



# Clinical characteristics of patients with diabetes mellitus and fatty liver diagnosed by liver/spleen Hounsfield units on CT scan

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## Abstract

**Objective:** The leading cause of liver injuries in diabetes mellitus may be associated with fatty liver. We aimed to elucidate the relationship between fatty liver and diabetes characteristics.

**Methods:** Retrospectively, 970 patients with diabetes were analysed. Fatty liver was diagnosed when the liver/spleen Hounsfield unit ratio by computed tomography was below 0.9. Clinical diabetes characteristics were compared between patients with and without fatty liver.

**Results:** Of 970 patients (717 male and 253 female; mean age 64.4 years), 175 males (24.4%) and 60 females (23.7%) had fatty liver. None of the 28 patients with type 1 diabetes had fatty liver. In male patients with type 2 diabetes, age, visceral adipose tissue (VAT), albumin, alanine aminotransferase (ALT), and triglycerides were independently associated with fatty liver. In females, age and bilirubin were associated with fatty liver.

**Conclusions:** Fatty liver is associated with type 2 diabetes characteristics, including younger age and elevated VAT, albumin, ALT, and triglycerides in males and younger age and elevated bilirubin levels in females.

## Keywords

Fatty liver, diabetes mellitus, visceral adipose tissue

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## Introduction

The prevalence of diabetes mellitus is rapidly increasing worldwide. In 2011, there were 366 million patients diagnosed with diabetes mellitus globally, and this number

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is expected to increase to 552 million by 2030.<sup>1</sup> The prevalence of diabetes mellitus in the western Pacific region was estimated to be 8.6% in 2013, and there were reported to be approximately 7.2 million patients with diabetes mellitus in Japan according to the International Diabetes Federation Diabetes Atlas.<sup>2</sup> The leading cause of death in patients with diabetes mellitus in Japan is malignant neoplasia (34.1%) followed by vascular diseases, including diabetic nephropathy, ischemic heart diseases, and cerebrovascular diseases (26.8%). The most common malignancy was liver cancer (8.6%), and liver cirrhosis was the cause of death in 5.6% of all deaths.<sup>3</sup> In Japan, hepatitis viruses are reportedly negative in the majority of patients with chronic liver disease and diabetes mellitus.<sup>4</sup> In addition, there is significant evidence demonstrating a high prevalence of fatty liver in patients with diabetes mellitus.<sup>5-8</sup> Subsequently, a large proportion of deaths from liver injuries in patients with diabetes mellitus could be associated with fatty liver. In fact, hepatocellular carcinoma related to obesity and diabetes mellitus is rapidly increasing in Japan.<sup>9</sup> Thus, it is critical to assess fatty liver and associated factors in patients with diabetes mellitus.

Many researchers have evaluated the modalities used to estimate steatosis. The gold standard for diagnosis of fatty liver is liver biopsy. However, because of the invasiveness of the procedure and sampling variability, liver biopsy is not suitable for a screening examination to detect fatty liver.<sup>10</sup> Abdominal echo examination is widely used as a clinical screening test to detect the presence of fatty liver and estimate its severity.<sup>11</sup> Recently, researchers have reported the usefulness of the liver/spleen Hounsfield unit (L/S) ratio calculated using computed tomography (CT) in patients with diabetes mellitus.<sup>12,13</sup> For example, Yoneda et al.<sup>13</sup> reported that serum adiponectin

levels are correlated with the L/S ratio. Visceral adipose tissue (VAT) measured by CT is also a reportedly valuable predictor of precancerous lesions, such as colorectal adenoma and Barrett's oesophagus, and other malignancies, including hepatocellular carcinoma.<sup>14-16</sup> However, the association between the L/S ratio and clinical characteristics of diabetes mellitus, such as VAT, subcutaneous adipose tissue (SAT), body mass index (BMI), and alcohol consumption, remains unclear. Thus, in this study, we aimed to elucidate the relationship between fatty liver and diabetes mellitus characteristics.

