ORIGINAL ARTICLE

Insulin-like growth factor-1 is inversely associated with liver fibrotic markers in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Insulin-like growth factor-1 (IGF-1) regulates mitochondrial function, oxidative stress, inflammation, stellate cells and insulin sensitivity in the liver, and it might be associated with liver fibrosis from non-alcoholic steatohepatitis. In contrast, type 2 diabetes mellitus is closely associated with the progression from non-alcoholic fatty liver to non-alcoholic steatohepatitis and cirrhosis, so careful evaluation of liver fibrosis is required for patients with type 2 diabetes mellitus. Therefore, we examined the relationship between IGF-1 and liver fibrosis markers in type 2 diabetes patients without obvious alcoholic consumption and determined whether IGF-1 is associated with fibrosis of non-alcoholic fatty liver disease.

Materials and Methods: We selected 415 patients with type 2 diabetes without obvious alcohol consumption, who were admitted to Uwajima City Hospital between May 2013 and December 2016. We collected and analyzed clinical data to determine correlations between IGF-1 or IGF-1 standard deviation score and fibrosis-4 index or 7S domain of type IV collagen.

Results: Multiple linear regression analysis showed that the fibrosis-4 index was inversely correlated with IGF-1 and IGF-1 standard deviation score. Furthermore, the 7S domain of type IV collagen was also inversely correlated with IGF-1 and IGF-1 standard deviation score.

Conclusions: IGF-1 was inversely correlated with liver fibrosis markers in type 2 diabetes mellitus patients without obvious alcoholic consumption. Measuring serum IGF-1 levels might help clinicians to identify type 2 diabetes mellitus patients with advanced non-alcoholic steatohepatitis.

INTRODUCTION

Liver fibrosis is one of the most important factors of liver disease progression[1,2], and advanced liver fibrosis is associated with hepatocellular carcinoma and liver failure. In non-alcoholic fatty liver disease (NAFLD), similarly, advanced fibrosis is reported to be a predictor of poor prognosis of liver-related diseases and cardiovascular diseases[3–6], it is therefore desirable to identify high-risk groups for advanced liver diseases among patients, easily.

Type 2 diabetes mellitus is frequently complicated with NAFLD. The prevalence of NAFLD in Japanese patients depends on blood glucose level; 27% in the subgroup with normal fasting glucose, 43% in impaired glucose and 62% in newly diagnosed diabetes[7]. Additionally, the presence of diabetes in NAFLD patients is a significant predictor of moderate-to-severe fibrosis[8,9], and paired biopsies showed that the prevalence of diabetes in NAFLD patients with fibrosis progression was higher than in those with non-progressed NAFLD[10]. Therefore, type 2 diabetes mellitus not only often complicates NAFLD, but is also closely associated with the progression from non-alcoholic fatty

liver (NAFL) to non-alcoholic steatohepatitis (NASH) and cirrhosis, and careful evaluation of advanced NASH is required for patients with type 2 diabetes mellitus. In contrast, insulin-like growth factor-1 (IGF-1) levels are also associated with glucose intolerance[11–13], and glucose intolerance might affect the levels of IGF-1.

IGF-1 is a peptide growth factor and is predominantly secreted from hepatocytes[14]. IGF-1 plays important roles, including stimulating cell growth and proliferation, inhibiting programmed cell death, and affecting various tissues[15–18]. In the liver, IGF-1 regulates mitochondrial function, oxidative stress, inflammation and insulin sensitivity[19–22]. Previous studies have shown that low levels of IGF-1 are observed in patients with various chronic liver diseases. In NAFLD, IGF-1 levels were associated with liver fibrosis markers and the histological fibrosis of NAFLD[23,24]. However, it is unknown whether IGF-1 correlates with liver fibrosis and liver fibrosis markers among type 2 diabetes mellitus patients, although type 2 diabetes mellitus has a high risk of advanced NASH and affects serum IGF-1 levels[25].

In the present study, we examined the relationship between IGF-1, including IGF-1 standard deviation scores (SDS), and liver fibrosis. We evaluated liver fibrosis by the fibrosis-4 (FIB-4) index (which is a non-invasive panel used to predict the fibrosis stage of several liver diseases)[26,27] and 7S domain of type IV collagen (IV-7S) in type 2 diabetes mellitus patients without obvious alcoholic consumption.

