

Type 2 diabetes mellitus is associated with the fibrosis severity in patients with nonalcoholic fatty liver disease in a large retrospective cohort of Japanese patients

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Abstract

Background The prevalence of nonalcoholic fatty liver disease (NAFLD) and metabolic syndrome have been increasing worldwide. The associations between metabolic factors and the histologic severity of NAFLD have not yet been clarified. Therefore, we studied the relationships between relevant metabolic factors and the histological severity of NAFLD.

Methods In a cross-sectional multicenter study conducted in Japan, we examined 1,365 biopsy-proven NAFLD

patients. The frequencies of underlying lifestyle-related diseases and their relationships to the NAFLD histology were investigated.

Results The hepatic fibrosis stages (Stage 0/1/2/3/4) were 22.6/34.1/26.7/14.5/2.1 (%) in the male patients, and 16.2/31.7/23.9/21.6/6.6 (%) in the female patients. Dyslipidemia was present in 65.7% (hypertriglyceridemia, 45.3%; increased low-density lipoprotein cholesterol, 37.5%; decreased high density lipoprotein cholesterol, 19.5%) of patients. Hypertension was present in 30.2%, and diabetes mellitus (DM) in 47.3%. The fibrosis stage increased with age, especially in postmenopausal females. The body mass index was positively correlated with the fibrosis stage. Deterioration of glucose control was positively correlated

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with the fibrosis stage, this correlation being more prominent in females. Multivariate analysis identified age and DM as significant risk factors for advanced fibrosis. No significant correlation of the fibrosis stage was observed with hypertension. There was a negative correlation between the serum triglyceride levels and the fibrosis stage. **Conclusions** DM appeared to be a significant risk factor for advanced fibrosis in patients with NAFLD, and would therefore need to be properly managed to prevent the progression of NAFLD.

Keywords NAFLD · Histology · Diabetes mellitus · Retrospective study

Abbreviations

NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
IR	Insulin resistance
DM	Diabetes mellitus
NAFL	Nonalcoholic fatty liver
BMI	Body mass index
CT	Computed tomography
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
GGT	Gamma glutamyl transpeptidase
ChE	Cholinesterase
HDL	High density lipoprotein
LDL	Low-density lipoprotein
FPG	Fasting plasma glucose
HbA1c	Hemoglobin A1c
FFA	Free fatty acid
CRP	C-reactive protein
IRI	Immunoreactive insulin
HOMA-IR	Homeostasis model assessment-insulin resistance
SD	Standard deviation
IGT	Impaired glucose tolerance
NGT	Normal glucose tolerance

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most commonly encountered chronic liver disease in the world. According to Japanese annual health check reports, 9–30 % of Japanese adults suffer from NAFLD [1–3]. Since it is now known that almost 10–20 % of individuals with NAFLD have nonalcoholic steatohepatitis (NASH), the prevalence of NASH is estimated to be 1–3 % in the adult Japanese population, similar to the prevalence reported from Western countries.

Nonalcoholic fatty liver disease includes a wide spectrum of liver diseases, ranging from nonalcoholic fatty liver

(NAFL), a benign and non-progressive condition, to NASH, which can progress to liver cirrhosis and hepatocellular carcinoma even in the absence of a history of significant alcohol consumption [4–7]. Furthermore, NASH is considered to be the hepatic manifestation of metabolic syndrome, and has been shown to be associated with obesity, insulin resistance (IR) and abnormalities of glucose and lipid metabolism [8–16]. Importantly, the rates of nonalcoholic fatty liver (NAFL) and NASH are expected to continue to grow with the developing pandemic of obesity and diabetes mellitus, to become global public health concerns.

Owing to the difficulties in diagnosing NAFLD (NAFL and/or NASH) and referral bias, it has been difficult to determine the prognostic factors in patients with NAFLD. NAFLD is a complex disease with multiple etiopathogenetic factors, including obesity, type 2 DM, dyslipidemia, hypertension, and other diseases associated with metabolic dysregulations. Recent reports have suggested that DM is an independent risk factor for NAFLD [17–19]. Despite the high prevalence and potentially serious nature of this disease, relatively little is known about the metabolic factors that might be associated with the histological severity of NAFLD.

The purpose of this study was to conduct a retrospective investigation of the association between metabolic factors and the histologic severity of NAFLD in a large cohort of Japanese patients with NAFLD.