## Methods

### Patients

To evaluate the association between fatty liver diagnosed by CT and clinical characteristics of diabetes mellitus, 1264 patients who underwent abdominal CT (for clinical indications, such as abdominal pain and abnormal findings by ultrasound examination) and VAT evaluation at our institution between January 2008 and March 2014 were retrospectively reviewed. We then excluded patients who fulfilled the following criteria: (i) absence or insufficiency of biochemical examinations ( $n = 21$ ), (ii) positive laboratory confirmation of hepatitis B surface antigen and anti-hepatitis C virus antibodies ( $n = 26$ ), (iii) history of splenectomy ( $n = 3$ ), and (iv) not fulfilling the diagnostic criteria of diabetes mellitus according to the 2010 Japan Diabetes Society criteria ( $n = 244$ ).<sup>17</sup> Finally, the remaining 970 patients with diabetes mellitus were analysed. Type 1 diabetes mellitus was diagnosed according to the Japan Diabetes Society criteria, i.e. based on the clinical history and positive detection of anti-glutamic acid decarboxylase antibodies, anti-islet cell cytoplasmic antibodies, anti-insulin auto-antibody, and anti-insulinoma-associated

antigen-2. Hypertension, hyperlipidaemia, and hyperuricemia diagnoses were based on the need for medical treatment. The study design was approved by the Ethics Committee at the Institute for Adult Diseases, Asahi Life Foundation and conforms to the Declaration of Helsinki. Patient records were anonymized prior to analysis. The decision of the committee was that the requirement for written informed consent was waived unless the patients refused to allow us to use the data for analysis after de-identification. To date, no patient has refused.

### *Clinical and laboratory evaluation*

Demographic parameters, including age, diabetes mellitus duration (defined as duration from patients' first visit to our hospital to the CT evaluation in this examination), sex, body mass index (BMI), history of smoking [Brinkman index (BI) = daily amounts of tobacco (pieces/day) × period of smoking (years)], and alcohol intake (g/day), and the treatments administered were recorded. Venous blood samples were obtained after overnight fasting. Haemoglobin A1c (HbA1c; levels were converted and expressed by National Glycohemoglobin Standardization Program values), platelet count, albumin, bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transferase ( $\gamma$ -GT), aspartate amino-transferase (AST), alanine amino-transferase (ALT), triglyceride, total cholesterol (total-C), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using standard laboratory techniques.

### *CT*

Patients were subjected to abdominal plain CT (Asteion Super4 Edition or Alexion/Advance Edition, TOSHIBA Medical Systems, Tochigi, Japan) in the helical

mode with 7 mm or thinner slice thickness after overnight fasting. CT numbers (Hounsfield units) were measured at three points respectively in the liver and spleen, avoiding blood vessels and heterogeneous areas. The mean numbers were used to calculate the L/S ratio. We defined the criterion of fatty liver diagnosis as  $< 0.9$  of the L/S ratio. VAT, SAT, and waist circumferences were determined at the umbilical level by Fat Scan software program (Fat Scan, East Japan Institute of Technology, Ibaraki, Japan).

### *Statistical analysis*

All statistical analyses were performed using JMP10 software (SAS Institute, Cary, NC, USA). The Mann-Whitney  $U$  test was used to compare means of continuous variables. Comparisons of nominal variables were conducted by the  $\chi^2$  test or Fisher's exact test as appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were used as a measure of association and were adjusted by unconditional logistic regression models. A two-sided  $p$ -value of  $< 0.05$  was considered statistically significant. The parameters that had statistical significance ( $p < 0.05$ ) in the univariate analysis were selected for multivariate analysis. Patients with type 2 diabetes mellitus were divided into an ALT normal or ALT high group, and the ratio of patients with fatty liver in each group was calculated. The patients were also divided according to triglyceride, BMI, VAT, SAT, and alcohol consumption levels, and the ratio of patients with fatty liver was calculated. The normal levels of these factors were defined as follows: ALT  $< 40$  U/ml; triglycerides  $< 150$  mg/dl; BMI  $< 25$  kg/m<sup>2</sup>; VAT  $< 100$  cm<sup>2</sup>; SAT  $< 100$  cm<sup>2</sup>; and alcohol consumption, male,  $< 30$  g/day and female,  $< 20$  g/day. Heavy drinker is defined both in male and female as alcohol consumption  $\geq 60$  g/day. Heavy smoking was defined as BI  $\geq 800$ .

## Results

### *Characteristics of patients with diabetes mellitus according to sex and fatty liver*

The baseline clinical characteristics are summarized in Table 1. A total of 970 patients (717 male and 253 female) were examined. The median age and BMI in male and female patients with diabetes mellitus was 65 and 67 years and 23.9 and 24.5 kg/m<sup>2</sup>, respectively. Of these, 175 male (24.4%) and 60 female (23.7%) patients with diabetes mellitus were diagnosed with fatty liver by the L/S ratio calculated using CT. There were 17 male and 11 female patients with type 1 diabetes mellitus. None of the patients with type 1 diabetes mellitus had fatty liver.

