continuous hepatic dysfunction in 2005. Laboratory and physical data showed BMI 26.6 kg/m^2, AST 30, ALT 65 (Table 1). Liver biopsy showed steatohepatitis (Stage 3, Grade 3). In cases 5 and 6, there was no common tendency of lifestyle or food preference. Figure 4 shows the family tree of Cases 5 and 6. DM and arteriosclerotic disease were found in 3 other relatives. In SNP analysis, both cases shared the SNPs of the MTP and MnSOD genes, which are reportedly related to NASH (Table 2).

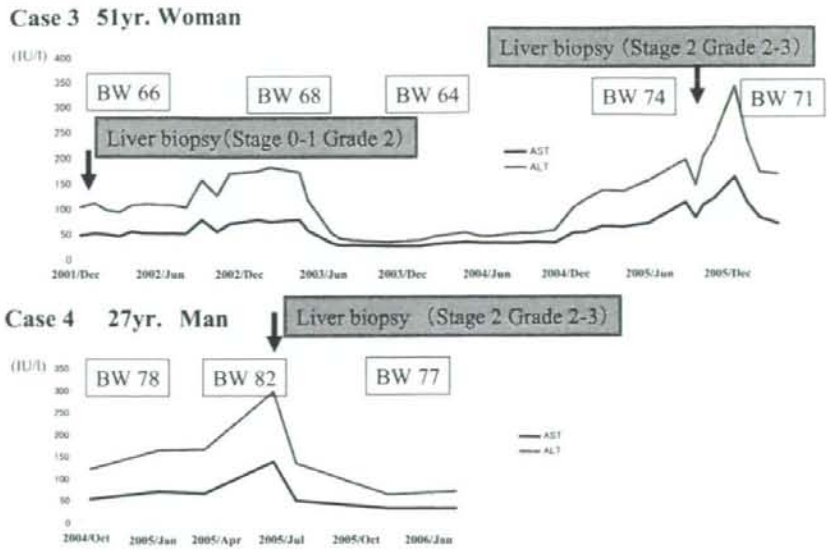

Figure 2. Clinical courses of cases 3 and 4 as well as the serum AST/ALT levels and body weight are shown. In case 3, the fibrosis stage progressed for 4 years. BW: body weight (kg).

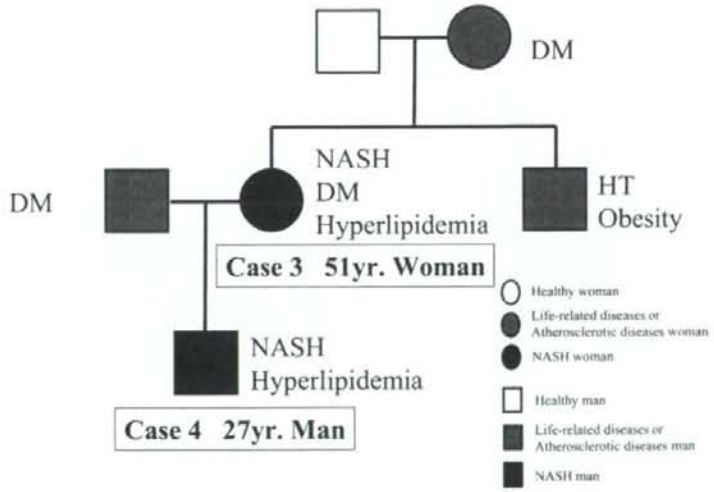

Figure 3. The family tree of family #2. Cases 3 and 4 were diagnosed with NASH. The mother of case 3 and the father of case 4 had type 2 diabetes mellitus (DM).

Discussion

We encountered three families in which NASH occurred in two siblings, or in both parent and child, similar to other lifestyle-related diseases. In these families, DM and other lifestyle-related diseases were frequently observed. The NASH-associated SNP sites that were found in each family were different, but all patients had some SNPs that are reportedly related to NASH or DM. For example, in family # 1, two SNP sites of adiponectin were shared between siblings, and each patient had other genetic SNPs. In family # 2, both patients shared an adiponectin gene SNP associated with DM, an economizing genotype of the β3-adrenergic receptor gene, a genotype of MTP that decreases the release capacity of very low-density lipoprotein (VLDL), and SNPs related to high TNF-α production. In family #3, both patients shared SNPs of the MnSOD gene and MTP gene, which are associated with oxidative stress. It was not confirmed whether these SNPs actually contributed to the occurrence and progression of NASH in our patients. For example, case 2 did not show insulin resistance, raising suspicion as to whether the adiponectin gene contributed to the pathogenesis of NASH in this case. The biological function