Patients and methods

Patient population

A total of 1,365 biopsy-proven NAFLD patients seen between 2001 and 2012 were enrolled from institutes affiliated with the Japan Study Group of NAFLD (JSG-NAFLD), represented by the following nine hepatology centers in Japan: Hiroshima University, Kyoto Prefectural University of Medicine, Yokohama City University, Kochi Medical School, Saga Medical School, Osaka City University, Nara City Hospital, Kurume University, and Saiseikai Suita Hospital. A portion of the patients (76.8 %; 1,048 out of 1,365) had also been involved in the previous JSG-NAFLD study [20, 21]. Informed consent was obtained from each patient, and the study was conducted in conformity with the ethical guidelines of the 7th revision of the Declaration of Helsinki (in October 2008) [22] and the approval of the ethics and research committees of the hospitals. In all patients, the current and past daily alcohol intake was less than 20 g per day; details regarding alcohol consumption were obtained independently by at least two physicians and confirmed by close family members. None

of the patients were receiving any medications that could cause NASH. Among the patients, those with the following disorders were excluded: secondary causes of steatohepatitis, drug-induced liver disease, alcoholic liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, α 1-antitrypsin deficiency, hemochromatosis, Wilson's disease, and biliary obstruction. [23].

Study design

A complete physical examination was performed on each patient within 1 month prior to the liver biopsy, as reported previously [24]. The body mass index (BMI) was calculated as the weight (kg) divided by height (m)-squared. Obesity was defined as a BMI of greater than 25, according to the criteria of the Japan Society for the Study of Obesity [25]. Computed tomography (CT) was used to determine the visceral fat area at the level of the umbilicus [26], as previously reported [24]. Dyslipidemia was diagnosed based on serum cholesterol levels higher than 220 mg/dl and/or high-density lipoprotein cholesterol levels lower than 40 mg/dl and/or triglyceride levels over 150 mg/dl. Hypertension was diagnosed if the patient was on antihypertensive medication and/or had a resting recumbent blood pressure of $\geq 130/85$ mmHg on at least two occasions. Hyperuricemia was diagnosed based on serum uric acid levels higher than 7.0 mg/dl. DM was diagnosed according to the 2006 World Health Organization (WHO) criteria [27].

Venous blood samples were taken in the morning following overnight fasting for 12 h. The laboratory evaluation in all patients included a blood cell count, hemoglobin, platelet count; and the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST/ALT ratio, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), cholinesterase (ChE), total bilirubin, direct bilirubin, albumin, total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), immunoreactive insulin (IRI), ferritin, uric acid, free fatty acid (FFA), and hyaluronic acids, were measured periodically during the treatment using the standard techniques of clinical chemistry laboratories.

Insulin resistance was calculated by the homeostasis model assessment-insulin resistance (HOMA-IR) using the following formula: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/ml}) \times \text{plasma glucose (mg/dl)}/405$ [28].

Pathology

Patients enrolled in this study underwent percutaneous liver biopsy under ultrasonic guidance after obtaining

informed consent. Formalin-fixed, paraffin-embedded liver sections were stained routinely with hematoxylin-eosin, silver reticulin, and Masson trichrome. All the specimens were examined by an experienced pathologist who was unaware of the clinical and biochemical data of the patients. Histological diagnosis for NAFLD was performed according to the methods of Matteoni et al. [6]. Grading and staging was classified according to Brunt et al. [29] and Kleiner et al. [30], as previously reported. In brief, steatosis was graded as follows: grade 1 (5–33 % of hepatocytes affected), grade 2 (34–66 % of hepatocytes affected), or grade 3 (> 66 % of hepatocytes affected). Necroinflammation was graded from grade 0 (absent) to 3 (1, occasional ballooned hepatocytes and no or very mild inflammation; 2, ballooning of hepatocytes and mild-to-moderate portal inflammation; 3, intra-acinar inflammation and portal inflammation). Fibrosis was staged from grade 0 (absent) to 4 (1, perisinusoidal/pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis).

Statistical analyses

The data were statistically analyzed using R software, version 3.0.0. Continuous variables were expressed as mean \pm standard deviation (SD). Qualitative data are expressed as numbers, with percentages shown in parentheses.

Statistically significant differences in the quantitative data were determined using the *t* test or Mann–Whitney *U* test. Multivariate analysis was carried out by logistic regression. Differences were considered to be statistically significant at *P* values of less than 0.05.