Most of the parameters, except duration of diabetes mellitus, BMI, albumin, triglycerides, and LDL-C, were significantly different between male and female patients without fatty liver. Age, duration of diabetes mellitus, waist circumference, SAT, and ALP levels were significantly higher in female patients with fatty liver than in male patients with fatty liver. In contrast, albumin, bilirubin,  $\gamma$ -GT, ALT, and triglyceride levels were significantly higher in male patients with fatty liver compared with those in female patients with fatty liver. There were no significant differences in BMI, L/S ratio, platelet count, VAT, or HbA1c, AST, total-C, HDL-C, or LDL-C levels between male and female patients with fatty liver. A total of 50 male (28.5%) and four female (6.67%) heavy smokers (BI  $\geq$  800) and 40 male (22.8%) and four female (6.67%) heavy drinkers (alcohol intake  $\geq$  60 g/day) were diagnosed with fatty liver.

### *Medications for patients with diabetes mellitus*

The medications administered to patients with diabetes mellitus are also displayed in Table 1. Insulin was administered to 259

male (36.1%) and 120 female (47.4%) patients. Medications for hypertension, hyperlipidaemia, and hyperuricemia were administered to 557 (57.4%), 495 (51.0%), and 79 (8.1%) patients with diabetes mellitus, respectively. Insulin was more likely to be administered to female than male patients without fatty liver. In contrast, other diabetic medications, except dipeptidyl peptidase-4-inhibitors and glucagon-like peptide-1 agonists, were more likely to be administered to male than female patients without fatty liver. Dipeptidyl peptidase-4-inhibitors, glucagon-like peptide-1 agonists, and hyperlipidaemia medications were more likely to be administered to female than to male patients with fatty liver. The rate of administration of the other medications was not significantly different between male and female patients with fatty liver.

### *Associated risk factors for fatty liver in patients with diabetes mellitus*

Next, we investigated the associated factors for fatty liver in 942 patients with type 2 diabetes mellitus (700 male and 242 female). A univariate analysis was performed to compare male patients with and without fatty liver (Table 2). There was a significant difference in most clinical factors except ALP and alcohol consumption. Using multivariate logistic regression in male patients with diabetes mellitus, age [OR (95% CI), 0.96 (0.94–0.99),  $p=0.0359$ ], VAT [1.01 (1.00–1.01),  $p=0.0108$ ], albumin [4.23 (1.74–10.2),  $p=0.0014$ ], ALT [1.02 (1.00–1.05),  $p=0.0255$ ], and triglycerides [1.01 (1.00–1.01)  $p=0.0339$ ] were independent associated factors for fatty liver (Table 2). In male patients with diabetes mellitus, no medications examined were associated with fatty liver by multivariate analysis.

Univariate and multivariate analyses were also performed to compare female patients with and without fatty liver (Table 3). Using multivariate logistic