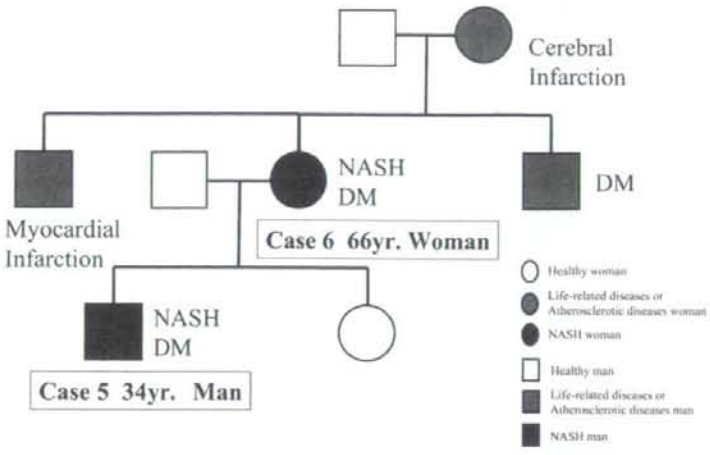

Figure 4. The family tree of family #3. Cases 5 and 6 were diagnosed with NASH. The mother and brothers of case 6 had type 2 diabetes mellitus (DM) or atherosclerotic diseases.

of adiponectin has been associated with not only insulin resistance, but also anti-inflammatory and anti-liver fibrosis, and it has been demonstrated that adiponectin is associated with the occurrence and progression of NASH (18, 19). In addition, Hara et al reported that adiponectin SNP might influence the production of adiponectin (3). These data suggest that adiponectin and these SNPs are associated with NASH. Among the SNPs which are reported to be related to the pathogenesis and progression of NASH, only adiponectin SNPs were shared in Cases 1 and 2. Therefore, we suggest that adiponectin SNPs are associated with NASH. In the future, SNP analysis and investigation of lifestyles in other family members who have not developed NASH will clarify which factors are more important.

Abdelmalek et al reported that NAFLD patients were more likely to have DM and that insulin resistance and DM occurred frequently in their first-degree relatives. In addition, a trend toward insulin resistance was noted in the mothers, but not the fathers, of patients with NAFLD (11). In our three families, all mothers had DM and/or NASH. In addition, in families #2 and #3, the grandmothers had DM or atherosclerotic diseases. Yatsuji et al reported that DM was more prevalent in older female NASH patients and suggested the possibility that female NASH patients might have stronger genetic factors (20). At any rate, it is necessary to pay close attention to children of mothers with DM or NASH.

Familial aggregation has been reported for DM, insulin resistance, and arteriosclerotic diseases (1, 2, 11). Here, in three family members, DM, HT, and arteriosclerotic diseases were frequently observed. These data support the hypothesis that NASH is associated with genetic background of these diseases and is part of the metabolic syndrome (12).

Case 5 was at stage 3 in young adulthood. In addition, the fibrosis of Case 3 progressed in the four years after diagnosis. Genetic background and similar eating habits or lifestyles might accelerate the progress of NASH. Considering the rapid progression of NASH in these patients, in addition to their family histories, amelioration of living habits and pharmacotherapy should be started from a young age.

Even when NASH patients had a genetic background for the disease, their liver function improved when body weight was controlled. These results suggested that adiposis based on living habits is the origin of NASH. We investigated the lifestyle and food preference by questionnaires. In cases 1 and 2, both liked to eat snacks and ice cream. In cases 3 and 4, there was no common tendency of lifestyle or food preference. Cases 5 and 6 also showed no common tendency. More detailed investigations will be necessary, because the possibility could not be denied that a similar lifestyle might be the cause of NASH among members of the same family. As overeating is encouraged in contemporary society, it is believed that the incidence of NASH will increase in the future. It is also recommended for the entire family of a NASH patient to undergo medical examinations.

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Original Article

Prevalence of esophagogastric varices in patients with non-alcoholic steatohepatitis

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Aim: In non-alcoholic steatohepatitis (NASH), fibrosis begins around the central veins, as also happens with alcoholic liver disease, so the symptoms of portal hypertension may be due to central vein occlusion. The aim of this study was to define the prevalence of esophagogastric varices and the clinical outcome after endoscopic treatment in NASH patients with severe fibrosis.

