表 3　飲酒カテゴリ別身体・血液検査・栄養素摂取特性（女性 n=571）

	生涯非飲酒者	過去飲酒者	機会飲酒者	現在飲酒者（≦23g エタノール/日）	現在飲酒者（>23g エタノール/日）	P 値
人数（人）	68	22	125	334	22	
年齢（歳）	49.0	50.6	49.9	49.0	47.2	0.112
BMI (kg/m²)	22.9	22.9	23.3	23.2	22.9	0.857
収縮期血圧（mmHg）	112.3	110.5	115.2	113.9	120.0	0.126
拡張期血圧（mmHg）	68.7	67.0	70.9	70.5	76.5	0.007
中性脂肪（mg/dL）	116.0	106.5	113.5	109.8	95.4	0.672
総ｺﾚｽﾃﾛｰﾙ（mg/dL）	207.1	192.0	202.8	202.7	189.2	0.086
LDL ｺﾚｽﾃﾛｰﾙ(mg/dL)	132.8	115.1	126.1	123.1	103.3	0.076
HDL ｺﾚｽﾃﾛｰﾙ(mg/dL)	58.0	57.9	56.7	61.5	64.6	0.001
HbA1c (%)	4.67	4.56	4.59	4.59	4.43	0.005
γ-GTP (IU/L)	19.6	19.1	23.7	21.8	46.2	< 0.001
現在喫煙者（%）	7.4	9.1	5.6	7.8	40.9	< 0.001
活動時間(中・強度)(時間)	2.1	2.1	2.5	2.8	1.5	0.263
教育年数（年）	11.4	11.7	11.4	11.7	11.9	0.442
栄養摂取量						
アルコール（g/日）	0.0	0.0	0.0	4.3	36.4	< 0.001
エネルギー（kcal/日）	1732	1645	1782	1821	1907	0.016
栄養摂取量，摂取密度（アルコール由来分エネルギー除く）						
エネルギー（kcal/日）	1731	1645	1780	1789	1677	0.126
タンパク質（%kcal）	16.2	16.1	16.1	16.3	18.4	0.001
総脂質（%kcal）	27.2	24.8	25.8	27.1	29.8	0.001
炭水化物（%kcal）	56.6	59.1	58.0	56.5	51.9	< 0.001
ナトリウム(mg/1,000kcal)	2339	2430	2355	2373	2852	< 0.001
食物繊維(g/1,000kcal)	8.8	9.8	9.6	8.8	7.4	< 0.001

P 値は，連続変数は ANOVA、割合（%）は chi-square 検定による。

＜研究③＞

表４　メタボリックシンドロームの有無別身体特性、平均値±１標準偏差

		メタボリック シンドロームあり	メタボリック シンドロームなし	P*
人数	名	248	820	
年齢	歳	65.5 ±9.0	63.6 ±10.2	0.005
一日アルコール量	g/日	25.7 ±27.5	22.6 ±27.2	0.113
腹囲	cm	92.4 ±5.7	83.3 ±7.4	＜0.001
BMI	kg/m^2	25.9 ±2.5	22.9 ±2.8	＜0.001
収縮期血圧	mmHg	145.4 ±16.9	133.5 ±18.7	＜0.001
拡張期血圧	mmHg	84.1 ±10.6	78.3 ±10.7	＜0.001
中性脂肪	mg/dl	178.0 ±94.0	111.0 ±69.0	＜0.001
HDL コレステロール	mg/dl	51.0 ±13.0	61.0 ±17.0	＜0.001
空腹時血糖	mg/dl	115.0 ±27.0	98.0 ±16.0	＜0.001

* t 検定（連続変量）、有意確率：＜0.05

図１　一日アルコール摂取量別６群毎のメタボリックシンドローム有病率

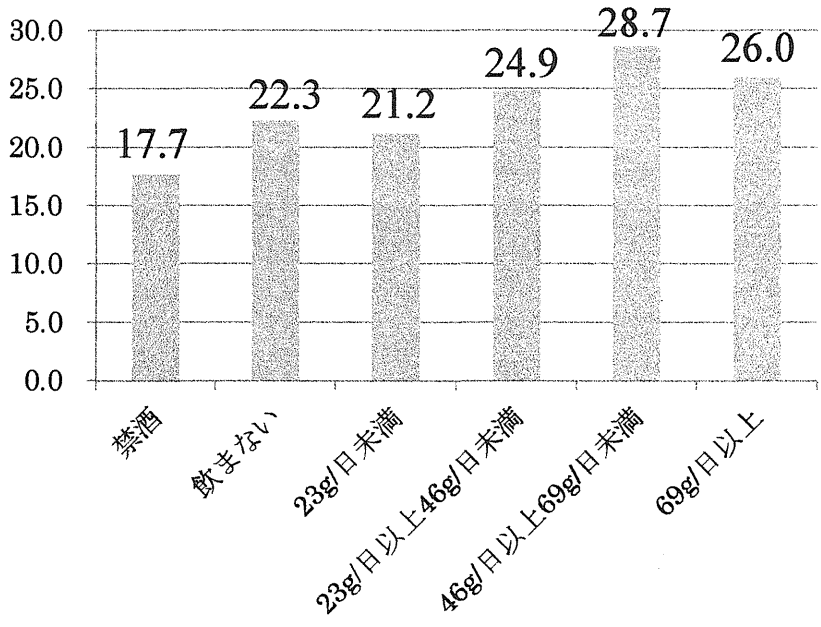
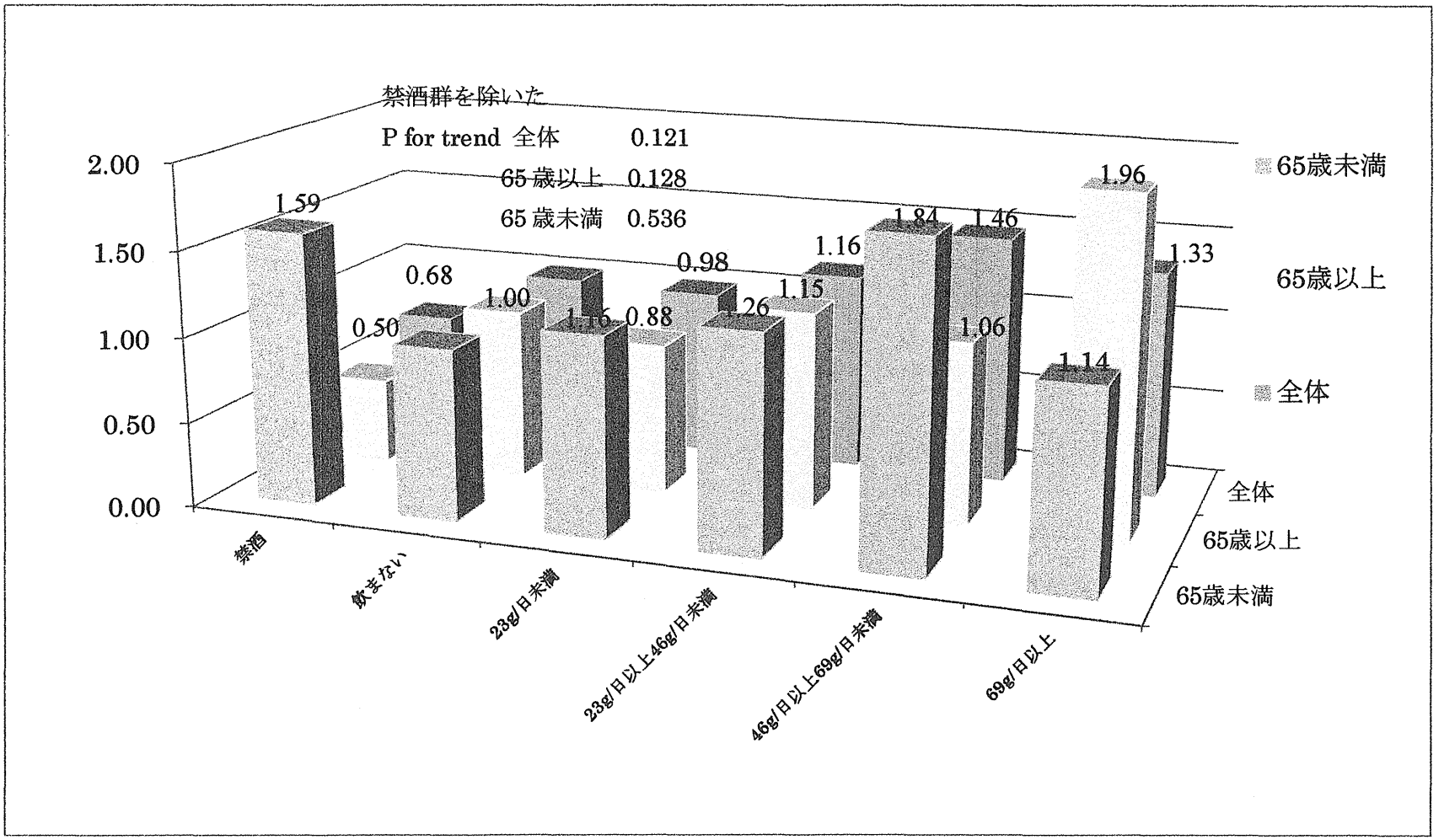