**Table 1.** Baseline characteristics of the 970 patients with diabetes mellitus according to sex and fatty liver.

Total (n = 970)	Male (n = 717)		Female (n = 253)		p <sup>†</sup>
	Non-fatty liver (n = 542)	Fatty liver (n = 175)	Non-fatty liver (n = 193)	Fatty liver (n = 60)	
	Age (years)	67 (61–72)	59 (49–67)	68 (62–75)	
Duration of diabetes mellitus (years)	13 (6–19)	6 (1–13)	8 (3–13)	20 (12–23)	<0.0001*
BMI (kg/m <sup>2</sup> )	23.3 (21.7–25.3)	26.0 (24.1–28.2)	23.5 (21.7–25.3)	26.9 (24.2–30.6)	0.1039
W/waist (cm)	86.8 (81.1–92.8)	93.6 (89.4–98.8)	88.9 (82.2–96.7)	96.6 (88.6–105)	0.0406*
L/S ratio	1.09 (1.02–1.16)	0.75 (0.59–0.84)	1.11 (1.02–1.20)	0.76 (0.59–0.83)	0.8258
SAT (cm <sup>2</sup> )	123 (95.5–164)	166 (129–203)	177 (139–238)	224 (169–304)	<0.0001*
VAT (cm <sup>2</sup> )	111 (82–151)	150 (121–187)	96.3 (60–137)	132 (101–173)	0.0668
HbA1c (%)	7.4 (6.8–8.0)	7.7 (7.1–8.8)	7.1 (6.6–8.2)	7.3 (6.6–8.7)	0.0539
Platelet (10 <sup>3</sup> /μl)	20.2 (17.6–23.1)	21.1 (18.1–24.6)	22.1 (18.9–25.3)	22.4 (19.1–25.9)	0.0747
Albumin (g/dl)	4.3 (4.1–4.5)	4.5 (4.4–4.6)	4.3 (4.0–4.5)	4.3 (4.1–4.5)	<0.0001*
Bilirubin (g/ml)	0.6 (0.5–0.8)	0.7 (0.6–0.9)	0.5 (0.4–0.7)	0.6 (0.5–0.7)	0.0395*
ALP (U/l)	209 (174–252)	210 (167–254)	230 (193–279)	240 (187–277)	0.0188*
γ-GT (U/l)	30 (20–47)	53 (33–100)	19 (15–31)	35.5 (24–51)	<0.0001*
AST (U/l)	21 (18–26)	29 (22–43)	20 (17–23)	27.5 (22–36)	0.3111
ALT (U/l)	20 (15–27)	39 (26–63)	18 (13–24)	30 (22–47)	0.0307*
Triglycerides (mg/dl)	112 (84–155)	164 (116–236)	113 (82–158)	129 (106–179)	0.0369*
TC (mg/dl)	187 (169–205)	198 (175–223)	191 (180–211)	191 (179–213)	0.5875
HDL-C (mg/dl)	52 (44–62)	47 (41–55)	58 (46–69)	48.5 (44–59)	0.1508
LDL-C (mg/dl)	104 (87.0–123)	113 (93–138)	102 (89–119)	112 (100–127)	0.8516
Heavy smoker, n (%)	213 (39.2%)	50 (28.5%)	11 (5.69%)	4 (6.67%)	0.0002*
Heavy drinker, n (%)	103 (19.0%)	40 (22.8%)	1 (0.51%)	4 (6.67%)	0.0040*
Hypertension, n (%)	312 (57.6%)	93 (53.1%)	118 (61.1%)	34 (56.6%)	0.5798
Hyperlipidemia, n (%)	256 (47.2%)	76 (39.3%)	124 (64.2%)	39 (65.0%)	0.0039*
Hyperuricemia, n (%)	54 (9.96%)	15 (8.57%)	9 (4.66%)	1 (1.66%)	0.0780
Type 1 diabetes mellitus, n (%)	17 (3.13%)	0 (0%)	11 (5.69%)	0 (0%)	-
Use of insulin, n (%)	211 (38.9%)	48 (27.4%)	102 (52.8%)	18 (30.0%)	0.7021
Use of sulfonylurea, n (%)	262 (48.3%)	91 (52.0%)	77 (39.8%)	27 (45.0%)	0.3906
Use of nateglinide, n (%)	30 (5.53%)	7 (4.00%)	2 (1.03%)	1 (1.66%)	0.6833

(continued)

Table 1. Continued.

Total (n = 970)	Male (n = 717)		Female (n = 253)		p <sup>†</sup>
	Non-fatty liver (n = 542)	Fatty liver (n = 175)	Non-fatty liver (n = 193)	Fatty liver (n = 60)	
Use of $\alpha$ -GI, n (%)	93 (17.1%)	31 (17.7%)	22 (11.3%)	12 (20.0%)	0.6927
Use of biguanide, n (%)	230 (42.4%)	100 (57.1%)	65 (33.6%)	35 (58.3%)	0.8721
Use of thiazolidine, n (%)	44 (8.1%)	17 (9.7%)	6 (3.1%)	3 (5.0%)	0.2587
Use of DPP-4, GLP-1, n (%)	36 (6.6%)	21 (12.0%)	16 (8.2%)	12 (20.0%)	0.0344*
Use of ARB, n (%)	224 (41.3%)	72 (41.1%)	88 (45.5%)	27 (45.0%)	0.6015
Use of ACE-I, n (%)	34 (6.2%)	7 (4.0%)	8 (4.1%)	2 (3.3%)	0.8163
Use of $\alpha/\beta$ -blocker, n (%)	39 (7.1%)	7 (4.0%)	11 (5.6%)	0 (0%)	0.1956
Use of CCB, n (%)	197 (36.3%)	64 (36.5%)	78 (40.4%)	19 (31.6%)	0.4927
Use of diuretics, n (%)	51 (9.4%)	8 (4.5%)	17 (8.8%)	6 (10.0%)	0.1252
Use of nitrate, n (%)	20 (3.6%)	5 (2.8%)	10 (5.1%)	3 (5.0%)	0.4242
Use of aspirin, n (%)	83 (15.3%)	15 (8.5%)	29 (15.0%)	9 (15.0%)	0.1558

Data shown are median (25<sup>th</sup>–75<sup>th</sup> percentile) or n (%).