Results

Patient characteristics

A total of 1,365 biopsy-proven patients with NAFLD were enrolled in this study. The demographic and clinical characteristics of the male and female NAFLD patients are shown in Supplemental Table 1. Of the total, 709 were males. The mean age of the patients was 51.0 ± 14.9 years (45.7 ± 15.1 and 56.8 ± 12.4 years for males and females, respectively). Whereas no significant differences were observed in the BMI, blood pressure, waist circumference, and visceral fat area between the male and female patients, the subcutaneous fat area and L/S ratio were significantly higher in the female patients. Statistically significant differences were observed in the white blood cell count, hemoglobin, and serum levels of transaminases, AST to ALT ratio, LDH, ALP, GGT, ChE, total and direct bilirubin, albumin, triglycerides, HDL cholesterol, fasting

Table 1 Prevalences of metabolic abnormalities in NAFLD patients

Variable	Percentage
BMI \geq 25	73.0
Hypertension	39.9
Dyslipidemia	65.7
Hypertriglyceridemia	45.3
Hyper-LDL cholesterolemia	37.5
Hypo-HDL cholesterolemia	19.5
DM	47.3
Hyperuricemia	30.2

glucose, HbA1c, ferritin, uric acid, and hyaluronic acid between the male and female patients, as shown in Supplemental Table 1.

The frequencies of the metabolic abnormalities in the NAFLD patients are shown in Table 1. Obesity, as defined by the criteria of the Japan Society for the Study of Obesity, was seen in 73.0 % of the NAFLD patients, hypertension was found in 39.9 %, dyslipidemia in 65.7 % (hypertriglyceridemia, 45.3 %; hyper-LDL cholesterolemia, 37.5 %; hypo-HDL cholesterolemia, 19.5 %), type 2 diabetes in 47.3 %, and hyperuricemia in 30.2 % of the patients.

Distribution of the metabolic factors by the histological findings

The fibrosis stages (Stage 0/1/2/3/4) were 22.6/34.1/26.7/14.5/2.1 (%) in males, and 16.2/31.7/23.9/21.6/6.6 (%) in females, respectively. The distribution of the fibrosis stage in the different age groups in both genders is shown in Supplementary Fig. 1. Whereas the percentage of patients with advanced fibrosis (Stage 3 and 4) increased gradually with age in both genders, significant increase was seen after the age of 60 years in the females.

The prevalences of obesity (BMI \geq 25) for each fibrosis stage are shown in Supplementary Fig. 2. The percentages of patients with obesity for each fibrosis stage (Stage 0/1/2/3/4) were 61.3/73.3/79.9/86.4/80.0 (%) in males, and 57.1/72.9/74.4/75.9/74.4 (%) in females, respectively. The prevalence of obesity showed a linear increase with progression of the fibrosis stage in the male NAFLD patients. However, no such increase was observed in the female NAFLD patients between Stage 1 and Stage 4.

The prevalences of dyslipidemia for each fibrosis stage are shown in Figs. 1 and 2. The percentages of patients with hypertriglyceridemia for each fibrosis stage (Stage 0/1/2/3/4) were 56.3/57.7/54.8/51.0/26.7 (%) in males, and 34.0/39.5/39.1/30.2/12.2 (%) in females, respectively. The percentages of patients with hyper-LDL cholesterolemia for each fibrosis stage (Stage 0/1/2/3/4) were 38.6/36.2/

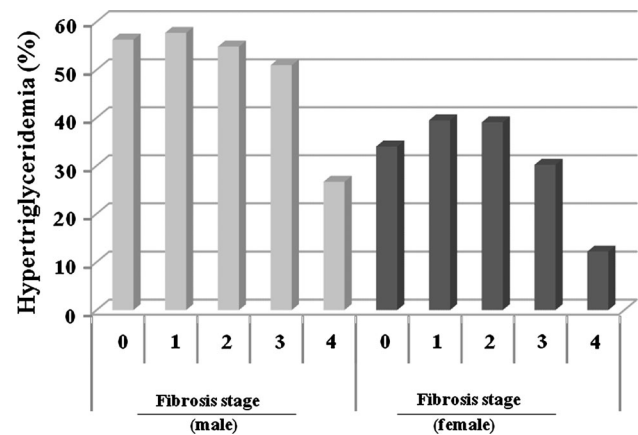


Fig. 1 Prevalence of hypertriglyceridemia for each stage of fibrosis. The horizontal axis shows the fibrosis stage and the longitudinal axis shows the percentage of patients with hypertriglyceridemia