METHODS

The present study, which utilized a cross-sectional and partially longitudinal study design, included 415 patients who were aged at least 20 years, had been diagnosed with type 2 diabetes mellitus, and were admitted to Uwajima City Hospital for diabetes treatment between May 2013 and December 2016. Patients who met the following criteria were excluded: daily consumption of ≥30 or ≥20 g of alcohol for men and women; the presence of other liver diseases (e.g., viral or autoimmune hepatitis, primary biliary cirrhosis, metabolic, or genetic liver disease); a cancer diagnosis; or a history of corticosteroids drug use. Of 415, 160 patients had available data from 3 years after their first hospitalization, and these patients were included in the longitudinal observational analysis.

Participants' height and bodyweight at the time of hospitalization were measured without wearing shoes or outer clothing and were used to calculate body mass index (BMI). Data on the patients' exercise and smoking habits were obtained using a questionnaire. The administration of statins and oral hypoglycemic agents, such as glimepiride, metformin, α-glucosidase inhibitors, glinides, pioglitazone, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RAs) and sodium–glucose cotransporter 2 inhibitor (SGLT2i) was determined from medical records. The participants' visceral fat areas were determined from computed tomography images at the navel level with a SYNAPSE VINCENT volume analyzer (FUJIFILM Corporation, Tokyo, Japan).

On the morning after admission, blood pressure was determined using an automated sphygmomanometer after a 15-min resting period, and blood samples were collected after a 12-h fast to evaluate the levels of fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), total cholesterol, triglycerides (TG), high-density (HDL-C) and low-density lipoprotein cholesterol, aspartate (AST) and alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), growth hormone (GH), and IGF-1, as well as platelet count. Serum levels of GH and IGF-1 were measured with Elecsys hGH (Roche Diagnostics K.K., Tokyo, Japan) and IGF-1 assay "Daiichi" (Fujirebio Inc., Tokyo, Japan), respectively. A METABO Rhythm (Eli Lilly Japan K.K., Kobe, Japan) was used to determine the IGF-1 SDS (i.e., IGF-1 level corrected for age and sex)[28]. The IV-7S levels were determined in 176 patients. The diagnosis of type 2 diabetes mellitus was based on The Japan Diabetes Society criteria[29]. The FIB-4 index was calculated as follows: age (years) × AST [U/L] / (platelets [10^9/L] × $\sqrt{\text{ALT [U/L]}}$)[27]. Advanced liver fibrosis was defined when the FIB-4 index was >3.25[26]. Additionally, for those participants in the longitudinal study, FIB-4 index was examined in outpatients using serum after a 12-h fast 3 years after the initial hospitalization.

The protocol for this study was approved by the institutional review board of Uwajima City Hospital (Approval ID no. 163-91, University Hospital Medical Information Network ID: UMIN000021519). This study conformed to the ethical guidelines of the 1983 revision of the Declaration of Helsinki (original publication: 1975). All participants provided written informed consent before participating.

Statistical analysis

To evaluate the correlation between FIB-4 or IV-7S and each clinical variable, Spearman's correlation coefficients were used. If the *P*-value was ≤0.2, the factor was chosen as an independent variable. Mann–Whitney's test was used to compare liver fibrosis markers, IGF-1 and IGF-1 SDS between groups medicated with each agent or not. Each medication was selected as an independent factor if the medication had significant associations with FIB-4 index or IV-7S. Additionally, it was also used to compare the differences of IGF-1 and IGF-1 SDS in the patients with liver fibrosis and without fibrosis of different sexes. Multiple linear regression analysis was then carried out to obtain the association between the FIB-4 index or IV-7S and IGF-1 or IGF-1 SDS. On analysis of the FIB-4 index, model 1 was adjusted for the following factors: sex, BMI, FPG, HbA1c, VFA, SBP, TG, HDL-C, γ-GTP and GH. Model 2 was adjusted for factors that were found to be an independent variable in univariate analyses, such as BMI, FPG, HbA1c, VFA, TG, HDL-C, γ-GTP, glimepiride, glinides and statins. Similarly, on analysis of IV-7S, model 1 was adjusted for the following factors: age, BMI, FPG, HbA1c, VFA, SBP, TG, HDL-C, AST, ALT, γ-GTP, platelets and GH. Model 2 was adjusted for factors that were found to be an independent variable in univariate analyses, such as age, VFA, AST, ALT, γ-GTP and platelets.

The Steel–Dwass test was used to examine the FIB-4 index of each quartile based on IGF-1 and IGF-1 SDS among the different sexes. All statistical analyses were calculated using JMP version 12.0 software (SAS Institute Japan Inc., Tokyo, Japan). P-values <0.05 were considered significant.