図２　アルコール摂取量によるメタボリックシンドローム有病リスクの調整オッズ比



年齢、喫煙習慣（喫煙の有無、禁煙の有無）を調整

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厚生労働科学研究費補助金（循環器疾患・糖尿病等生活習慣病対策総合研究事業）
我が国における飲酒の実態把握およびアルコールに関連する生活習慣病と
その対策に関する総合的研究
（研究代表者　樋口　進）

平成24年度分担研究報告書
人間ドック受診者における飲酒習慣と生活習慣病との関連の研究

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研究要旨：今までに、飲酒と脂肪肝や脂質異常との関連を解析し、適度な飲酒は脂肪肝抑制や脂質代謝異常の改善に役立つ可能性を報告している。今回の研究では、健診受診者を対象に、脂肪肝と糖尿病との関連について検討を行った。2011年に人間ドックを受診した11153名（男性6882例/女性4271例）を対象とした横断研究では、脂肪肝は糖尿病と独立した危険因子であった。また、2006年度に糖尿病のなかった受診者（5346例）を対象とすると、脂肪肝は2011年度の糖尿病発症に寄与する独立した危険因子であった（オッズ比は男性が1.73、女性が4.13）。さらに、飲酒は糖尿病発症に抑制的に作用する傾向であった。以上のことから、適度な飲酒は直接もしくは脂肪肝抑制を介して糖尿病発症を抑制する可能性がある。

研究協力者
今村也寸志：鹿児島県厚生連病院内科

A．研究目的

今までに、飲酒と脂肪肝や脂質異常との関連を解析し、適度な飲酒は脂肪肝抑制や脂質代謝異常の改善に役立つ可能性を報告している。今回の研究では、健診受診者を対象に、脂肪肝と糖尿病との関連について検討した。

B．研究方法

2011年度に人間ドックを受診した男性6882例、女性4271例を対象とした。そのうち、HBs抗原とHCV抗体がいずれも陰性、糖尿病、脂質異常、高血圧の加療歴のない男性3705例、女性2549例をサブ解析した。糖尿病の診断は空腹時血糖126 mg/dl以上、もしくはHbA1c（JDS）6.1%以上と定義した。また、これらの対象者のうち、2006年に糖尿病が無かった男性3352例、女性1994例を対象として縦断研究を行った。

（倫理面への配慮）

臨床情報は匿名化してあるものを用い、個人情報保護に努めた。解析結果や臨床情報等は厳重に保管し、解析はネットワークから遮断されたコンピュータを用いた。

C．研究結果

11153名を対象とした横断研究では、脂肪肝は糖尿病の独立した危険因子（表1、オッズ比は男性が1.97、女性が3.12）であった。また、ウイルスマーカー陰性、生活習慣病の治療歴のない対象者のみの6254例の検討でも同様の結果であった。さらに、縦断研究でも脂肪肝は糖尿病発症の危険因子であった（オッズ比[95% CI]は男性が1.95 [1.24-3.09]、女性が2.30[0.98-5.38]）。一方、男性では飲酒は飲酒量に関わらず糖尿病発症に抑制的に作用し、女性では少量の飲酒が糖尿病発症に抑制的に作用する傾向であった（表1）。

D．考察

本年度は横断研究および縦断研究を用いて、糖尿病発症に寄与する因子を解析し、脂肪肝は糖尿病発症に関連する独立した危険因子であ

ることを示した。一方、糖尿病発症に飲酒は抑制的に作用する傾向であった。

糖尿病は脂肪肝の危険因子であり、今回示した横断研究の結果からは糖尿病と脂肪肝のどちらが誘因となっているかは判断できない。しかし、本研究では2006年に脂肪肝が存在すると、2011年に糖尿病になりやすいことを示した。このことから、糖尿病は脂肪肝の危険因子であるとともに、脂肪肝は糖尿病の発症に寄与する因子であり、脂肪肝の改善が糖尿病発症予防につながると考えられた。

一方、飲酒は糖尿病や脂肪肝の発症に抑制的に作用する可能性が報告されていることから、飲酒者は糖尿病になりにくい可能性がある。我々の検討でも、男性では飲酒者、女性では少量飲酒者（エタノール換算で20g/日以下）は糖尿病になるリスク（オッズ比）が低い傾向であった。多量飲酒者は脂肪肝や慢性膵炎を発症し、糖尿病発症を促進する可能性はあるが、適度な飲酒は糖尿病発症抑制と関連する可能性があり、多数例でのさらなる検討が必要である。

E．研究発表

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F．知的財産権の出願・登録状況

1．特許取得

なし。

2．実用新案登録

なし。

3．その他

なし。

表 1

2011年度人間ドック受診者の解析による糖尿病(FBS ≥ 126 mg/dl, A1c ≥ 6.1 %)のリスク評価

Variables	Men (n=6882)	Men* (n=3705)	Women (n=4271)	Women**(2549)
Age				
30-39	1 (reference)	1 (reference)	1 (reference)	1 (reference)
40-49	2.38 [1.49-3.94]	2.15 [1.13-4.55]	0.52 [0.21-1.33]	0.37 [0.12-1.11]
50-59	5.03 [3.23-8.27]	5.17 [2.81-10.7]	1.38 [0.68-3.20]	1.06 [0.47-2.71]
60-69	7.64 [4.85-12.70]	7.83 [4.17-16.41]	2.32 [1.14-5.40]	1.35 [0.59-3.51]
70-79	7.35 [4.53-12.54]	7.86 [3.86-17.42]	3.02 [1.43-7.16]	1.88 [0.72-5.29]
BMI				
Underweight	1.17 [0.67-1.92]	1.38 [0.67-2.57]	0.70 [0.29-1.44]	1.25 [0.46-2.87]
Normal weight	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Overweight	1.59 [0.95-1.41]	1.21 [0.89-1.65]	1.58 [1.11-2.25]	1.77 [1.00-3.10]
Obesity	1.70 [1.40-2.07]	1.66 [1.21-2.28]	1.52 [1.05-2.19]	1.20 [0.63-2.26]
Hypertension	1.39 [1.19-1.62]	0.94 [0.72-1.21]	2.19 [1.65-2.91]	2.24 [1.39-3.55]
Dyslipidemia	1.35 [1.16-1.58]	1.14 [0.89-1.45]	1.44 [1.09-1.91]	0.89 [0.56-1.41]
Fatty liver	1.97 [1.66-2.32]	2.33 [1.78-3.05]	3.12 [2.29-4.26]	4.25 [2.51-7.20]
Smoking status				
Never smoking	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Former smoking	1.22 [1.02-1.46]	1.30 [0.96-1.76]	1.46 [0.77-2.61]	1.25 [0.42-3.02]
Current smoking	1.42 [1.16-1.74]	1.63 [1.19-2.26]	0.65 [0.19-1.64]	0.38 [0.02-1.88]
Alcohol consumption				
Non	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Less than 20 g/day	0.82 [0.67-1.02]	0.78 [0.57-1.09]	0.75 [0.55-1.01]	0.62 [0.37-1.01]
20 g/day or more	0.76 [0.60-0.96]	0.79 [0.56-1.13]	1.24 [0.41-3.01]	1.10 [0.17-4.06]