Methods: The subjects were 72 patients with clinicopathologically confirmed NASH who had bridging fibrosis (F3) or cirrhosis (F4) determined by the examination of liver biopsy specimens, and who underwent upper gastrointestinal endoscopy. The prevalence and pattern of endoscopically detected varices at the time of liver biopsy were evaluated. The results of NASH patients ($n = 11$) with endoscopically treated esophageal varices were compared to those with alcoholic ($n = 67$) and hepatitis C virus-associated cirrhosis ($n = 152$).

Results: Esophagogastric varices were detected in 34 out of the 72 (47.2%) patients; esophageal varices in 25 (34.7%) and gastric varices in nine (12.5%), while six of these patients had variceal bleeding. In NASH patients, the cumulative recurrence-free probability at 24 months after endoscopic treatment was 63.6%, the bleeding-free probability was 90.9%, and the 5-year survival was 100%. Only one out 11 patients died of liver failure at 70 months after treatment.

Conclusion: About half of NASH patients with severe fibrosis had esophagogastric varices. The clinical status and course of the varices do not necessarily improve after endoscopic treatment. NASH patients with esophagogastric varices need to be followed up carefully, like patients with other chronic liver diseases.

Key words: esophageal varices, gastric varices, liver cirrhosis, NASH, portal hypertension

INTRODUCTION

WITH WESTERNIZATION OF the Japanese diet, lifestyle-related diseases such as obesity, hypertension, hyperlipidemia and diabetes mellitus have become a problem in Japan. The incidence of one liver condition related to lifestyle, non-alcoholic fatty liver disease (NAFLD), has been increasing and has attracted attention.[1–3] NAFLD comprises mainly simple fatty liver that is considered benign, and some patients develop non-alcoholic steatohepatitis (NASH), which is a clinicopathological entity characterized by the development of histopathological changes in the liver resembling those induced by excessive alcohol intake in non-alcohol abusers. NASH is generally considered to be a mild condition, but it can sometimes progress rapidly, causing cirrhosis, and may even lead to the development of hepatocellular carcinoma (HCC).[4–6] In NASH, fibrosis begins around the central veins, as is the case for alcoholic liver disease,[7,8] so symptoms of portal hypertension may be the first to appear at the stage of bridging fibrosis (F3), before the onset of cirrhosis (F4), due to central vein occlusion. Although several studies have analyzed the natural history of NAFLD and cirrhosis due to NASH,[1,6,9,10] none of the published studies has focused on the esophagogastric varices of NASH with severe fibrosis, which includes bridging fibrosis and cirrhosis. Variceal bleeding is the most serious complication in portal hypertension. At least two-thirds of cirrhotic patients develop esophageal varices during their lifetime. Severe upper gastrointestinal bleeding as a complication of portal hypertension develops in about 30–40% of patients with cirrhosis. In patients with cirrhosis, variceal bleeding is associated with significant

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Table 1 Characteristics of NASH patients with severe fibrosis

	All ($n = 72$)	Bridging fibrosis ($n = 25$)	Cirrhosis ($n = 47$)
Male/Female	35/37	13/12	22/25
Age (years)	62.0 (16–89)	57.0 (18–81)	64.0 (16–89)
Obesity (BMI > 25 kg/m^2)	56 (77.8%)	17 (68.0%)	39 (83.0%)
Diabetes	32 (44.4%)	10 (40.0%)	22 (46.8%)
Hyperlipidemia	37 (51.4%)	13 (52.0%)	24 (51.1%)
Hypertension	21 (29.2%)	5 (20.0%)	16 (34.0%)
Child–Pugh classification A/B/C	52/17/3	22/1/2	30/16/1
Concomitant HCC	7 (9.7%)	1 (4.0%)	6 (12.8%)
AST (IU/L)	49.0 (9–392)	72.5 (9–392)	45.0 (12–145)
ALT (IU/L)	47.0 (5–740)	77.0 (5–740)	38.0 (9–147)
Platelet count ($\times 10^4$/mm^3)	13.7 (3.7–45.1)	19.4 (5.7–45.1)	11.8 (3.7–22.3)

Data are number of patients (percentage) or median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis.

morbidity, mortality and health care costs.[11–14] Thus, prevention of variceal bleeding is critically important.

The aim of this study was to define the prevalence of esophagogastric varices and the clinical outcomes after endoscopic treatment in NASH patients with severe liver fibrosis.