RESULTS

Baseline characteristics

Table 1 summarizes the baseline characteristics of the participants. The mean age, BMI and HbA1c were 61.9 years, 25.8 kg/m^2 and 9.8%, respectively, and the mean liver enzymes were mildly high (Table 1). The administration rates of oral hypoglycemic agents and statins were 58.8 and 38.1%,

respectively. The FIB-4 index and the IV-7S were 1.6 ± 1.1 and 4.0 ± 1.1 ng/mL, respectively. Patients taking glimepiride, glinides and statins had higher FIB-4 indices than those who did not take these drugs (Table S1). However, there was no significant difference on IV-7S between patients who were taking hypoglycemic agents compared with those who were not (Table S1). The mean IGF-1 was 118.6 ng/mL and the mean IGF-1 SDS was −0.54 (Table 1). On IGF-1 and IGF-1 SDS, there was no significant difference between patients who were taking hypoglycemic agents compared with those who were not (Table S2). In sex-specific comparisons, the mean age of men was significantly lower than that of women. In addition, the current smoker ratio, the mean levels of HDL-C, γ-GTP and

Table 1 | Baseline characteristics of patients (n = 415)

Characteristics	Total (n = 415)		Men (n = 248)		Women (n = 167)		P-value
	Mean ± SD	Participants (%)	Mean ± SD	Participants (%)	Mean ± SD	Participants (%)	
Age (years)	61.9 ± 12.6		60.3 ± 12.0		64.3 ± 13.1		<0.0002
BMI (kg/m^2)	25.8 ± 4.8		25.6 ± 4.7		26.1 ± 4.9		0.37
FPG (mg/dL)	164.3 ± 53.2		164.2 ± 54.2		164.6 ± 51.7		0.88
HbA1c (%)	9.8 ± 2.1		9.9 ± 2.2		9.8 ± 1.9		0.80
Regular exercise		63 (15.2)		52 (21.0)		11 (6.6)	0.049
Current smoker		62 (14.9)		57 (23.0)		5 (3.0)	<0.0001
Glimepiride		127 (30.6)		70 (28.2)		57 (34.1)	0.23
Metformin		75 (18.1)		43 (17.3)		32 (19.2)	0.70
α-GI		61 (14.7)		36 (14.5)		25 (15.0)	0.89
Glinides		6 (1.4)		4 (1.6)		2 (1.1)	1.00
DPP4i		153 (36.9)		83 (33.5)		70 (41.9)	0.10
Pioglitazone		23 (5.5)		17 (6.9)		6 (3.6)	0.19
SGLT2i		4 (0.1)		3 (1.2)		1 (0.6)	0.65
GLP-1 RA		2 (0.5)		0 (0.0)		2 (1.2)	0.16
Statins		158 (38.1)		90 (36.2)		68 (40.7)	0.41
VFA (cm^2)	142.6 ± 68.2		149.2 ± 75.9		133.8 ± 55.3		0.12
SBP (mmHg)	129.9 ± 20.2		128.3 ± 19.1		132.2 ± 21.5		0.20
DBP (mmHg)	75.4 ± 13.2		75.4 ± 13.2		75.3 ± 13.1		0.76
T-chol (mg/dL)	189.6 ± 45.7		186.7 ± 45.1		193.9 ± 46.3		0.11
TG (mg/dL)	165.9 ± 136.3		172.6 ± 152.8		156.0 ± 107.1		0.11
HDL-C (mg/dL)	49.9 ± 14.0		47.4 ± 13.3		53.6 ± 14.2		<0.0001
LDL-C (mg/dL)	118.3 ± 40.5		117.0 ± 40.1		120.3 ± 41.0		0.44
AST (U/L)	27.8 ± 18.8		27.3 ± 18.1		28.6 ± 19.8		0.52
ALT (U/L)	32.3 ± 27.3		31.6 ± 23.5		33.2 ± 32.1		0.76
γ-GTP (IU/L)	57.7 ± 92.9		68.3 ± 113.6		42.2 ± 44.5		<0.0001
Platelets ($\times 10^4/\mu$L)	22.7 ± 7.7		21.6 ± 7.3		24.2 ± 8.0		0.0001
FIB-4 index	1.60 ± 1.08		1.64 ± 1.19		1.54 ± 0.91		0.96
IV-7S (ng/mL)	4.04 ± 1.12		4.02 ± 1.11		4.08 ± 1.14		0.81
GH (ng/mL)	0.55 ± 1.36		0.64 ± 1.56		0.43 ± 0.99		0.39
IGF-1 (ng/mL)	118.6 ± 50.2		128.2 ± 52.9		104.3 ± 42.3		<0.0001
IGF-1 SDS	−0.54 ± 1.42		−0.48 ± 1.46		−0.6 ± 1.36		0.34