Data are expressed as odd's ratios [95% confidence intervals]. Logistic regression analysis was carried out using variables in this table.
*/**, subjects are limitted to those who were HBs-Ag (-), HCV-Ab (-), and without medication for hypertension and/ or dyslipidemia.

研究成果の刊行に関する一覧

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Hosoyamada K, Uto H, Imamura Y, Hiramine Y, Toyokura E, Hidaka Y, Kuwahara T, Kusano K, Saito K, Oketani M, Ido A, Tsubouchi H.	Fatty liver in men is associated with high serum levels of small, dense low-density lipoprotein cholesterol.	Diabetol Metab Syndr.	34	4	2012

J Gastroenterol
DOI 10.1007/s00535-012-0653-5

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinicopathological features of liver injury in patients with type 2 diabetes mellitus and comparative study of histologically proven nonalcoholic fatty liver diseases with or without type 2 diabetes mellitus

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Abstract
Background The Japan Society of Diabetes Mellitus reported that the leading cause of death in patients with diabetes mellitus (DM) was chronic liver disease; however, there are limited studies investigating the cause of liver injury in these patients. Our study aimed to clarify the clinicopathological features of liver injury and the characteristics of nonalcoholic fatty liver disease (NAFLD) in DM patients.
Methods In total, 5,642 DM patients and 365 histologically proven NAFLD patients were enrolled. Clinical and laboratory parameters and liver biopsy results were, respectively, recorded and analyzed for the two sets of patients.
Results Positivity rates for Hepatitis B surface antigens (HBsAg) and anti-hepatitis C virus antibodies (anti-HCV Ab) were 1.7 and 5.1 %, respectively. The proportion of drinkers consuming 20–59 g and ≥60 g alcohol daily was 14.9 and 4.3 %, respectively. The percentage of DM patients with elevated serum alanine aminotransferase (ALT) levels (≥31 IU/L) was 28.6 %. Alcohol consumption had no significant effect on serum ALT levels. Seventy-two percent of HBsAg-positive patients were serum hepatitis B virus (HBV)-DNA negative, whereas 10 % exhibited high levels of the same (>4.0 log copies/ml).

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Thirty-eight percent of anti-HCV Ab-positive patients were serum HCV-RNA negative. Among the NAFLD patients, the frequencies of NASH and advanced stage NASH were significantly higher in male DM patients than in male patients without DM.

Conclusions Although HBsAg- and anti-HCV Ab-positivity rates were high in our Japanese DM patients, a majority of liver injuries could be associated with NAFLD/nonalcoholic steatohepatitis.

Keywords Nonalcoholic fatty liver disease · Nonalcoholic steatohepatitis · Diabetes mellitus · Hepatitis virus carrier · Alcoholic liver disease · Nationwide study

Abbreviations

HCC	Hepatocellular carcinoma
NAFLD	Nonalcoholic fatty liver disease
DM	Diabetes mellitus
NASH	Nonalcoholic steatohepatitis
HBV	Hepatitis B virus
HCV	Hepatitis C virus
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
GGT	Gamma glutamyl transpeptidase
FPG	Fasting plasma glucose
HOMA-IR	The homeostasis model assessment of insulin resistance index
HBsAg	Hepatitis B surface antigen
anti-HBc Ab	Anti-hepatitis B core antibody
anti-HCV Ab	Anti-hepatitis C virus antibody
HBV-DNA	Hepatitis B virus-deoxyribonucleic acid
HCV-RNA	Hepatitis C virus-ribonucleic acid
OR	Odds ratio
CI	Confidence interval

Introduction

As per the International Diabetes Federation, the number of diabetes mellitus (DM) sufferers rose to 366 million in 2011, representing 8.3 % of the global adult population, which is increasing in every country [1]. Worldwide, hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer mortality [2]. HCC largely occurs in patients with chronic liver disease. Persistent hepatitis C virus (HCV) or hepatitis B virus (HBV) infections are the main causes of HCC; however, non-HCV- and non-HBV-associated HCC cases are increasing in Japan [3].

In 2007, the Japan Society of DM reported that the most frequent cause of death among 18,385 DM patients who died in hospitals during 1991–2000 was malignancy (34.1 %), followed by ischemic heart disease (10.2 %) and cerebrovascular disease (9.8 %) [4]. Among the malignancies, HCC showed the highest frequency (8.6 %), followed by lung (5.3 %), pancreatic (4.8 %), and gastric cancer (3.5 %). Furthermore, the frequency of deaths caused by liver cirrhosis was 4.7 %, and in total, 13.3 % DM patients died of liver diseases. The cancer death rate in that study was quite different from that reported in the general Japanese population, in which lung (5.7 %), gastric (4.7 %), and colon (2.5 %) cancer occur with high frequencies [5]. Moreover, the death rate from liver diseases (13.3 %) was three times higher than that in the general Japanese population (HCC 3.2 %, liver cirrhosis 1.5 %, total 4.7 %) [6]. However, the incidences of HBV and HCV infection and the details of alcohol intake were not analyzed in that report.

The Japan Nonalcoholic Steatohepatitis (NASH) Study Group was founded in 2007 to investigate the cause of death in DM patients, the genetic factors in nonalcoholic fatty liver disease (NAFLD) patients, and the background of NASH-HCC patients [7]. This study focused on clarifying the cause of liver injury in Japanese DM patients and investigating the histological distribution of NAFLD in patients with and without DM.

Patients and methods

Patients

In total, 5,642 DM patients (3,238 males, 2,404 females) who visited nine DM clinics belonging to the Japan NASH Study Group (Saiseikai Suita Hospital; Kagoshima University Graduate School of Medical and Dental Sciences; Graduate School of Medicine, The University of Tokyo; Kanazawa University Graduate School of Medical Science; Department of Medicine, Asahikawa Medical College; Yamagata University Faculty of Medicine; Kyoto Prefectural University of Medicine; Okayama Saiseikai General Hospital; Fukui-ken Saiseikai Hospital) between January 2008 and December 2009 were enrolled in this observational study.

Three hundred and sixty-five NAFLD patients (182 males, 183 females) who visited Saiseikai Suita Hospital were enrolled in the histopathological study.

The study protocol was approved by the Human Ethics Committee of each participating hospital. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Clinical and laboratory assessment

Demographic parameters, including age, sex, height, weight, and body mass index (BMI), and comorbidities, including alcohol consumption, hypertension, and dyslipidemia, were

recorded for all subjects in addition to the treatment administered for DM and the frequency of HCC occurrence. Clinical laboratory tests were conducted to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), albumin, total cholesterol, triglyceride (TG), ferritin, uric acid, hemoglobin A1c, fasting plasma glucose (FPG), and insulin levels. The homeostasis model assessment of insulin resistance (HOMA-IR) index; platelet (PLT) count; and hyaluronic acid, type IV collagen 7S, hepatitis B surface antigen (HBsAg), anti-hepatitis B core antibody (anti-HBc Ab), anti-HCV antibody (anti-HCV Ab), HBV-DNA, and HCV-RNA levels were also measured.