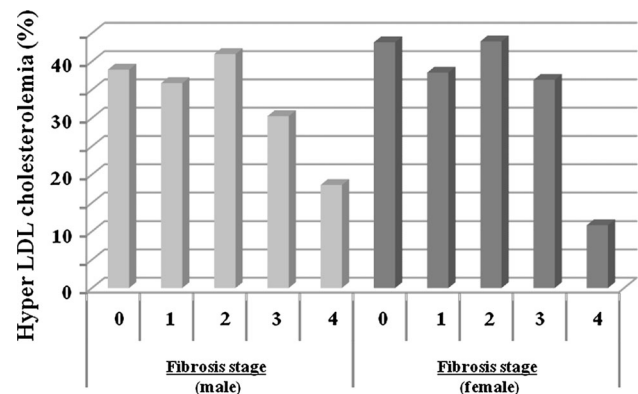


Fig. 2 Prevalence of hyper-LDL cholesterolemia for each stage of fibrosis. The horizontal axis shows the fibrosis stage and the longitudinal axis shows the percentage of patients with hyper-LDL cholesterolemia

41.3/30.4/18.2 (%) in males, and 43.4/38.0/43.6/36.8/11.1 (%) in females, respectively. The prevalence rates of dyslipidemia (hypertriglyceridemia and hyper-LDL cholesterolemia) decreased with progression of the fibrosis stage, especially in Stage 4.

The prevalence of hypertension for each fibrosis stage was shown in Fig. 3. The percentages of patients with hypertension for each fibrosis stage (Stage 0/1/2/3/4) were 17.9/34.0/40.3/51.4/42.9/35.3 (%) in males, and 35.3/50.0/47.7/50.0/23.9 (%) in females respectively.

The prevalences of impaired glucose tolerance, including DM, for each fibrosis stage are shown in Fig. 4. The percentages of patients with DM for each fibrosis stage (Stage 0/1/2/3/4) were 23.7/32.8/53.7/65.8 (%) in males, and 34.7/45.2/60.9/64.7 (%) in females, respectively. The percentages of patients with impaired glucose tolerance (IGT) in each fibrosis stage (Stage 0/1/2/3/4) were 6.6/18.5/17.6/16.2 (%) in males, and 15.3/10.6/14.1/14.1 (%) in females, respectively. The percentages of patients with

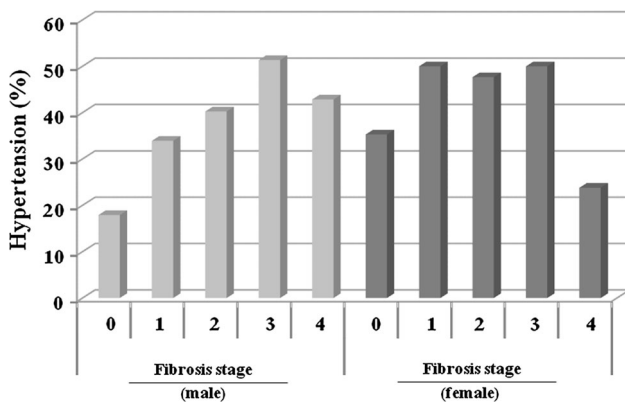


Fig. 3 Prevalence of hypertension for each stage of fibrosis. The horizontal axis shows the fibrosis stage and the longitudinal axis shows the percentage of patients with hypertension

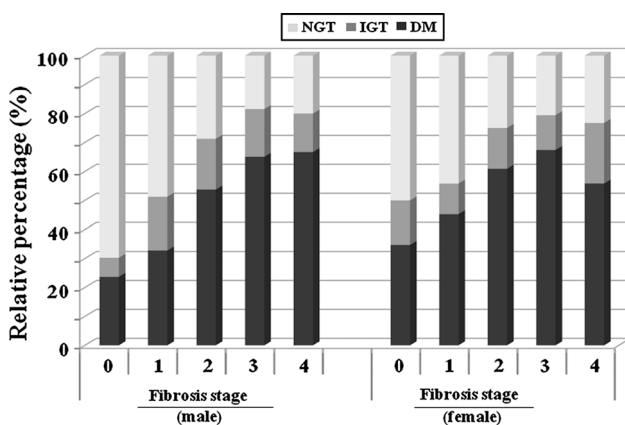


Fig. 4 The glucose tolerance pattern for each fibrosis stage in male and female NAFLD patients. The horizontal axis shows the fibrosis stage and the longitudinal axis shows the normal glucose tolerance, impaired glucose tolerance, or DM

normal glucose tolerance (NGT) were 69.7/48.7/28.7/17.9 (%) in males, and 50.0/44.2/25.0/21.2 (%) in females, respectively. The percentage of patients with DM increased with progression of the fibrosis stage in both male and female NAFLD patients.