Data were expressed as mean ± standard deviation or frequency (percentage). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; DPP4i, dipeptidyl peptidase 4 inhibitors; FIB-4, fibrosis-4; FPG, fasting plasma glucose; GH, growth hormone; GLP-1 RA, glucagon like peptide-1 receptor agonists; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; IGF-1, insulin-like growth factor-1; IV-7S, 7S domain of type IV collagen; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SD, standard deviation; SDS, standard deviation score; SGLT2i, sodium glucose cotransporter 2 inhibitors; T-chol, total cholesterol; TG, triglyceride; VFA, visceral fat area; α-GI, α-glucosidase inhibitors; γ-GTP, gamma glutamyl transpeptidase.

IGF-1, and platelet counts were significantly different between men and women (Table 1).

Clinical variables correlated with FIB-4 index and IV-7S

A positive correlation was observed between the FIB-4 index and the serum levels of γ-GTP, and an inverse correlation was observed between FIB-4 index and BMI, FPG, HbA1c, serum levels of IGF-1, and IGF-1 SDS (Table 2, Figure 1a). In contrast, IV-7S showed a positive correlation with age, VFA, FIB-4 index and serum levels of AST, ALT and γ-GTP; and an inverse correlation with platelets, IGF-1 and IGF-1 SDS (Table 2; Figure 1b). In men, the FIB-4 index and IV-7S were inversely correlated with IGF-1 SDS ($r = -0.33$, $P < 0.0001$ and $r = -0.43$, $P < 0.0001$, respectively; Figure S1a,b). In contrast, only IV-7S was inversely correlated with IGF-1 SDS ($r = -0.41$, $P = 0.01$) in women (Figure S1c,d).

Correlation between FIB-4 index and IGF-1

The results of the multiple linear regression analysis with FIB-4 index as the dependent variable are shown in Tables 3, S3 and S4. Model 1 showed that the FIB-4 index was inversely correlated with IGF-1 (β −0.32, B −0.007, 95% confidence interval [CI] −0.009, −0.005, $P < 0.0001$) and IGF-1 SDS (β −0.22, B −0.17, 95% CI −0.24, −0.09, $P < 0.0001$). Similarly, model 2 showed that the FIB-4 index was inversely correlated with IGF-1

Table 2 | Correlations between each liver fibrotic marker and clinical variables

	FIB-4 index ($n = 415$)		IV-7S ($n = 176$)	
	r	P-value	r	P-value
Age	NA	NA	0.22	0.004
BMI	−0.10	0.045	0.09	0.24
FPG	−0.14	0.007	−0.08	0.32
HbA1c	−0.15	0.003	−0.006	0.94
VFA	0.10	0.06	0.16	0.04
SBP	−0.04	0.39	−0.06	0.44
TG	−0.07	0.19	−0.10	0.21
HDL-C	0.07	0.13	0.03	0.72
AST	NA	NA	0.37	<0.0001
ALT	NA	NA	0.21	0.006
γ-GTP	0.34	<0.0001	0.35	<0.0001
Platelets	NA	NA	−0.28	0.0001
FIB-4 index	NA	NA	0.48	<0.0001
GH	0.03	0.57	0.09	0.21
IGF-1	−0.38	<0.0001	−0.43	<0.0001
IGF-1 SDS	−0.26	<0.0001	−0.43	<0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, fibrosis-4; FPG, fasting plasma glucose; GH, growth hormone; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; IGF-1, insulin-like growth factor-1; IV-7S, 7S domain of type IV collagen; NA, not applicable; SBP, systolic blood pressure; SDS, standard deviation score; TG, triglyceride; VFA, visceral fat area; γ-GTP, gamma glutamyl transpeptidase.
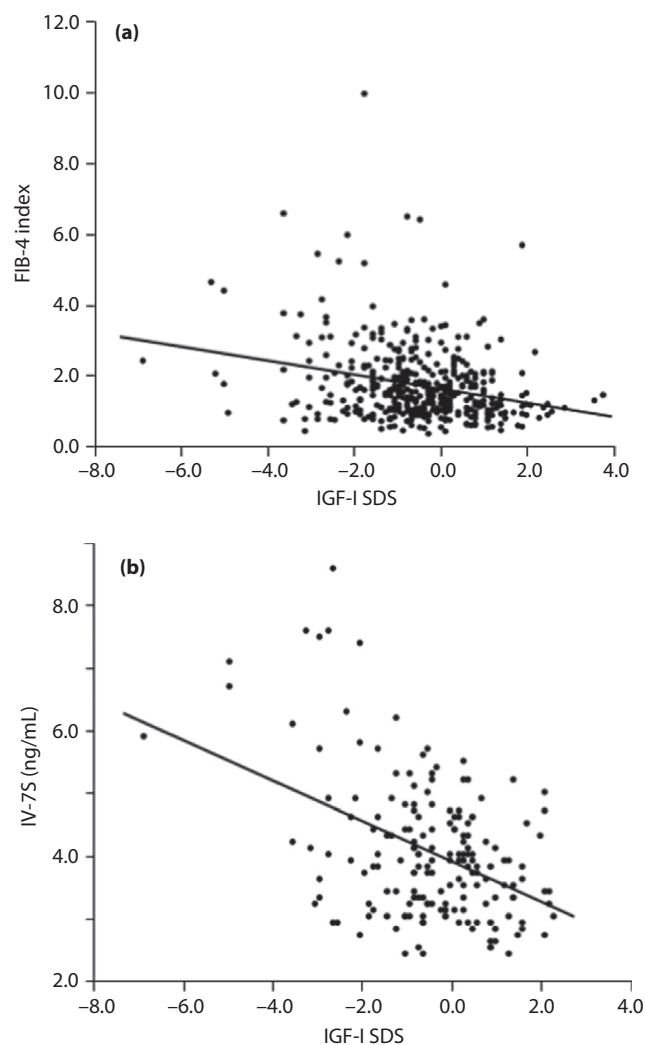