Blood samples were procured in the morning after overnight fasting. HOMA-IR was only calculated for subjects with FPG <140 mg/dL. HBV-DNA levels were measured by PCR (Amplicor HBV-DNA kit, Roche Diagnostics) or real-time PCR (TaqMan HBV-DNA kit, Roche Diagnostics) for HBsAg-positive, whereas HCV-RNA levels were measured by PCR (Amplicor HCV-RNA kit, version 2.0, Roche Diagnostics) or real-time PCR (TaqMan HCV-RNA kit, Roche Diagnostics) for anti-HCV Ab-positive patients.

Histopathological examination

In total, 365 patients (177 non-DM and 188 DM) at Saiseikai Suita Hospital fulfilled the criteria for NAFLD, namely serum HBsAg and anti-HCV Ab negativity, no alcohol consumption, and the absence of autoimmune liver diseases or hereditary liver injury. These patients underwent an ultrasound-guided liver biopsy using a 16G needle.

Specimens were fixed in formalin, embedded in paraffin, and subjected to hematoxylin–eosin, Masson trichrome, and Perl's iron staining. Histological features of samples were interpreted according to a method described by Matteoni et al [8]. NASH stage was classified according to Brunt's classification [9].

Statistical analysis

All statistical analyses were performed using SPSS for Windows (SPSS Japan Inc.). Data were summarized by frequency for categorical variables and mean ± standard deviation (SD) for continuous variables. The Chi-square test was used to determine the differences between categorical variables. Student's t test was used to compare means of continuous valuables with equal variance, and the Mann–Whitney U test was used for non-normally distributed variables. The Cochran–Armitage test was used to study the trend of continuous variables. Forward stepwise logistic regression analysis was used to identify independent variables related to elevated serum ALT (≥31 IU/L) levels. A p value of <0.05, obtained by a two-tailed test, was considered statistically significant.

Since there is no official report on the HBV and HCV carrier rate in the general Japanese population, we utilized blood donor data for comparison with our patients [10].

Results

Baseline characteristics

The mean age and BMI of male and female DM patients was 62.2 and 64.8 years and 24.5 and 24.7 kg/m^2, respectively (Table 1). Hypertension and dyslipidemia occurred in 51.0 and 63.3 % of DM patients, respectively. Respective DM treatment types in DM patients were as follows: no medication, 20.5 %; oral drugs, 47.7 %; insulin, 28.9 %; and oral drugs and insulin, 2.8 % (Table 2).

Mean ALT level was significantly higher in males (30.6 IU/L) than in females (Table 1). Abnormal serum ALT levels (≥31 IU/L) were found in 28.6 % of DM patients (males 32.8 %, females 23.0 %). When the healthy upper limit of abnormal serum ALT level in females was defined as 20 IU/L according to Prati et al.'s [11] criteria, the frequency of abnormal ALT (≥21 IU/L) levels in females was 43 %. The mean PLT count was 20.8 × 10^4/μL in males and 21.9 × 10^4/μL in females. Mean values of other clinical laboratory tests are shown in Table 1.

Prevalence of HBV and HCV infection and drinking and their effects on liver function tests

HBsAg positivity was detected in 1.7 % of DM patients (M 1.8 %, F 1.6 %) (Table 2); this was significantly higher than that (total 0.9 %, M 1.0 %, F 0.7 %) detected in 1.7 million blood donors aged >40 years ($p < 0.001$). For both sexes, the HBsAg detection rate was significantly higher in DM patients than in blood donors in the 50- to 59- and 60- to 69-year age groups ($p < 0.05$) (Fig. 1). There were no significant differences in serum AST, ALT, and GGT levels between HBsAg-positive and HBsAg-negative DM patients of both sexes.

Seventy-two percent of HBsAg-positive patients (M 69 %, F 79 %) demonstrated HBV-DNA negativity (<2.6 log copies/ml) (Table 3). Of the HBsAg-positive patients, only 10 % showed high serum HBV-DNA levels (≥4.0 log copies/ml); these could be HBV infection-induced liver injury cases. Mean values of age, serum ALT level, and PLT counts in HBV-DNA-negative HBV carriers were 63.6 years, 25.3 IU/L, and 20.5 × 10^4/μL, respectively. HBV-DNA-negative HBV carriers were older and exhibited lower ALT levels and higher PLT counts; however, the differences were not significant.

Table 1 Backgrounds of diabetes mellitus patients (1)

Characteristic	Total subjects		Males		Females		
	n	M ± SD	*n*	M ± SD	*n*	M ± SD	*p*
Age (years)	5,510	63.3 ± 12.7	3,164	62.2 ± 12.5	2,346	64.8 ± 12.9	<0.001
BMI (kg/m^2)	5,173	24.6 ± 4.7	2,987	24.5 ± 4.2	2,186	24.7 ± 5.2	0.629
Aspartate aminotransferase (IU/L)	5,568	26.4 ± 17.2	3,188	27.1 ± 18.0	2,380	25.5 ± 15.9	<0.001
Alanine aminotransferase (IU/L)	5,569	28.2 ± 24.5	3,190	30.6 ± 26.9	2,379	24.9 ± 20.5	<0.001
GGT (IU/L)	5,476	48.3 ± 72.5	3,131	59.6 ± 86.7	2,345	33.1 ± 42.9	<0.001
Albumin (g/dL)	5,031	4.2 ± 0.4	2,869	4.2 ± 0.5	2,162	4.1 ± 0.4	<0.001
Platelet (×10^4/μL)	5,419	21.3 ± 6.1	3,112	20.8 ± 6.0	2,307	21.9 ± 6.1	<0.001
Fasting plasma glucose (FPG; mg/dL)	5,123	152.7 ± 61.7	2,945	156.0 ± 63.9	2,178	148.3 ± 58.2	<0.001
HbA1c (%)	5,479	7.2 ± 1.7	3,143	7.2 ± 1.7	2,336	7.2 ± 1.6	0.744
HOMA-IR (FPG <140)	1,005	2.55 ± 2.60	570	2.51 ± 2.59	435	2.61 ± 2.60	0.209
Total cholesterol (mg/dL)	5,260	195.1 ± 39.5	3,016	191.6 ± 40.0	2,244	199.6 ± 38.5	<0.001
Triglycerides (mg/dL)	5,443	136.3 ± 102.7	3,119	145.1 ± 111.9	2,324	124.5 ± 87.5	<0.001
Hyaluronic acid (ng/mL)	559	74.5 ± 98.6	319	59.3 ± 73.0	240	94.6 ± 122.1	<0.001
Type 4 collagen 7S (ng/mL)	474	4.9 ± 2.0	269	4.8 ± 2.0	205	4.9 ± 1.9	0.544
Ferritin (ng/mL)	1,838	142.0 ± 157.0	1,084	171.9 ± 174.9	754	99.1 ± 114.1	<0.001
Uric acid (mg/dL)	3,645	5.4 ± 1.5	2,043	5.7 ± 1.4	1,602	4.9 ± 1.4	<0.001

Results are shown as mean ± SD

GGT gamma glutamyl transpeptidase, *HOMA-IR* homeostasis model assessment of insulin resistance

Table 2 Backgrounds of diabetes mellitus patients (2)