METHODS

OF 82 PATIENTS with clinicopathologically confirmed NASH who had bridging fibrosis (F3) or cirrhosis (F4), determined by examination of liver biopsy specimens between January 1993 and March 2006 at Tokyo Women's Medical University, 72 patients (35 men and 37 women, median age: 62.0 years; 25 with bridging fibrosis and 47 with cirrhosis) who underwent upper gastrointestinal endoscopy were enrolled in this study (Table 1). The diagnosis of NASH was based on the following criteria: (i) intake of less than 100 g of ethanol per week, as confirmed by the attending physician and family members who were in close contact with the patient; and (ii) appropriate exclusion of other liver diseases, such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction and metabolic liver diseases.[8,15–19] To rule out viral hepatitis as a cause of liver pathology, patients were excluded if they were positive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C virus (anti-HCV), or hepatitis C RNA (HCV-RNA) by polymerase chain reaction. None of the patients had a history of jejunoileal bypass surgery.

The prevalence and pattern of esophagogastric varices detected by endoscopy at the time of liver biopsy were evaluated, together with management of the patient subsequent to the detection of varices. Esophageal and gastric varices were classified according to the criteria proposed by the Japanese Society for Portal Hypertension.[20]

The cumulative recurrence-free probability, cumulative bleeding-free probability and survival probability were evaluated in 11 NASH patients with endoscopically treated esophageal varices (the NASH group). The two control groups for comparison were: (i) 67 patients with alcoholic cirrhosis (the alcoholic group), whose esophageal varices were treated endoscopically during the period from January 1993 to March 2006; and (ii) 152 patients with HCV-associated cirrhosis (the HCV cirrhosis group). Patients with Child–Pugh class C for liver function at the start of endoscopic treatment and patients with HCC were excluded from the study.

Liver histopathology

All liver biopsy specimens were examined using the following staining methods: hematoxylin–eosin, Mallory, silver reticulin, Victoria blue stain for copper binding protein and Perls iron stain for hemosiderosis. Tissue sections were assessed by one investigator (EH) blinded to the clinical and biochemical data of the patients. Fibrosis was scored using a 5-grade scale: F0, normal connective tissue; F1, foci of perivenular or pericellular fibrosis in zone 3; F2, perivenular or pericellular fibrosis confined to zones 3 and 2, with or without portal/periportal fibrosis; F3, bridging or septal fibrosis; and

F4, cirrhosis. Steatosis was graded mild to severe: mild (affecting 10–29% of hepatocytes); moderate (30–69% of hepatocytes); and severe (>70% of hepatocytes). Another investigator graded inflammation as mild, moderate, or severe based on the overall impression after evaluating the specimens for ballooning degeneration, Mallory bodies, giant mitochondria, disarray of hepatocytes, lobular and portal inflammation, focal necrosis, Councilman bodies, lipogranulomas and pigmented macrophages. Inflammation and steatosis was graded on a scale of 1–3: 1, mild; 2, moderate; and 3, severe.[8,15–19] We classified F0–F2 as mild fibrosis, whereas bridging fibrosis (F3) and cirrhosis (F4) were classified as severe fibrosis.

Endoscopic procedure

The endoscopic apparatus was a Q260 electronic endoscope (Olympus Optical, Tokyo, Japan). For endoscopic variceal ligation, the ligator was a pneumatically activated ligation device (Sumitomo Bakelite, Tokyo, Japan) or the Speedband Superview Super 7 (Boston Scientific Microvasive, Boston, MA, USA). Ligation was performed along the varices from the squamo-columnar junction according to the method of Stiegmann *et al.*[21,22] Up to eight ligations were applied per treatment session and ligation was repeated at 1-week intervals. As sclerosant, 5% ethanolamine oleate (5% EO, Oldamin; Grelan Pharmaceutical, Tokyo, Japan) was mixed with iopamidol and used for intravariceal injection sclerotherapy. A 23-gauge needle (5-mm long, Sumitomo Bakelite) was used for esophageal variceal sclerotherapy. When performing sclerotherapy, a transparent hood was fitted to the tip of the endoscope. In order to ensure treatment of the feeding vessels, intravariceal injection of contrast medium containing 5% EO was confirmed under fluoroscopic control.[23] The amount of sclerosant injected per treatment session was up to 20 mL and sclerotherapy was repeated after an interval of one week. In some patients, sclerotherapy with perivariceal injection of 1% polidocanol (Aethoxysklerol; Kreussler, Wiesbaden, Germany) or mucosal fibrosis therapy by argon plasma coagulation was used.[24,25] Treatment was terminated after confirming reduction of the varices to F1RC(−) or milder. Endoscopy was performed to evaluate esophageal and gastric varices before and 1 week, 3 months and 6 months following the endoscopic procedure. Furthermore, patients were followed up by endoscopy every 3 or 6 months and recurrence was defined as the development of F1 with red color sign or more, or the occurrence of bleeding. In the present study, the criteria used for prophylactic treatment of esophageal varices were as follows: (i) esophageal varices at risk of bleeding, varices classified as either red color-positive F2 form or F3; (ii) severe red color-positive F1 form; and (iii) a tendency to increase in size and red color sign within a period of 6 months.