Factors associated with advanced fibrosis

Factors associated with advanced fibrosis were examined (Table 2). NAFLD patients with advanced fibrosis were older, more likely to be female, and obese. The BMI, visceral fat area, and liver/spleen (L/S) ratio were significantly higher in NAFLD patients with advanced fibrosis. Furthermore, significant increases of the serum level of AST, AST/ALT ratio, ALP, GGT, total and direct bilirubin, fasting glucose, HbA1c, IRI, HOMA-IR, ferritin, FFA, and hyaluronic acid, and decreases of hemoglobin, platelet count, ChE, albumin, total cholesterol, triglycerides, LDL

cholesterol, and uric acid were observed in the patients with advanced fibrosis. In cases with high fasting plasma glucose levels, HOMA-IR does not reflect insulin resistance exactly, and was assumed to be a reference level. To investigate the factors that might be related to the progression to advanced fibrosis, univariate analysis was performed between NAFLD patients with advanced fibrosis and those with no or mild fibrosis, as shown in Table 3. The results of the analysis revealed obesity (BMI ≥ 25), hypertension, hypotriglyceridemia, hyper-LDL cholesterol, DM, and hyperuricemia as risk factors for advanced fibrosis. Multivariate analysis identified older age, low serum triglyceride and DM as risk factors for advanced fibrosis.

Discussion

Many factors have been reported to be implicated in the pathogenesis of NAFLD, including obesity, DM, dyslipidemia and hypertension. However, it is still unclear how the metabolic factors might affect the pathogenesis and progression of NAFLD [11, 20, 31–34]. Therefore, identifying the risk factors for the deterioration of NAFLD would be useful for designing therapeutic strategies not only for the liver itself, but also for these metabolic diseases. Whereas a large number of papers have reported the differences in the clinical features between NAFL and NASH, comparisons of the clinical features by the histological severity are scarce. In this study, we retrospectively investigated the associations between metabolic factors and the histologic severity of NAFLD in a large cohort of 1,365 biopsy-proven NAFLD patients, considered as one of the largest-scale studies in the world to date.

The first important finding of our study was that the severity of fibrosis advanced gradually with age in the male patients with NAFLD, while it increased only in those women over 60 years of age. This gender difference may be attributable to menopause in females [35, 36].

The second important finding of our study was the association between obesity and fibrosis severity in NAFLD patients. We compared the prevalence of obesity and the histological severity of NAFLD. As shown in Supplementary Fig. 2, whereas the prevalence of obesity increased with the progression of fibrosis in males, the prevalence remained at approximately 70 % in all age groups of females.

It has been reported that 42–72 % of patients with NAFLD, including NASH, have dyslipidemia [37, 38]. Consistent with these reports, dyslipidemia was present in 65.7 % of patients in our study, including hypertriglyceridemia in 45.3 %, increased serum low-density lipoprotein cholesterol in 37.5 %, and decreased serum high-density

Table 2 Comparison for the demographic and clinical characteristics between patients with mild (Stage 0–2) and advanced (Stage 3, 4) fibrosis with NAFLD