Characteristic	Total subjects		Males		Females		
	n	Positive (%)	*n*	Positive (%)	*n*	Positive (%)	*p*
Hepatitis B surface antigen	4,867	83 (1.7 %)	2,796	50 (1.8 %)	2,071	33 (1.6 %)	0.604
Anti-hepatitis B core antibody	3,211	958 (29.8 %)	1,824	572 (31.4 %)	1,387	386 (27.8 %)	0.030
Anti-hepatitis C virus antibody	4,877	247 (5.1 %)	2,812	144 (5.1 %)	2,065	103 (5.0 %)	0.834
Alcohol	4,443		2,554		1,889		<0.001
<20 g/day		3589 (80.8 %)		1769 (69.3 %)		1820 (96.3 %)	
20–59 g/day		661 (14.9 %)		609 (23.8 %)		52 (2.8 %)	
≥60 g/day		193 (4.3 %)		176 (6.9 %)		17 (0.9 %)	
Hypertension	4,936	2515 (51.0 %)	2,841	1419 (49.9 %)	2,095	1096 (52.3 %)	0.100
Dyslipidemia	5,423	3434 (63.3 %)	3,091	1882 (60.9 %)	2,332	1552 (66.6 %)	<0.001
Diabetes mellitus (intervention)	5,227		3,013		2,214		0.002
None		1072 (20.5 %)		629 (20.9 %)		443 (20.0 %)	
Oral drugs		2495 (47.7 %)		1489 (49.4 %)		1006 (45.4 %)	
Insulin		1513 (28.9 %)		810 (26.9 %)		703 (31.8 %)	
Oral drugs + insulin		147 (2.8 %)		85 (2.8 %)		62 (2.8 %)	
Hepatocellular carcinoma	4,700	67 (1.4 %)	2,696	48 (1.8 %)	2,004	19 (0.9 %)	0.017

Anti-HCV Ab positivity was detected in 5.1 % (M 5.1 %, F 5.0 %) of DM patients; this rate was significantly higher than that (total 1.0 %, M 1.1 %, F 1.0 %) in blood donors of every age group of both sexes ($p < 0.001$), except for females aged 50–59 years (Table 2; Fig. 2). ALT and GGT levels were significantly higher in male anti-HCV Ab-positive patients than in their negative counterparts ($p < 0.001, p < 0.05$) (Fig. 2). For both sexes,

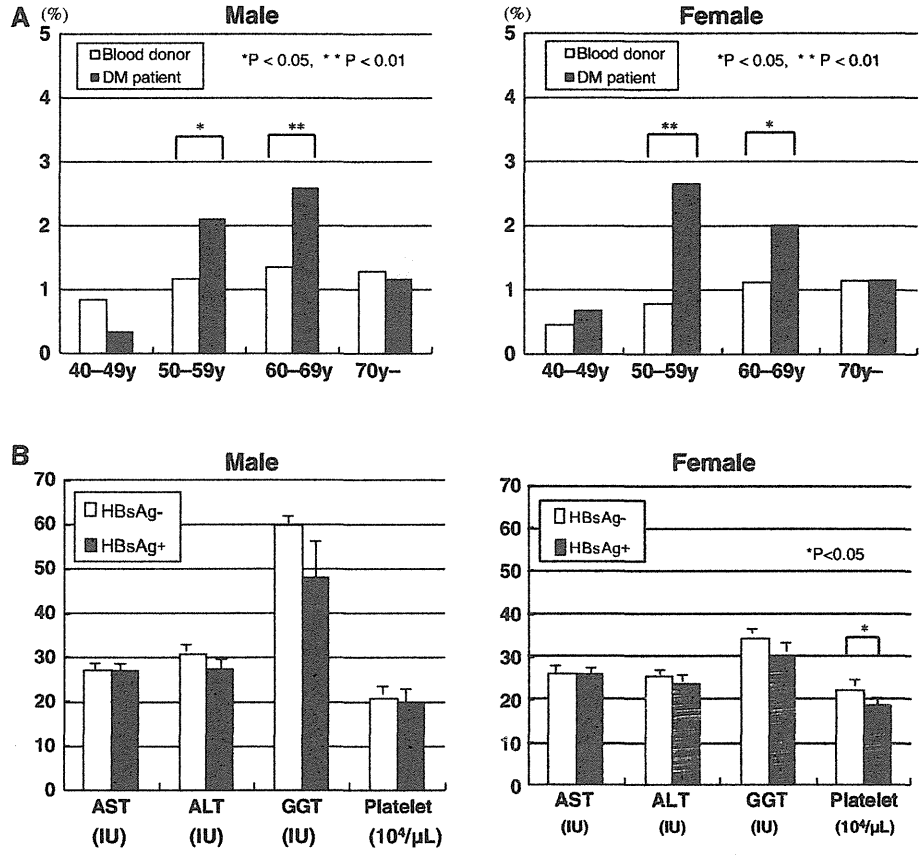

Fig. 1 Prevalence of HBV infection and the effect of HBV infection on laboratory tests in DM patients. **a** Prevalence of HBV infection in blood donors and DM patients. **b** The effect of HBV infection on laboratory tests in DM patients. There were no significant differences in serum AST, ALT, and GGT levels between HBsAg-positive and HBsAg-negative DM patients of both sexes. *Error bars* SD

Table 3 Serum HBVDNA and HCVRNA levels, age, serum ALT level, and platelet (PLT) counts in HBsAg-positive patients and anti-HCV Ab-positive patients

	% (*n*)	Mean age (years)	Mean ALT levels (IU/L)	Mean PLT count($\times 10^4$/μL)
Serum HBV-DNA[a]				
Negative (<2.6 log copy/ml)	72 (29)	63.6	25.3	20.5
Positive (=2.6 log copy/ml)	28 (11)	55.6	28.0	18.5
=2.6<4.0	18 (7)	61.9	26.6	18.9
=4.0	10 (4)	50.0	30.5	17.8
Serum HCV-RNA[b]				
Negative (<2.7 log IU/ml)	38 (57)	67.2	28.2	17.7
Positive (=2.7 log IU/ml)	62 (91)	67.4	51.7	15.3
=2.7<5.0	3 (4)	65.1	28.0	16.4
=5.0	59 (87)	67.5	52.7	15.2

[a] Results are presented as either frequency or mean in 40 HBsAg-positive patients

[b] Results are presented as either frequency or mean in 148 anti-HCV Ab-positive patients

the PLT count was significantly lower in anti-HCV Ab-positive DM patients than in their negative counterparts ($p < 0.001$).

Thirty-eight percent of anti-HCV Ab-positive patients (M 36 %, F 42 %) demonstrated HCV-RNA negativity (Table 3), and 96 % of HCV-RNA-positive patients exhibited high serum HCV-RNA levels (≥5.0 log IU/ml). Serum ALT levels in anti-HCV Ab-positive patients with HCV-RNA positivity and those with HCV-RNA negativity were 51.7 ± 39.7 and 28.2 ± 18.1 IU/L, respectively, whereas those in anti-HCV Ab-negative patients were 27.7 ± 22.8 IU/L. Serum ALT levels were significantly higher in HCV-RNA-positive patients than in HCV-RNA-negative patients ($p < 0.001$).