Statistical analysis and ethical considerations

Results are expressed as number of patients and median (range). Univariate analysis was conducted using the Mann–Whitney *U*-test to assess the significance of differences in continuous variables between the varices positive group and the varices negative group. The cumulative recurrence-free probability, the cumulative bleeding-free probability and the survival probability were determined for each group using the Kaplan–Meier method and were compared by the log–rank test. A *P*-value of less than 0.05 was defined as indicating a statistically significant difference. Written informed consent was obtained from all patients before liver biopsy and endoscopic procedures.

RESULTS

Prevalence of esophagogastric varices in NASH patients with severe fibrosis

ESOPHAGOGASTRIC VARICES WERE detected in 34 out of the 72 (47.2%) patients, while esophageal varices were noted in 25 patients (34.7%). The varices were F1 in 12 patients, F2 in seven patients and F3 in six patients. Four of these patients presented with bleeding. Gastric varices were noted in nine (12.5%) patients; being F1 in one patient, F2 in three patients and F3 in five patients, while two patients presented with bleeding. In relation to liver histopathology, esophagogastric varices were found in four out of 25 (16.0%) patients who had bridging fibrosis (F3) and in 30 out of 47 (63.8%) patients with cirrhosis (F4). Table 2 shows the details of these patients. Esophageal varices were treated by endoscopic sclerotherapy in five patients and ligation in seven patients. Three patients underwent balloon-occluded retrograde transvenous obliteration (B-RTO) of gastric varices,[26] while one patient each underwent endoscopic obliteration with N-butyl-2-cyanoacrylate,[27] endoscopic clipping and Hassab's operation.

Comparison of subjects with and without esophagogastric varices

The median age of the patients with varices was 64.5 years and that of patients without varices was

Table 2 Prevalence of esophagogastric varices in NASH patients with severe fibrosis

	All ($n = 72$)	Bridging fibrosis ($n = 25$)	Cirrhosis ($n = 47$)
Esophageal varices	25 (34.7%)	4 (16.0%)	21 (44.7%)
Variceal form			
F1	12	1	11
F2	7 (2)†	1 (1)†	6 (1)†
F3	6 (2)†	2 (1)†	4 (1)†
Red color sign (-/+/++/+++)	9/5/8/3	1/1/2/0	8/4/6/3
Gastric varices	9 (12.5%)	0 (0.0%)	9 (19.1%)
Variceal form			
F1	1	0	1
F2	3 (1)†	0	3 (1)†
F3	5 (1)†	0	5 (1)†
Red color sign (-/+)	6/3	0/0	6/3
No varices	38 (52.8%)	21 (84.0%)	17 (36.2%)

†Number of bleeding cases. Data are number of patients (percentage).
NASH, non-alcoholic steatohepatitis.

61.5 years (P = NS). Among 38 patients without varices, 34 (89.5%) were classified as Child–Pugh class A. Among 34 patients with varices, 18 patients (52.9%) were Child–Pugh class A and 15 patients (44.1%) Child–Pugh class B. Thus, patients with varices were more likely to be class B. Transaminases tended to be lower in patients with varices than in patients without varices. The median platelet count of the patients with varices was $9.6 \times 10^4/mm^3$, while that of patients without varices was $17.9 \times 10^4/mm^3$. Patients with varices had a significantly lower platelet count than those without varices ($P < 0.0001$) (Table 3).

Results of treatment of esophageal varices in NASH patients

The cumulative recurrence-free probability at 24 months after endoscopic treatment of esophageal varices was 63.6% in the NASH group, 45.7% in the alcoholic group and 73.3% in the HCV group (Fig. 1). The cumulative bleeding-free probability at 24 months was 90.9% in the NASH group, 78.8% in the alcoholic group and 89.7% in the HCV group (Fig. 2). The 5-year survival probability was 100% in the NASH group, 82.8% in the alcoholic group and 75.9% in the HCV group (Fig. 3). There were no significant differences in these rates between the NASH group and the alcoholic group or between the NASH group and the HCV group. In the NASH group, only one patient died of liver failure at 70 months after treatment of the varices. In the alcoholic group, three patients each died of liver failure and variceal bleeding. In the HCV group, 23 patients died of liver failure and 13 patients died of HCC that developed during the course of follow-up (Table 4).