Heavy smoker, Brinkman Index  $\geq 800$ ; Heavy drinker, alcohol consumption  $\geq 60$  g/day; BMI, body mass index; LS ratio, liver/spleen Hounsfield unit ratio; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; HbA1c, haemoglobin A1c; ALP, alkaline phosphatase;  $\gamma$ -GT, gamma-glutamyl transferase; AST, aspartate amino-transferase; ALT, alanine amino-transferase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol;  $\alpha$ -GI,  $\alpha$ -glucosidase inhibitor; DPP-4, inhibitors of dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1 receptor agonists; ARB, angiotensin II receptor blocker; ACE-I, angiotensin converting enzyme inhibitor; CCB, calcium channel blocker

<sup>†</sup>p-value for comparison between male and female with fatty liver

\*statistically significant ( $p < 0.05$ )

**Table 2.** Associated factors for fatty liver: Male patients with type 2 diabetes mellitus.

Male (n = 700)	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Age (year)	0.92 (0.90–0.94)	<0.0001*	0.96 (0.94–0.99)	0.0359*
Duration of diabetes mellitus (year)	0.92 (0.90–0.94)	<0.0001*	0.98 (0.95–1.01)	0.2915
BMI (kg/m <sup>2</sup> )	1.40 (1.30–1.50)	<0.0001*	1.12 (0.97–1.30)	0.1478
Waist (cm)	1.12 (1.09–1.14)	<0.0001*	0.99 (0.91–1.07)	0.9303
SAT (cm <sup>2</sup> )	1.01 (1.01–1.02)	<0.0001*	1.00 (0.99–1.01)	0.8384
VAT (cm <sup>2</sup> )	1.01 (1.01–1.02)	<0.0001*	1.01 (1.00–1.01)	0.0108*
HbA1c (%)	1.31 (1.15–1.50)	<0.0001*	1.05 (0.85–1.29)	0.6255
Platelet (10 <sup>3</sup> /μl)	1.04 (1.00–1.07)	0.0262*	1.01 (0.96–1.06)	0.6176
Albumin (g/dl)	7.72 (3.91–15.2)	<0.0001*	4.23 (1.74–10.2)	0.0014*
Bilirubin (g/ml)	2.14 (1.22–3.76)	0.0023*	1.56 (0.68–3.57)	0.2909
ALP (U/l)	0.99 (0.99–1.00)	0.8533		
γ-GT (U/l)	1.00 (1.00–1.01)	<0.0001*	0.99 (0.99–1.00)	0.9997
AST (U/l)	1.06 (1.04–1.08)	<0.0001*	1.01 (0.98–1.05)	0.1850
ALT (U/l)	1.05 (1.04–1.07)	<0.0001*	1.02 (1.00–1.05)	0.0255*
Triglycerides (mg/dl)	1.00 (1.00–1.01)	<0.0001*	1.01 (1.00–1.01)	0.0339*
TC (mg/dl)	1.01 (1.01–1.02)	<0.0001*	0.99 (0.97–1.02)	0.7055
HDL-C (mg/dl)	0.97 (0.96–0.98)	0.0002*	1.00 (0.97–1.03)	0.7811
LDL-C (mg/dl)	1.01 (1.01–1.02)	0.0001*	1.01 (0.99–1.04)	0.1865
Smoking (BI)	0.99 (0.99–1.00)	0.0225*	1.00 (0.99–1.00)	0.5420
Alcohol (g/day)	1.00 (0.99–1.01)	0.8026		
Hypertension	0.83 (0.59–1.17)	0.2340		
Hyperlipidemia	0.85 (0.60–1.20)	0.3144		
Hyperuricemia	0.84 (0.46–1.54)	0.5099		
Use of insulin	0.59 (0.40–0.86)	0.0217*	1.06 (0.62–1.81)	0.8047
Use of sulfonylurea	1.15 (0.82–1.62)	0.3800		
Use of nateglinide	0.71 (0.30–1.64)	0.4248		
Use of α-GI	1.03 (0.66–1.62)	1.0000		
Use of biguanide	1.80 (1.28–2.55)	0.0022*	1.17 (0.73–1.88)	0.4994
Use of thiazolidine	1.21 (0.67–2.19)	0.5881		
Use of DPP-4, GLP-1	1.91 (1.08–3.38)	0.0312*	1.68 (0.77–3.67)	0.1864
Use of ARB	0.99 (0.70–1.40)	0.8943		
Use of ACE-I	0.62 (0.27–1.43)	0.2270		
Use of α/β-blocker	0.53 (0.23–1.22)	0.1129		
Use of CCB	1.01 (0.70–1.43)	0.9638		
Use of diuretics	0.46 (0.21–0.99)	0.0339*	0.50 (0.20–1.27)	0.1472
Use of nitrate	0.58 (0.19–1.71)	0.3542		
Use of aspirin	0.51 (0.29–0.92)	0.0194*	0.77 (0.037–1.61)	0.4946