The proportion of DM patients consuming >60 g and 20–59 g alcohol daily was 4.3 % (M 6.9 %, F 0.9 %) and

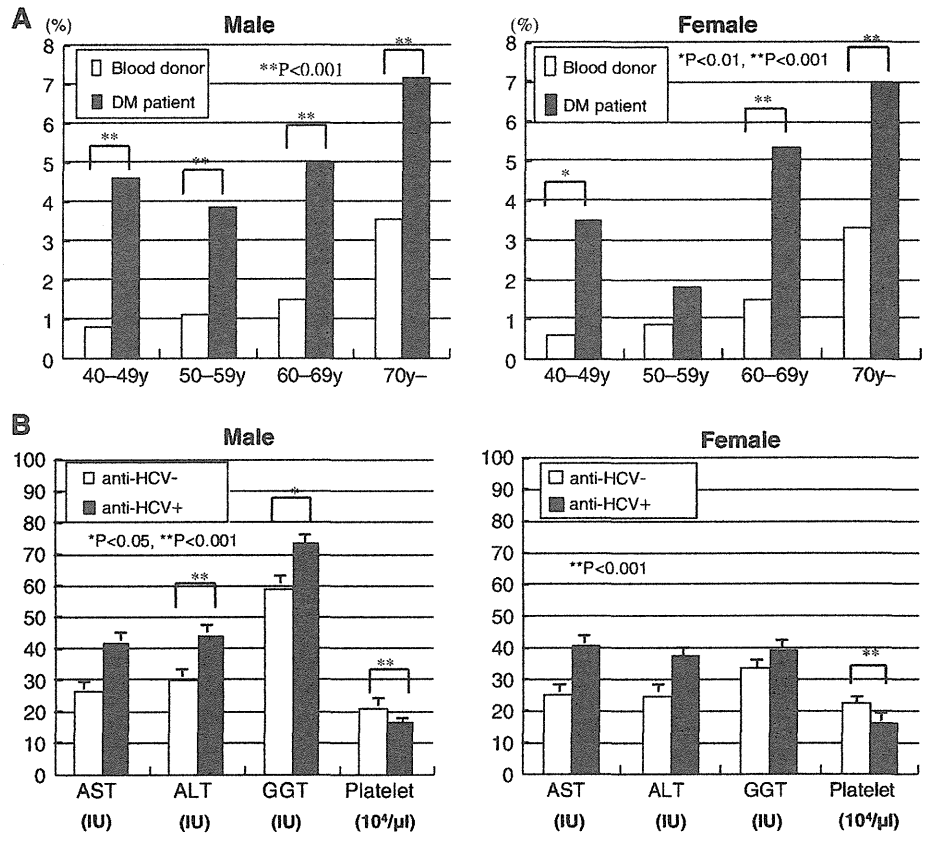

Fig. 2 Prevalence of HCV infection and the effect of HCV infection on laboratory tests in DM patients. **a** Prevalence of HCV infection in blood donors and DM patients. **b** The effect of HCV infection on laboratory tests in DM patients. *GGT* gamma glutamyl transpeptidase. ALT and GGT levels were significantly higher in male anti-HCV Ab-positive patients than in their negative counterparts. *Error bars* SD

14.9 % (M 23.8 %, F 2.8 %), respectively (Table 2). The highest percentage of drinkers were males in the 60- to 69-year age group and females in the <40-year age group. Male drinkers consuming >60 g alcohol daily had significantly higher serum AST and GGT levels compared with nondrinkers (patients consuming <20 g of daily alcohol intake) ($p < 0.001$). Serum ALT levels in drinkers consuming >60 g alcohol daily were comparable with those in nondrinkers. Drinkers of both sexes consuming 20–59 g alcohol daily had significantly higher serum GGT levels ($p < 0.001$) (Fig. 3).

Factors related to serum ALT levels

With increasing age in both sexes, the number of DM patients with elevated serum ALT levels and high BMI decreased, whereas those with decreased PLT counts increased. The number of DM patients with elevated serum ALT levels increased with increasing BMI in both sexes (Fig. 4).

A forward stepwise logistic regression model yielding odds ratios (ORs) and 95 % confidence intervals (CIs) was used to analyze the factors related to elevated serum ALT levels. The model included BMI, age, drinking status, HBsAg status, anti-HCV Ab status, PLT count, hypertension status, and dyslipidemia status as independent variables. The odds ratio shown indicates the change in odds for one SD increase in each variable.

Multivariate analysis showed that age (M: OR 0.674, CI 0.613–0.741; $p < 0.001$; F: OR 0.767, CI 0.683–0.861; $p < 0.001$), PLT count (M: OR 0.806, CI 0.732–0.886; $p < 0.001$, F: OR 0.714, CI 0.632–0.808, $p < 0.001$), anti-HCV Ab status (M: OR 1.321, CI 1.218–1.433; $p < 0.001$; F: OR 1.232, CI 1.117–1.359; $p < 0.001$), and BMI (M: OR 1.509, CI 1.374–1.657; $p < 0.001$; F: OR 1.487, CI 1.330–1.663; $p < 0.001$) were significantly associated with elevated serum ALT levels (Table 4).

For both sexes, AST and ALT levels were similar in drinkers consuming 20–59 g alcohol daily and those consuming <20 g alcohol daily (Fig. 3). After eliminating HBV-positive patients and/or HCV carriers and heavy drinkers consuming >60 g alcohol daily, the number of male, female, and total DM patients with elevated serum ALT levels were 33.4, 23.3, and 28.3 %, respectively. These values were comparable with those in all DM patients, including those with hepatitis and/or those consuming alcohol (M 32.8 %, F 23.0 %, total 28.6 %).

Liver histology in DM patients

The median age of histologically proven, DM- ($n = 87$) and non-DM-associated ($n = 95$) male NAFLD patients

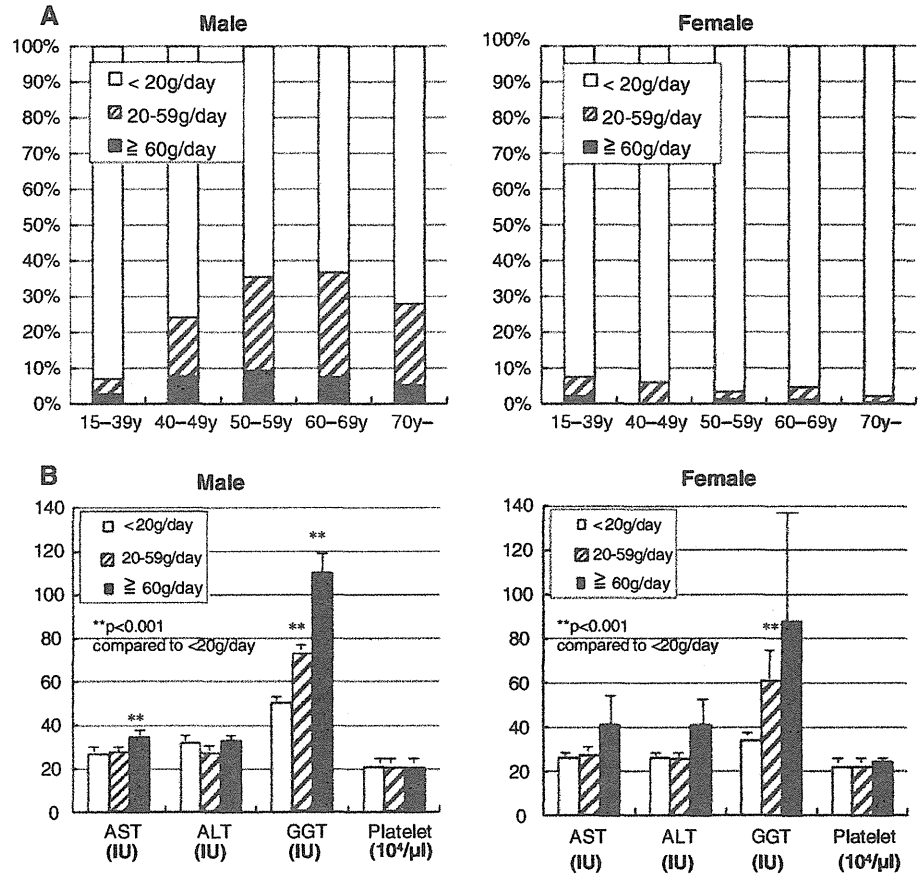

Fig. 3 Drinking habits and the effect of alcohol consumption on laboratory tests in DM patients. **a** Drinking habits in individual age. **b** The effect of alcohol consumption on laboratory tests in DM patients. *GGT* gamma glutamyl transpeptidase. Serum ALT levels in drinkers consuming >60 g alcohol daily were comparable with those in nondrinkers. *Error bars* SD