DISCUSSION

IN THE PRESENT study, we determined the incidence and morphological features of esophagogastric varices in NASH patients with bridging fibrosis (F3) or cirrhosis (F4). Of 72 NASH patients who were diagnosed as having severe fibrosis by liver biopsy, esophagogastric varices were detected in 34 (47.2%) patients, esophageal varices in 25 (34.7%) patients and gastric varices in nine (12.5%) patients. Six of these patients presented with variceal bleeding. Esophagogastric

Table 3 Comparison of subjects with and without varices

	Varices present ($n = 34$)	Varices absent ($n = 38$)	P-value
Male/Female	17/17	18/20	NS
Age (years)	64.5 (39–82)	61.5 (16–89)	NS
Child–Pugh classification (A/B/C)	18/15/1	34/2/2	<0.05
Concomitant HCC	2	5	NS
Liver histology (bridging fibrosis/cirrhosis)	4/30	21/17	<0.05
AST (IU/L)	44.5 (12–132)	52.0 (9–392)	NS
ALT (IU/L)	36.0 (9–212)	66.0 (5–740)	<0.05
Platelet count ($\times 10^4/mm^3$)	9.6 (3.7–21.9)	17.9 (5.2–45.1)	<0.0001

Data are number of patients or median (range). Mann–Whitney U-test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; NS, not significant.

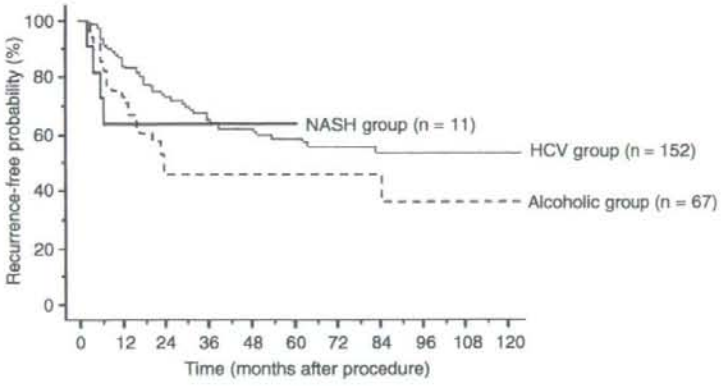

Figure 1 Kaplan–Meier analysis of cumulative recurrence-free curves. The cumulative recurrence-free probability at 24 months after endoscopic procedure for the non-alcoholic steatohepatitis (NASH) group (63.6%) was not significantly different from that of the alcoholic group (45.7%) or the hepatitis C virus (HCV) group (73.3%).

varices were found in four out of 25 (16.0%) patients who had bridging fibrosis versus 30 out of 47 (63.8%) patients who had cirrhosis. Thus, the rate of esophagogastric varices is higher in patients with cirrhosis than in those with fibrosis. Development of varices did not correlate with age and gender; no differences were detected in these variables between 34 patients with varices and 38 patients without varices. A high percentage of

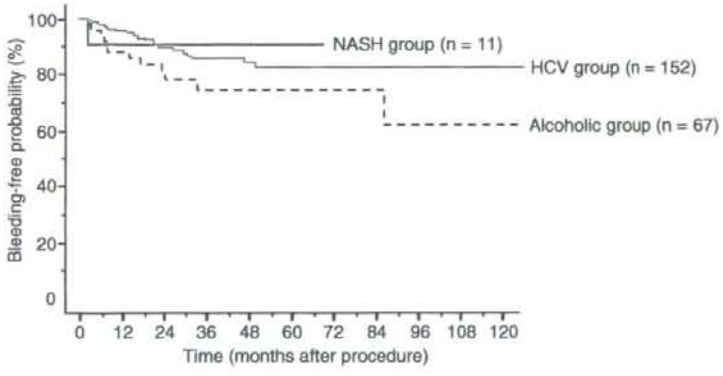

Figure 2 Kaplan–Meier analysis of cumulative bleeding-free curves. The cumulative bleeding-free probability at 24 months after endoscopic procedure for the non-alcoholic steatohepatitis (NASH) group (90.9%) was not significantly different from that of the alcoholic group (78.8%) or the hepatitis C virus (HCV) group (89.7%).