OR, odds ratio; CI, confidence interval; BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; HbA1c, haemoglobin A1c; ALP, alkaline phosphatase; γ-GT, gamma-glutamyl transferase; AST, aspartate amino-transferase; ALT, alanine amino-transferase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BI, Brinkman Index; α-GI, α-glucosidase inhibitor; DPP-4, inhibitors of dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1 receptor agonists; ARB, angiotensin II receptor blocker; ACE-I, angiotensin converting enzyme inhibitor; CCB, calcium channel blocker

\*statistically significant ( $p < 0.05$ )



**Table 3.** Associated factors for fatty liver: Female patients with type 2 diabetes mellitus.

Female (n = 242)	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Age (year)	0.96 (0.93–0.99)	0.0019*	0.92 (0.86–0.99)	0.0207*
Duration of diabetes mellitus (year)	1.24 (1.16–1.31)	<0.0001*	1.28 (1.18–1.39)	<0.0001*
BMI (kg/m <sup>2</sup> )	1.13 (1.07–1.20)	<0.0001*	1.26 (0.95–1.67)	0.1077
Waist (cm)	1.05 (1.02–1.07)	0.0001*	0.99 (0.82–1.18)	0.9137
SAT (cm <sup>2</sup> )	1.00 (1.00–1.01)	0.0017*	0.99 (0.98–1.01)	0.5159
VAT (cm <sup>2</sup> )	1.01 (1.01–1.02)	0.0001*	0.99 (0.97–1.01)	0.4879
HbA1c (%)	1.08 (0.91–1.29)	0.2869		
Platelet (10 <sup>3</sup> /μl)	1.01 (0.96–1.07)	0.4439		
Albumin (g/dl)	1.30 (0.54–3.11)	0.7974		
Bilirubin (g/ml)	3.53 (1.09–11.4)	0.0246*	7.36 (1.10–48.9)	0.0389*
ALP (U/l)	1.00 (0.99–1.00)	0.9162		
γ-GT (U/l)	1.00 (1.00–1.01)	<0.0001*	0.99 (0.98–1.00)	0.2334
AST (U/l)	1.06 (1.04–1.09)	<0.0001*	1.07 (0.97–1.18)	0.1507
ALT (U/l)	1.05 (1.03–1.07)	<0.0001*	1.00 (0.94–1.07)	0.8473
Triglycerides (mg/dl)	1.00 (1.00–1.01)	0.0310*	1.00 (0.99–1.01)	0.2129
TC (mg/dl)	1.00 (0.99–1.01)	0.5658		
HDL-C (mg/dl)	0.96 (0.94–0.98)	0.0070*	0.98 (0.93–1.02)	0.3730
LDL-C (mg/dl)	1.01 (1.00–1.02)	0.0105*	1.01 (0.98–1.03)	0.4886
Smoking (BI)	1.00 (0.99–1.00)	0.1817		
Alcohol (g/day)	0.98 (0.96–1.01)	0.5064		
Hypertension	0.83 (0.46–1.49)	0.4105		
Hyperlipidemia	1.03 (0.56–1.89)	0.9567		
Hyperuricemia	0.84 (0.46–1.54)	0.4583		
Use of insulin	0.59 (0.40–0.86)	0.0085*	0.27 (0.09–0.75)	0.0123*
Use of sulfonylurea	1.23 (0.68–2.21)	0.7148		
Use of nateglinide	1.61 (0.14–18.1)	0.7303		
Use of α-GI	1.94 (0.89–4.20)	0.0976		
Use of biguanide	2.75 (1.52–4.99)	0.0020*	2.25 (0.80–6.30)	0.1769
Use of thiazolidine	1.64 (0.39–6.76)	0.6937		
Use of DPP-4, GLP-1	2.76 (1.22–6.23)	0.0185*	2.24 (0.46–10.8)	0.3136
Use of ARB	0.97 (0.54–1.74)	0.8763		
Use of ACE-I	0.79 (0.16–3.86)	0.7199		
Use of α/β-blocker	-	0.0700		
Use of CCB	0.68 (0.39–1.26)	0.1439		
Use of diuretics	1.15 (0.43–3.06)	0.8799		
Use of nitrate	0.96 (0.25–3.62)	0.8828		
Use of aspirin	0.99 (0.44–2.24)	0.9427		

OR, odds ratio; CI, confidence interval; BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; HbA1c, haemoglobin A1c; ALP, alkaline phosphatase; γ-GT, gamma-glutamyl transferase; AST, aspartate amino-transferase; ALT, alanine amino-transferase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BI, Brinkman Index; α-GI, α-glucosidase inhibitor; DPP-4, inhibitors of dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1 receptor agonists; ARB, angiotensin II receptor blocker; ACE-I, angiotensin converting enzyme inhibitor; CCB, calcium channel blocker

\*statistically significant (p < 0.05)



regression in female patients with diabetes mellitus, age [OR (95% CI), 0.92 (0.86–0.99)  $p=0.0207$ ], diabetes mellitus duration [1.28 (1.18–1.39)  $p<0.0001$ ], bilirubin levels [7.36 (1.10–48.9),  $p=0.0389$ ], and insulin use [0.27 (0.09–0.75),  $p=0.0123$ ] were independent associated factors for fatty liver (Table 3). In female patients with diabetes mellitus, alcohol consumption was not associated with fatty liver by univariate analysis.