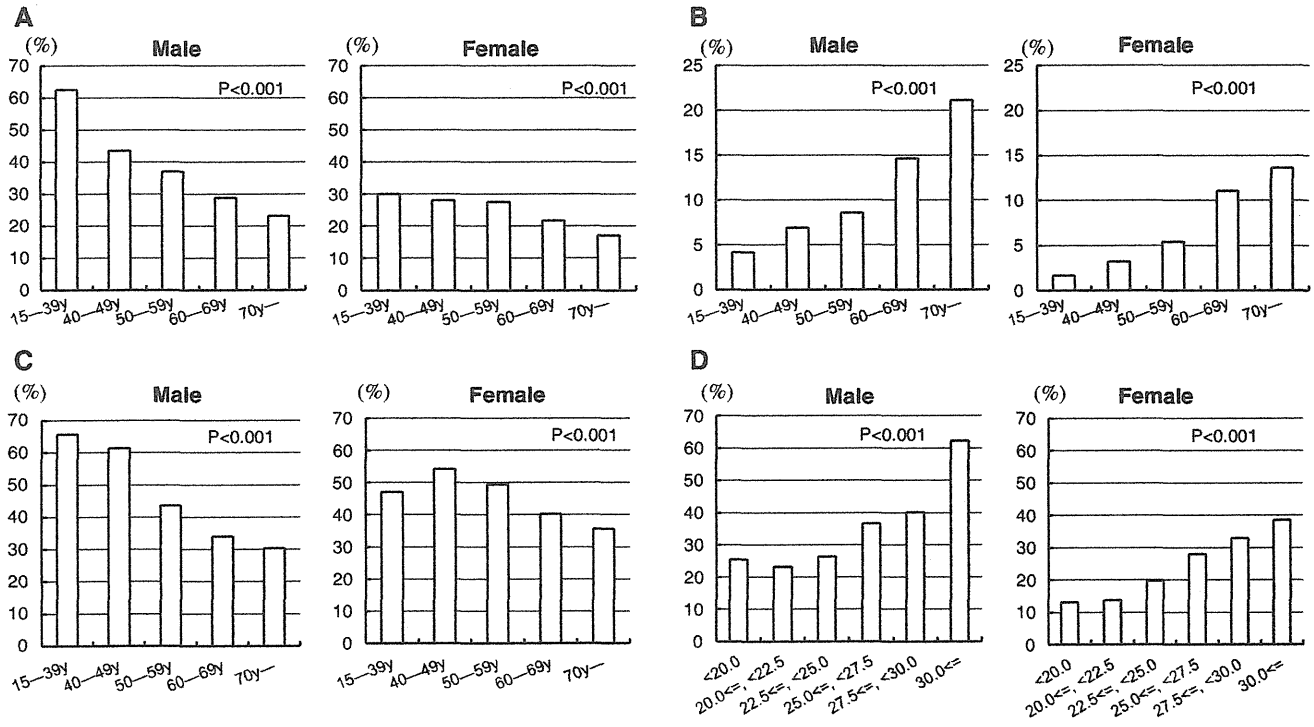

Fig. 4 Influence of age on the ratio of patients with elevated serum ALT level, decreased PLT count and abnormal BMI, and the relationship between BMI and the ratio of patients with elevated serum ALT level. **a** The ratio of patients with elevated serum ALT level (≥31 IU/L). **b** The ratio of patients with decreased PLT count (<15 × 10^4/μL). **c** The ratio of patients with abnormal BMI (≥25). **d** The relationship between BMI and the ratio of patients with elevated serum ALT level (≥31 IU/L)

Table 4 Multivariate analysis to identify independent variables related to elevated serum ALT level (≥31 IU/L)

	Regression coefficient	Standard error	Odds ratio	95 % confidence interval	*p*
Males					
Age	−0.394	0.048	0.674	0.613–0.741	<0.001
Platelet	−0.216	0.049	0.806	0.732–0.886	<0.001
Anti-hepatitis C virus	0.278	0.042	1.321	1.218–1.433	<0.001
Body mass index	0.411	0.048	1.509	1.374–1.657	<0.001
Females					
Age	−0.265	0.059	0.767	0.683–0.861	<0.001
Platelet	−0.336	0.063	0.714	0.632–0.808	<0.001
Anti-hepatitis C	0.208	0.050	1.232	1.117–1.359	<0.001
Body mass index	0.397	0.057	1.487	1.330–1.663	<0.001

was 60 and 45 years, respectively; in corresponding females, the median age was 66 ($n = 101$) and 61 years ($n = 82$), respectively. No significant difference was noted in BMI between DM and non-DM NAFLD patients (M 26.0 and 27.5 kg/m^2, respectively; F 26.0 and 27.0 kg/m^2, respectively). Male NAFLD patients without DM were significantly younger than those with DM ($p < 0.001$).

NAFLD patients were classified according to Matteoni's classification. Type 1, 2, 3, and 4 cases were 14 (16 %), 14 (16 %), 4 (5 %), and 55 (63 %), respectively, among male DM patients and 28 (29 %), 23 (24 %), 11 (12 %), and 33 (35 %), respectively, among male non-DM patients. Type 1, 2, 3, and 4 cases were 16 (16 %), 8 (8 %), 4 (4 %), and 73 (72 %), respectively, among female DM patients and 9 (11 %), 9 (11 %), 7 (9 %), and 57 (69 %), respectively, among female non-DM patients (Fig. 5). The frequency of Type 4 NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.001$).The rate of Type 4 NASH was high in both female DM and non-DM patients.

In total, 244 (M 103, F 141) NASH patients were classified according to Brunt's classification. The number of patients with stage 0 (Matteoni Type 3), 1, 2, 3, and 4 were 2 (3 %), 27 (46 %), 10 (17 %), 17 (29 %), and 3 (5 %), respectively, among male DM patients and 10 (23 %), 16 (36 %), 8 (18 %), 9 (21 %), and 1 (2 %), respectively, among male non-DM patients. Stage 0, 1, 2, 3, and 4 cases were 4 (5 %), 25 (33 %), 24 (31 %), 13 (17 %), and 11 (14 %), respectively, among female DM patients and 4 (6 %), 28 (44 %), 20 (31 %), 10 (16 %), and 2 (3 %), respectively, among female non-DM patients (Fig. 6). The frequency of advanced stage NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.05$).The rate of Stage 4 NASH was higher in female DM patients than in female non-DM patients; however, the difference was not significant ($p = 0.198$).

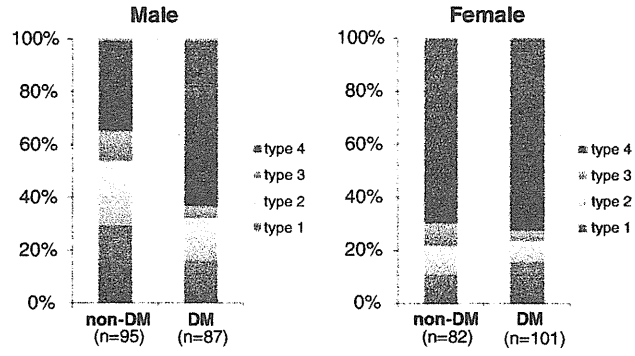

Fig. 5 Distribution of Matteoni's type classification in individual status of glucose metabolism among NAFLD patients. The frequency of type 4 NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.001$). The rate of type 4 NASH was high in both female DM and non-DM patients