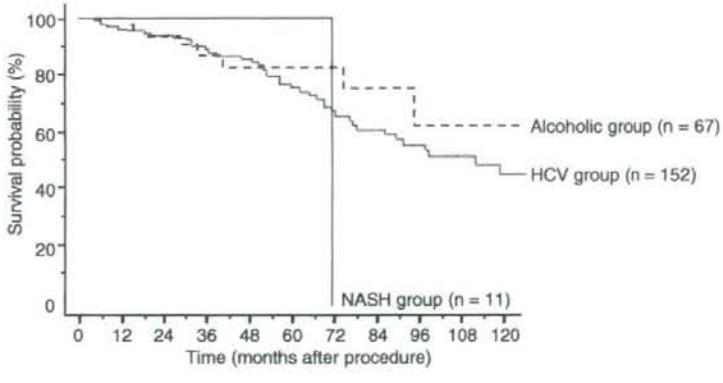

Figure 3 Kaplan–Meier analysis of survival curves. The survival probability at 5 years after endoscopic procedure for the non-alcoholic steatohepatitis (NASH) group (100%) was not significantly different from that of the alcoholic group (82.8%) or the hepatitis C virus (HCV) group (75.9%).

Table 4 Characteristics and prognosis of NASH, alcoholic and HCV patients with esophageal varices treated by endoscopic procedures

	NASH group ($n = 11$)	Alcoholic group ($n = 67$)	HCV group ($n = 152$)
Male/Female	4/7	64/3	82/70
Age (years)	68.0 (45–82)	56.0 (22–72)	62.5 (29–83)
Child-Pugh classification (A/B)	3/8	25/42	44/108
Variceal form (F1/F2/F3)	1/4/6	4/40/23	16/88/48
Red color sign (−/+/++/+++)	0/1/7/3	0/14/35/18	2/32/78/40
Endoscopic treatment (ligation/sclerotherapy)	8/3	45/22	91/61
Indication for treatment (prophylaxis/bleeding)	8/3	38/29	125/27
Follow-up period (months)	21.0 (8–70)	28.0 (3–157)	45.5 (3–159)
Mortality	1 (9.1%)	8 (11.9%)	46 (30.3%)
Causes of death			
Liver failure	1	3	23
HCC	0	1	13
Variceal bleeding	0	3	1
Others, unknown	0	1	9

Data are number of patients (percentage) or median (range).
HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis.

patients with varices had cirrhosis, which meant that they had advanced liver disease on histopathological examination. The median platelet count of patients with varices (9.6×10^4/mm^3) was significantly lower than that of patients without varices (17.9×10^4/mm^3).

Sanyal *et al.*[28] evaluated 1016 patients with HCV infection who had advanced fibrosis. They found that 16% of patients with bridging fibrosis and 39% of those with cirrhosis had esophageal varices ($P < 0.0001$), while 2% of patients with bridging fibrosis and 11% of those with cirrhosis had medium to large esophageal varices. They reported that the risk of esophageal varices in such patients increased along with a decrease of the platelet count, an increase of bilirubin and a higher international normalized ratio (INR), and that the risk of medium to large varices was negligible in the patients with platelet counts >150 000/mm^3. We compared the risk of esophagogastric varices in our NASH patients and those with HCV infection reported by Sanyal *et al.*,[28] since the risk of development of esophagogastric varices (approximately 16%) was similar in the two groups with bridging fibrosis. Esophagogastric varices occurred more frequently in our NASH patients who had cirrhosis and more than half of these patients had varices classified as F2 or more severe. More NASH patients had medium to large varices compared with patients suffering from HCV-associated cirrhosis. This is because NASH is characterized by fibrosis around the central veins that tends to cause portal hypertension. Zaman *et al.*[12] performed endoscopy in 98 patients to evaluate their eligibility for liver transplantation, and they found esophageal varices in 68% of the patients and gastric varices in 15%. They also reported that a platelet count of ≤88 000/mm^3 was a predictor for the presence of esophagogastric varices. Levy *et al.*[29] detected esophageal varices in 56 out of 113 patients (49.6%) with primary biliary cirrhosis and reported that the frequency of detection by screening endoscopy was 37% when 22 patients with variceal bleeding were excluded. A platelet count of ≤140 000/mm^3 was regarded as an independent predictor of esophageal varices.

Considered together, our results indicate that the incidence of esophagogastric varices in NASH patients with severe fibrosis is equivalent to or higher than that in patients with advanced fibrosis and cirrhosis caused by other liver diseases. In addition, NASH patients with severe fibrosis often developed bleeding. Kaneda *et al.*[30] reported that high serum hyaluronic acid and low platelet count are useful indicators of fibrosis in NASH and that the platelet count of patients with cirrhosis is ≤160 000/mm^3, with a median count of 130 000/mm^3. We suggest that platelet count can be a predictor of esophagogastric varices in NASH patients and that a count of <96 000/mm^3 could be a marker of cirrhosis in such patients, warranting endoscopy.