Variable	All cases (<i>n</i> = 1,365)	Stage 0–2 (<i>n</i> = 1,062)	Stage 3, 4 (<i>n</i> = 303)	<i>P</i> value	<i>P</i> value (after adjustment for age/sex)
Age	51 ± 14.9	49 ± 15.0	57 ± 12.8	<0.0001	
Gender (male/female)	709/656	591/471	118/185	<0.0001	
Clinical and anthropometric measure					
Body mass index (kg/m ²)	27.9 ± 4.8	27.7 ± 4.8	28.6 ± 4.7	0.0006	<0.0001
BMI ≥ 25 (%)	73.0	71.2	79.5	0.0054	<0.0001
Waist circumference (cm ²)	96.7 ± 13.5	96.1 ± 12.4	98.1 ± 15.4	0.2372	0.0239
Subcutaneous fat area (cm ²)	220.7 ± 103.9	221.1 ± 110.7	219.7 ± 87.1	0.4631	0.4865
Visceral fat area (cm ²)	151.9 ± 65.9	144.4 ± 56.8	168.5 ± 80.3	0.0025	0.0007
L/S ratio	0.75 ± 0.30	0.73 ± 0.29	0.81 ± 0.32	0.0013	0.3528
Blood pressure sys. (mmHg)	127 ± 16.9	127 ± 15.7	124 ± 21.3	0.5343	0.0867
Blood pressure dia. (mmHg)	77 ± 11.1	77 ± 11.0	76 ± 11.5	0.6701	0.6802
Laboratory studies					
White blood cells (/μl)	6,330 ± 1,616.9	6,348 ± 1,583.4	6,272 ± 1,717.7	0.7037	0.6377
Hemoglobin (g/dl)	14.5 ± 1.6	14.6 ± 1.6	14.2 ± 1.6	<0.0001	0.6617
Platelet count (× 10 ⁴ /μl)	22.4 ± 10.0	23.7 ± 10.4	18.0 ± 6.7	<0.0001	<0.0001
AST (IU/l)	57 ± 38.9	52 ± 36.0	72 ± 44.6	<0.0001	<0.0001
ALT (IU/l)	88 ± 60.3	87 ± 60.2	92 ± 60.4	0.1319	0.0003
AST/ALT	0.72 ± 0.3	0.67 ± 0.3	0.89 ± 0.4	<0.0001	<0.0001
LDH (IU/l)	210 ± 55.1	209 ± 56.7	213 ± 49.9	0.1446	0.5835
ALP (IU/l)	258 ± 111.0	250 ± 103.5	284 ± 130.1	<0.0001	0.0003
GGT (IU/l)	91 ± 103.4	88 ± 103.2	101 ± 103.4	<0.0001	0.0023
ChE (IU/l)	374 ± 106.5	383 ± 104.7	345 ± 107.3	<0.0001	0.0004
Bilirubin, total (mg/dl)	0.89 ± 0.39	0.86 ± 0.36	0.97 ± 0.45	0.0024	0.7731
Bilirubin, direct (mg/dl)	0.21 ± 0.16	0.19 ± 0.13	0.26 ± 0.22	<0.0001	0.2974
Albumin (g/dl)	4.46 ± 0.43	4.50 ± 0.39	4.29 ± 0.50	<0.0001	<0.0001
Total cholesterol (mg/dl)	209 ± 41.9	212 ± 41.5	200 ± 41.6	<0.0001	<0.0001
Triglyceride (mg/dl)	164 ± 102.6	170 ± 107.7	145 ± 79.7	<0.0001	0.0226
HDL cholesterol (mg/dl)	51 ± 15.7	51 ± 16.2	51 ± 13.9	0.3545	0.0297
LDL cholesterol (mg/dl)	130 ± 37.9	133 ± 37.2	123 ± 38.8	<0.0001	0.0009
Fasting plasma glucose (mg/dl)	114 ± 37.8	111 ± 36.1	123 ± 41.4	<0.0001	0.0001
HbA1c (NGSP) (%)	6.32 ± 1.2	6.26 ± 1.2	6.67 ± 1.4	<0.0001	0.0003
IRI (μIU/ml)	15.2 ± 18.5	13.7 ± 11.7	20.1 ± 31.4	<0.0001	<0.0001
HOMA-IR	4.89 ± 10.0	3.98 ± 5.6	6.84 ± 15.5	<0.0001	0.0012
Ferritin (ng/ml)	260.2 ± 475.8	255.8 ± 522.4	275.4 ± 254.5	0.5642	0.5421
Uric acid (mg/dl)	5.9 ± 1.5	6.0 ± 1.5	5.7 ± 1.3	0.0297	0.8563
Free fatty acid (μEq/l)	0.41 ± 0.3	0.36 ± 0.3	0.56 ± 0.3	<0.0001	<0.0001
Hyaluronic acid (ng/ml)	64.3 ± 168.9	42.2 ± 66.9	145.8 ± 329.9	<0.0001	<0.0001

Comparison between patients with mild (Stage 0–2) and advanced (Stage 3, 4) fibrosis using the Chi-square test for binary variables and logistic regression of group indicator on continuous variables

lipoprotein cholesterol in 19.5 % of patients. However, as the third important finding of our study, dyslipidemia tended to decrease in prevalence as the fibrosis stage progressed. Multivariate analysis revealed a negative correlation between the serum triglyceride levels and the fibrosis stage (OR = 0.5687, 95 % CI 0.394–0.821). This result

may reflect a deterioration of lipid metabolism with the progression of liver fibrosis towards liver cirrhosis.