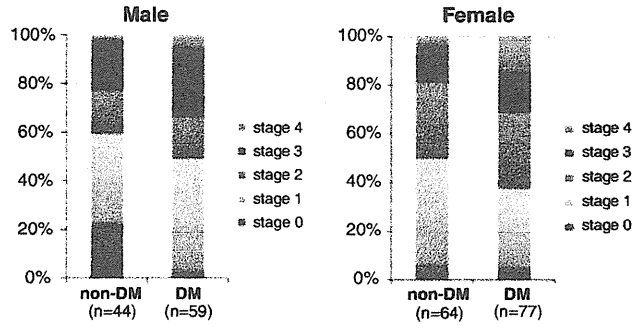

Fig. 6 Distribution of Brunt's stage in individual status of glucose metabolism among NASH patients. The frequency of advanced stage NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.05$). The rate of stage 4 NASH was higher in female DM patients than in female non-DM patients; however, the difference was not significant ($p = 0.198$)

HCC incidence in DM patients

In total, 67 (M 48, F 19) HCC cases (1.4 %) were reviewed (Table 2). HCC incidence was significantly higher in males

than in females. Five of 67 HCC patients consumed >60 g alcohol daily, and two of these five patients were anti-HCV Ab positive. HBsAg positivity, anti-HCV Ab positivity, and non-B non-C prevalence in the HCC patients was 8.6, 50.0, and 41.4 %, respectively. In a Japanese nationwide survey of 19,499 HCC patients [3], HBsAg positivity, anti-HCV Ab positivity, and non-B non-C prevalence was 15.0, 67.7, and 17.3 %, respectively. Non-B non-C prevalence was higher in our DM patients with HCC than in the nationwide HCC survey participants ($p < 0.001$). Mean PLT count in DM patients with HCC was as follows: HBsAg-positive patients, 12.4 ± 6.8; anti-HCV Ab-positive patients, 12.4 ± 5.6; and non-B non-C patients, 16.0 ± 7.0 ($\times 10^4/\mu L$); PLT count was significantly higher in the non-B non-C patients than in the anti-HCV-positive patients ($p < 0.05$). Mean BMI in these three patient groups was as follows: HBsAg-positive patients, 23.2 ± 5.1; anti-HCV Ab-positive patients, 22.8 ± 3.3; and non-B non-C patients, 27.2 ± 4.4 (kg/m^2); BMI was significantly higher in the non-B non-C patients than in the anti-HCV Ab-positive patients ($p < 0.001$).

Discussion

This is the first multicenter study, as per our knowledge, that clarifies the cause of liver injury in DM patients in Japan. Most Japanese HBV carriers are genotype C, acquired via perinatal vertical transmission or early childhood infection [12]. The HBV carrier rate in Japan is higher than that in western countries and significantly lower than that in other Asian countries [13]. In 1986, the Japanese government initiated a nationwide hepatitis B immunization program for infants born to HBV carrier mothers to prevent perinatal transmission. Consequently, the number of young serum HBsAg-positive individuals is extremely low. In our study, although the HBV carrier rate in DM patients was significantly higher than that in blood donors, 72 % of HBsAg-positive patients were serum HBV-DNA negative. Only 10 % of HBsAg-positive patients exhibited high serum HBV-DNA levels (≥4.0 log copies/ml), which is likely to induce hepatitis. These results indicate that a majority of DM patients who are HBV carriers may be asymptomatic.

Chronic hepatitis C may result in life-threatening complications, including cirrhosis and HCC. Worldwide, cirrhosis can be attributed to HBV (30 %) and HCV infection (27 %) [14]. The leading cause of cirrhosis among HBV and HCV sufferers and alcohol consumers varies with individual countries. A recent nationwide Japanese survey reported the etiology of cirrhosis in Japan as follows: HCV 60.9 %, HBV 13.9 %, alcoholism 13.6 %, primary biliary cirrhosis 2.4 %, NASH-related 2.1 %, and autoimmune hepatitis 1.9 % [15]. However, we must consider that hepatic triglycerides diminish with liver fibrosis progression in NASH patients (so-called "burned-out" NASH), resulting in difficulty in diagnosing NASH. Sixty-two percent of anti-HCV Ab-positive DM patients were HCV-RNA positive; these patients showed significantly higher serum ALT levels compared with HCV-RNA-negative patients. These results indicate that HCV infection is involved in the etiology of liver disease in DM patients.

There is no doubt that the positive rates of serum HBsAg and anti-HCV Ab in the general population are higher than in blood donors. Unfortunately, there were no data in the distribution of the rate of hepatitis virus carriers in each age group in Japan. In the present study, the positive rates of HBsAg and anti-HCV Ab in DM patients were significantly higher than that in blood donors. However, the present study demonstrated that most of HBsAg positive patients were negative for serum HBV DNA or had low serum HBV DNA levels and around one-third of anti-HCV Ab positive patients were negative for serum HCV RNA.

These results indicate the possibility that the frequency of hepatitis virus carriers in DM patients is higher than that in general population but no significant differences might be noted between DM patients and the general population.

Alcohol consumption is reportedly a significant factor associated with the risk of HCC development in patients with NASH-associated cirrhosis [16]. In our study, serum AST and ALT levels were comparable between drinkers consuming 20–59 g alcohol daily and nondrinkers. The ratio of heavy drinkers consuming >60 g alcohol daily was low (4.3 %) in our study. Moreover, drinking was not chosen as a variable related to elevated serum ALT levels. These results suggest that alcohol intake is not an important factor in the pathogenesis of liver disease in DM patients.

In our study, the frequency of anti-HCV Ab-positive DM patients was 5 %, whereas the serum HCV-RNA positivity rate in anti-HCV Ab-positive patients was 62 %. Therefore, the HCV carrier rate was calculated as 3 %. Since the proportion of HCV carriers and patients with elevated ALT levels were 3 % and up to 29 %, respectively, the influence of HCV infection is estimated to be no more than 10 % (3 % divided by 29 %) among DM patients with elevated ALT levels. There was no significant change in the number of DM patients with elevated ALT levels before and after elimination of HBV and/or HCV carriers and heavy drinkers. These results suggested that the major cause (up to 90 %) of liver injury in DM patients may be NAFLD.

In the present study, the frequency of advanced stage NASH was significantly higher in male DM patients than

in male non-DM patients. Neuschwander-Tetri et al. [17] reported that patients with advanced stage NASH were more likely to have DM. Mayaaki et al. [18] also examined the relationship between hepatic fibrosis stage and DM prevalence. In the mild fibrosis group, only 42 % were complicated with DM, whereas in the severe fibrosis group, the prevalence was as high as 71 % ($p = 0.020$). Lo et al. [19] reported that DM exacerbated diet-induced NASH fibrosis in mice. Therefore, DM may be an important factor in hepatic fibrosis development in NAFLD patients.

HCC frequency is significantly higher in obese and DM patients than in non-obese and non-DM patients [20, 21]. Recently, Tokushige et al. [22] reported on the backgrounds of Japanese HCC patients, and non-B non-C HCC accounted for 16 % of cases. A recent report has shown that NASH patients are likely to develop HCC in an earlier stage of fibrosis compared with chronic hepatitis C patients [23]. Our previous study analyzed 87 histologically proven NASH-HCC patients [24]; 37 % (20/54) of male HCC patients had a mild to moderate stage of liver fibrosis (F1 or F2); however, no female HCC patients were F1 stage, and only 15 % (5/33) were F2 stage. In the present study, DM patients with non-B non-C HCC exhibited a tendency to have higher PLT counts than those in DM patients with HCV-HCC, indicating that non-B non-C HCC is more likely to occur in DM patients with less advanced liver disease than in those with viral hepatitis.

In conclusion, HBsAg and anti-HCV Ab positivity rates were high; however, most of these patients were HBV-DNA negative or had low serum HBV-DNA levels. One-third of anti-HCV Ab-positive patients were HCV-RNA negative, and 4.3 % patients were drinkers whose ALT levels were comparable with those of nondrinkers. From these results, we conclude that up to 90 % of Japanese DM patients with liver injury may have NAFLD/NASH.

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Conflict of interest The authors declare that they have no conflicts of interest to disclose.

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