In general, the serum alanine aminotransferase (ALT) level tends to decrease with the progression of liver fibrosis (in cirrhosis). In our patients, the ALT level

varied widely during follow-up within a wide range (5–740 IU/L). Although the statistical analysis found ALT level to be a predictor of varices, we consider that further investigation including a larger sample is needed to analyze the reason for this finding.

The therapeutic outcome of endoscopically treated NASH patients with esophageal varices was compared with that of patients with alcohol- and HCV-associated cirrhosis. The NASH group included 11 patients, after excluding one patient with HCC from the 12 patients who underwent endoscopy. The control groups were the alcoholic group (67 patients) and the HCV group (152 patients). All patients were Child–Pugh A and B without HCC at the start of endoscopic treatment. The cumulative recurrence-free probability at 24 months after endoscopic treatment of esophageal varices was 63.6% for the NASH group, 45.7% for the alcoholic group and 73.3% for the HCV group. The cumulative bleeding-free probability after 24 months was 90.9% for the NASH group, 78.8% for the alcoholic group and 89.7% for the HCV group. Thus, the NASH group had better bleeding-free and recurrence-free probabilities than the alcoholic group, although the differences were not significant. Comparable results were noted for the NASH group and the HCV group. The 5-year survival probability was 100% for the NASH group, 82.8% for the alcoholic group and 75.9% for the HCV group. The NASH group showed better survival, although the difference was not significant. In the alcoholic group, three patients died of variceal bleeding associated with liver failure. Many patients with alcoholic cirrhosis cannot stop drinking, which leads to hepatopathy and gastrointestinal mucosal damage. These events tend to be recurrent and can readily cause bleeding, resulting in death from variceal hemorrhage in some cases. The largest number of deaths from liver failure was noted in the HCV group, and there were also many deaths due to HCC that occurred during the course of follow-up. In patients with HCV-associated cirrhosis, liver failure and HCC are important prognostic factors. In the NASH group, only one patient died of liver failure and the prognosis was better than that of alcoholic cirrhosis or HCV-associated cirrhosis. A number of reports described NASH patients who developed HCC, but the risk is considered to be lower than that for patients with HCV-associated cirrhosis. Thus, the main cause of death in NASH patients seems to be liver failure. In fact, Hui *et al.*[9] reported that liver failure was the most common cause of morbidity and mortality in patients with NASH-associated cirrhosis. HCC appears to be less common in these patients than in those with cirrhosis due to hepatitis C. Accordingly, the prognosis of NASH patients who develop cirrhosis appears to be similar to or better than that of patients with HCV-associated cirrhosis. Sanyal *et al.*[10] have also reported that compensated cirrhosis due to NASH is associated with a lower mortality rate than that due to HCV and that there is also a lower risk of ascites, hyperbilirubinemia and HCC. HCC can occur in patients with NASH-related cirrhosis, but the risk is lower than that reported for patients with cirrhosis due to hepatitis C. However, cardiovascular mortality is higher in NASH patients. Although our study included only a small group of patients and should thus be considered preliminary in nature, we can recommend that patients with cirrhosis should be followed up to monitor the progression of liver disease (function), development of HCC and other complications. Lifestyle modification including diet, physical activity and pharmacological treatment should be the first line and mainstay of management. NASH should be recognized as a part of the metabolic syndrome and managed in a multidisciplinary approach that addresses liver disease in the context of risk factors for diabetes and premature cardiovascular disease.

CONCLUSION

IN CONCLUSION, 47.2% of our NASH patients who were diagnosed with severe liver fibrosis had esophagogastric varices. In particular, 63.8% of the patients with cirrhosis (F4) had esophagogastric varices. More than half of NASH patients had varices classified as F2 or more severe. The survival probability of NASH was better than that associated with alcoholic cirrhosis or HCV-associated cirrhosis. However, the cumulative recurrence-free probability and bleeding probability of NASH were similar to those of alcoholic cirrhosis and HCV-associated cirrhosis. The clinical status and course of esophagogastric varices in NASH patients do not necessarily improve after endoscopic treatment, compared with alcoholic cirrhosis or HCV-associated cirrhosis. Therefore, NASH with esophagogastric varices needs to be followed up carefully, like other chronic liver diseases. We consider that NASH patients with treated varices will increase in the future and their natural history need to be defined by long-term follow-up.

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