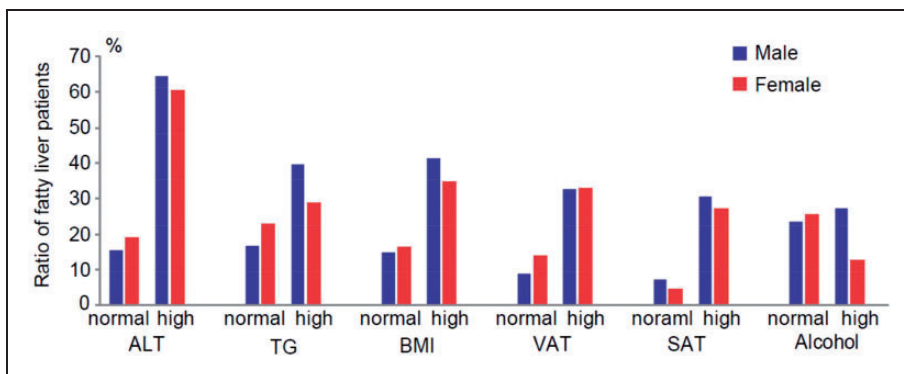
### *The ratio of fatty liver in diabetes mellitus according to ALT, triglyceride, BMI, VAT, SAT, and alcohol consumption levels*

Finally, we examined the incidence of fatty liver in patients with type 2 diabetes mellitus according to ALT, triglyceride, BMI, VAT, SAT, and alcohol consumption levels. Of the patients with type 2 diabetes mellitus, 25.0% (175/700) of male and 24.7% (60/242) of female patients had fatty liver. In the ALT normal group, 15.4% (89/576) of male and 19.1% (40/209) of female patients with type 2 diabetes mellitus were diagnosed with fatty liver. In the ALT high group, fatty liver

was diagnosed in 64.5% (86/124) of male and 60.6% (20/33) of female patients. In the normal BMI group, 14.9% (65/434) of male and 16.1% (21/130) of female patients were diagnosed with fatty liver, and in the high BMI group, 41.3% (110/266) of male and 34.8% (39/112) of female patients were diagnosed with fatty liver. In the normal alcohol consumption group, 23.5% (103/437) of male and 25.6% (58/226) of female patients were diagnosed with fatty liver, and in the high alcohol consumption group, 27.3% (72/263) of male and 12.5% (2/16) of female patients were diagnosed with fatty liver. The ratio of patients with fatty liver in each group is shown in Figure 1.

## Discussion

In this study, we aimed to elucidate the clinical characteristics of patients with diabetes mellitus that were diagnosed with fatty liver by L/S ratio using CT. Our results indicated that, in patients with type 2 diabetes mellitus, younger age, high VAT, and albumin, ALT, and triglyceride levels in



**Figure 1.** The incidence of fatty liver in patients with type 2 diabetes mellitus. The incidence of fatty liver in patients with type 2 diabetes mellitus according to indicated group is shown. The normal ranges are defined as follows: ALT < 40 U/ml; triglycerides < 150 mg/dl; BMI < 25 kg/m<sup>2</sup>; VAT < 100 cm<sup>2</sup>; SAT < 100 cm<sup>2</sup>; and alcohol consumption, male, < 30 g/day and female, < 20 g/day. ALT, alanine amino-transferase; TG, triglyceride; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue

males and younger age and high bilirubin levels in females were associated with fatty liver.