The fourth finding of our study was the recognition of a relationship between hypertension and the fibrosis severity in NAFLD patients. In our NAFLD population, hypertension was present in 30.2 %. Whereas no obvious trends in

Table 3 Multiple regression analysis to identify predictive factors for the advanced fibrosis

Variable	All cases	Stage 0–2	Stage 3, 4	Univariate odds ratio (95 % CI)	P value	Multivariate odds ratio (95 % CI)	P value
Age (mean)	51.0	49.2	57.5	1.042 (1.032–1.053)	<0.0001	1.036 (1.021–1.051)	<0.0001
Female (%)	48.1	44.4	61.1	1.967 (1.516–2.553)	<0.0001	1.180 (0.787–1.768)	0.423
BMI \geq 25 (%)	73.0	71.2	79.5	1.566 (1.149–2.133)	0.0045	1.568 (0.991–2.481)	0.0545
Hypertension (%)	39.9	38.0	47.3	1.468 (1.063–2.027)	0.0198	0.943 (0.641–1.387)	0.7640
Hypertriglyceridemia (%)	45.3	82.7	34.7	0.566 (0.432–0.739)	<0.0001	0.663 (0.453–0.970)	0.0343
Hyper-LDL cholesterolemia (%)	37.5	39.6	30.7	0.676 (0.496–0.920)	0.0129	0.885 (0.596–1.313)	0.5444
Hypo-HDL cholesterolemia (%)	19.5	19.7	18.9	0.836 (0.671–1.343)	0.7680	–	–
DM (%)	47.3	42.1	64.9	2.544 (1.948–3.320)	<0.0001	2.387 (1.603–3.553)	<0.0001
Hyperuricemia (%)	30.2	32.1	24.4	0.684 (0.485–0.965)	0.0308	1.058 (0.693–1.617)	0.793

the prevalence of hypertension were observed in females, comparison of the relationship between the prevalence of hypertension and the stage of fibrosis, except for Stage 4, revealed a tendency towards increase in the prevalence of hypertension with progression of the fibrosis stage. In general, blood pressure is considered to have an effect on the rate of progression of NAFLD. Systolic and diastolic blood pressures have been reported to be correlated with the liver fat content, and patients with systolic hypertension were reported to be correlated with the liver fat contents, and patients with systolic hypertension were reported to show a two-fold higher risk of development of NAFLD [39]. As shown in Fig. 3, the decrease in the rate of hypertension in NAFLD patients with Stage 4 liver fibrosis might be, at least in part, attributable to the hyperdynamic circulation, characterized by peripheral vasodilation and increased portal resistance, observed in patients with liver cirrhosis [40, 41].

Impaired glucose tolerance is well known to accompany NAFLD. While it appears clear that abnormal glucose tolerance, including DM, is a risk factor for NAFLD and vice versa, the relationship between abnormal glucose tolerance and the histological severity of NAFLD is still unknown. The fifth finding in our study was that the prevalence of DM increased with progression of the fibrosis stage (Fig. 4). Multivariate analysis identified DM as an independent risk factor for advanced fibrosis (OR = 2.8573, 95 % CI 1.941–4.207). In vitro, high glucose and high insulin concentrations, which are often observed in patients with NAFLD, were shown to stimulate connective tissue growth factor expression, which is known as one of the important mechanisms involved in the progression of hepatic fibrosis [42]. Furthermore, the cirrhotic condition is suspected to facilitate the development of hyperinsulinemia and hyperglycemia via the deteriorated liver function [43, 44]. Taken together, it would be reasonable to consider DM as both a cause and result of NAFLD [45].

In conclusion, we have reported the prevalences of lifestyle-related diseases, such as obesity, dyslipidemia, hypertension, and DM, in NAFLD patients according to the stage of fibrosis. Multivariate analysis identified DM as a significant risk factor for advanced fibrosis. Accordingly, impaired glucose tolerance, including DM, should be properly evaluated and managed for preventing the progression of NAFLD, even in the early stages of NASH.

Conflict of interest The authors declare that they have no conflict of interest.

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