Figure 1 | (a) Correlation between insulin-like growth factor-1 (IGF-1) standard deviation scores (SDS) and the fibrosis-4 (FIB-4) index ($n = 415$). IGF-1 SDS was inversely correlated with the FIB-4 index (r −0.26, $P < 0.0001$). (b) Correlation between IGF-1 SDS and 7S domain of type IV collagen (IV-7S; $n = 176$). IGF-1 SDS was inversely correlated with IV-7S (r −0.43, $P < 0.0001$)

(β −0.29, B −0.006, 95% CI −0.009, −0.004, $P < 0.0001$) and IGF-1 SDS (β −0.22, B −0.17, 95% CI −0.25, −0.10, $P < 0.0001$).

Correlation between IV-7S and IGF-1

The results of the multiple linear regression analysis with IV-7S as the dependent variable are shown in Tables 3, S5 and S6. Age-adjusted analysis showed that IV-7S was inversely correlated with IGF-1 (β −0.4, B −0.009, 95% CI −0.01, −0.006, $P < 0.0001$) and IGF-1 SDS (β −0.44, B −0.34, 95% CI −0.44, −0.24, $P < 0.0001$). Model 1 showed that IV-7S was inversely correlated with IGF-1 (β −0.24, B −0.006, 95% CI −0.009, −0.002, $P = 0.004$) and IGF-1 SDS (β −0.27, B −0.20, 95% CI −0.31, −0.09, $P = 0.0004$). Similarly, model 2 showed that

IV-7S was inversely correlated with IGF-1 (β −0.27, B −0.007, 95% CI −0.01, −0.003, P = 0.0004) and IGF-1 SDS (β −0.30, B −0.23, 95% CI −0.34, −0.13, P < 0.0001).

Analysis of FIB-4 index grouped by quartiles of IGF-1 and IGF-SDS on different sexes

The results of analysis of the FIB-4 index grouped by quartiles of IGF-1 and IGF-1 SDS are shown in Figure 2. The average levels of quartile 1 of IGF-1 were significantly lower than that of quartile 4 in both sexes (Figure 2a,c). In contrast, the average levels of quartile 1 of IGF-1 SDS in men were significantly higher than that of quartile 4, but there was no significant difference in women (Figure 2b,d).

Analysis of the levels of IGF-1 and IGF-1 SDS in patients with versus without advanced liver fibrosis of different sexes

Table S7 shows the results of the analysis of the levels of IGF-1 and IGF-1 SDS in patients with and without advanced liver fibrosis in different sexes. In men, the levels of IGF-1 and IGF-1 SDS with advanced liver fibrosis were significantly lower than that of the patients without advanced liver fibrosis. In women, the levels of IGF-1 SDS were not significantly different between those patients with advanced liver fibrosis compared with those without advanced liver fibrosis, although the levels of IGF-1 in patients with advanced liver fibrosis were significantly lower than that of those patients without advanced liver fibrosis.