The prevalence of fatty liver diagnosed by CT in patients with diabetes mellitus was 24.4% (175/717) in males and 23.7% (60/252) in females. Furthermore, we showed that there were no patients with type 1 diabetes mellitus diagnosed with fatty liver (28 patients). Jimba et al.<sup>6</sup> previously reported that the prevalence of patients with fatty liver diagnosed by ultrasonography in patients with newly diagnosed diabetes was as high as 62% (28/45). The prevalence may vary with the diagnosis modality and targeted patients. Our study patients had a relatively long history of diabetes mellitus (median, 6 and 20 years in male and female patients with fatty liver, respectively). The age of the study patients would also be important for steatosis. In this examination, multivariate analysis revealed that the prevalence of fatty liver decreased with advancing age both in males and females in agreement with a previous study.<sup>18</sup> The term “burned-out” denotes a significant reduction in hepatic adiposity with the natural course of non-alcoholic steatohepatitis and is a well-known phenomenon.<sup>19</sup> Our results may reflect the natural course of patients with fatty liver diagnosed by CT in the same manner. In females, although younger age was associated with increased fatty liver, longer duration of diabetes mellitus was a risk factor for fatty liver. The bidirectional relationship linking type 2 diabetes mellitus and fatty liver as previously reported<sup>20</sup> could be a possible explanation, and early onset of diabetes mellitus could be a possible risk factor for fatty liver in female patients with diabetes mellitus.

Interestingly, the associated factors for fatty liver were different between males and females.<sup>21</sup> By multivariate analysis, BMI, waist circumference, and SAT were not significant risk factors for fatty liver in males, but high VAT in males was a

significant risk factor, suggesting a critical role of VAT in metabolic syndrome, consistent with a previous report.<sup>22</sup> In contrast, VAT was not a significant risk factor for fatty liver in female patients with type 2 diabetes mellitus by multivariate analysis, in accordance with a previous report indicating a sex difference of the role of VAT in the pathogenesis of fatty liver.<sup>23</sup> Biochemical examinations revealed that high albumin, triglyceride, and ALT levels in males and high bilirubin levels in females were associated risk factors for fatty liver. In this study, the age of female patients was relatively high [median, 67 years (25<sup>th</sup>–75<sup>th</sup> percentile, 60–74)]. The progression of liver fibrosis is reportedly accelerated by postmenopausal status.<sup>24</sup> The increased risk of fibrosis in postmenopausal women could be a potential explanation for the observed sex differences. In this report, alcohol intake was not related to fatty liver in either males or females, consistent with a previous report on patients with diabetes mellitus.<sup>4,25</sup> Alcohol consumption may not be a critical factor in the pathogenesis of fatty liver in patients with diabetes mellitus.

Improvements in steatosis by various medications have previously been reported.<sup>26–29</sup> By univariate analysis in the present study, the ratio of administration of several anti-diabetes medications was statistically different between patients with fatty liver and those without. The only significant difference that remained after adjusting for clinical characteristics, including age and BMI, was insulin use in females. Although our multivariate analysis showed low incidence of fatty liver in female insulin users with type 2 diabetes mellitus, prescription selection depends on several factors, including patient history and extent of complications, which were not included in this study. Subsequently, further examinations are required to identify effective medications for fatty liver.

There are limitations to our study. First, it was a cross-sectional study. We could not

assess the effect of duration of anti-diabetic treatments. Thus, a future prospective study is required. Second, liver biopsies were not included. Fibrosis and hepatitis were not evaluated in this study. Third, some possibly influential information, such as exercise habits, diet, and obstructive sleep apnoea of the patients,<sup>30,31</sup> was not included.

Previous studies have reported that a high percentage (15%–21%) of Asian-Pacific patients with non-alcoholic fatty liver disease are non-obese (with BMI < 25.0 kg/m<sup>2</sup>).<sup>32</sup> In this study, 14.9% and 16.1% of fatty liver cases were diagnosed in male and female patients with type 2 diabetes mellitus with BMI < 25.0 kg/m<sup>2</sup>, respectively (Figure 1). In addition, the present study revealed that 15.4% of males and 19.1% of females with type 2 diabetes mellitus that were diagnosed with fatty liver had normal ALT levels. This is consistent with a previous report showing that non-alcoholic fatty liver disease can be observed in individuals with normal ALT values.<sup>33</sup>

## Conclusion

In conclusion, this study identified the factors associated with fatty liver diagnosed by L/S ratio using CT in patients with type 2 diabetes mellitus. Fatty liver is associated with clinical characteristics of type 2 diabetes mellitus, such as younger age, increased VAT, and elevated albumin, ALT, and triglyceride levels in males and younger age and elevated bilirubin levels in females.

## Abbreviations

CT, computed tomography; L/S ratio, liver/spleen ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; BMI, body mass index; ALP, alkaline phosphatase;  $\gamma$ -GT, gamma-glutamyl transferase; AST, aspartate amino-transferase; ALT, alanine amino-transferase; TC, total

cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

## Author's contributions

Conceived and designed the experiments: KS and HK. Performed the experiments: KS, HK, AT, and HA. Analysed the data: KS, KE, HK, SK, and KK. Wrote the paper: KS. All authors approved the final manuscript.

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The authors declare that there is no conflict of interest.

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