Longitudinal observational analysis between high IGF-1 level group versus low IGF-1 level group

Table 4 shows the results of the longitudinal observational analysis between the high IGF-1 level group versus low IGF-1 level group. The cut-off value of IGF-1 was defined as 96.0 ng/mL for advanced liver fibrosis (FIB-4 index >3.25; Table S8). The change of the FIB-4 index for 3 years in the patients in the low IGF-1 level group was significantly worse compared with the patients in the high IGF-1 level group (P = 0.049).

DISCUSSION

In the present study, we examined the relationship between IGF-1 and liver fibrosis markers in type 2 diabetes mellitus patients without obvious alcoholic consumption, and showed that IGF-1 was inversely correlated with FIB-4 index and IV-7S. This association remained significant after adjusting for potential confounders. Additionally, the liver fibrosis marker of the patients with low IGF-1 levels was worse than that of patients with high IGF-1 levels after 3 years.

Previously, several studies have shown the relationship between IGF-1 and liver fibrosis marker and histological liver fibrosis in NAFLD patients. Hribal et al.[23] showed that plasma IGF-1 levels inversely correlated with NAFLD fibrosis scores in 221 NAFLD patients, diagnosed by ultrasonography and with at least one cardiometabolic risk factor. Furthermore, they showed that the liver IGF-1 messenger ribonucleic acid levels were inversely associated with the fibrosis stage in 50 biopsy-proven NAFLD patients. Ichikawa et al. examined the plasma IGF-1 levels in 52 NAFLD patients, and reported that plasma low IGF-1 levels were associated with stage 2–3 by multivariate logistic regression analysis (stage 0–1 vs stage 2–3)[24]. Garcia-Galiano et al.[30] analyzed 36 NAFLD patients with morbid obesity, and identified IGF-1 <110 ng/mL as an independent predictor of NASH (NAFLD activity score 5–8) by the multivariate regression analysis. Additionally, Colak et al.[31]

Table 3 | Results of the multiple linear regression analysis of the fibrosis-4index and 7S domain of type IV collagen (n = 176) and the insulin-like growth factor-1 and insulin-like growth factor-1 standard deviation score

	Age-adjusted				Model 1				Model 2			
	β	B	95% CI (Low, High)	P value	β	B	95% CI (Low, High)	P value	β	B	95% CI (Low, High)	P value
FIB-4 index												
n = 415												
IGF-1	NA	NA	NA	NA	−0.32	−0.007	−0.009, −0.005	<0.0001	−0.29	−0.006	−0.009, −0.004	<0.0001
IGF-1 SDS	NA	NA	NA	NA	−0.22	−0.17	−0.24, −0.09	<0.0001	−0.22	−0.17	−0.25, −0.10	<0.0001
IV-7S												
n = 176												
IGF-1	−0.40	−0.009	−0.01, −0.006	<0.0001	−0.24	−0.006	−0.009, −0.002	0.004	−0.27	−0.007	−0.01, −0.003	0.0004
IGF-1 SDS	−0.44	−0.34	−0.44, −0.24	<0.0001	−0.27	−0.20	−0.31, −0.09	0.0004	−0.30	−0.23	−0.34, −0.13	<0.0001

The 95% confidence intervals (CI) were expressed as (Low, High). Fibrosis-4 (FIB-4) index: model 1 was adjusted for the following factors: sex, body mass index (BMI), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), visceral fat area (VFA), systolic blood pressure (SBP), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), gamma glutamyl transpeptidase (γ-GTP) and growth hormone (GH). Model 2 was adjusted for factors that were found to be an independent variable in univariate analyses, such as BMI, FPG, HbA1c, VFA, TG, HDL-C, γ-GTP, glimepiride, glinides and statins. IV-7S: Model 1 was adjusted for the following factors: age, sex, BMI, FPG, HbA1c, VFA, SBP, TG, HDL-C, AST, ALT, γ-GTP, platelets and GH. Model 2 was adjusted for factors that were found to be an independent variable in univariate analyses, such as age, VFA, AST, ALT, γ-GTP and platelets. ALT, alanine aminotransferase; AST, aspartate aminotransferase; B, partial regression coefficient; IGF-1, insulin-like growth factor-1; IV-7S, 7S domain of type IV collagen.
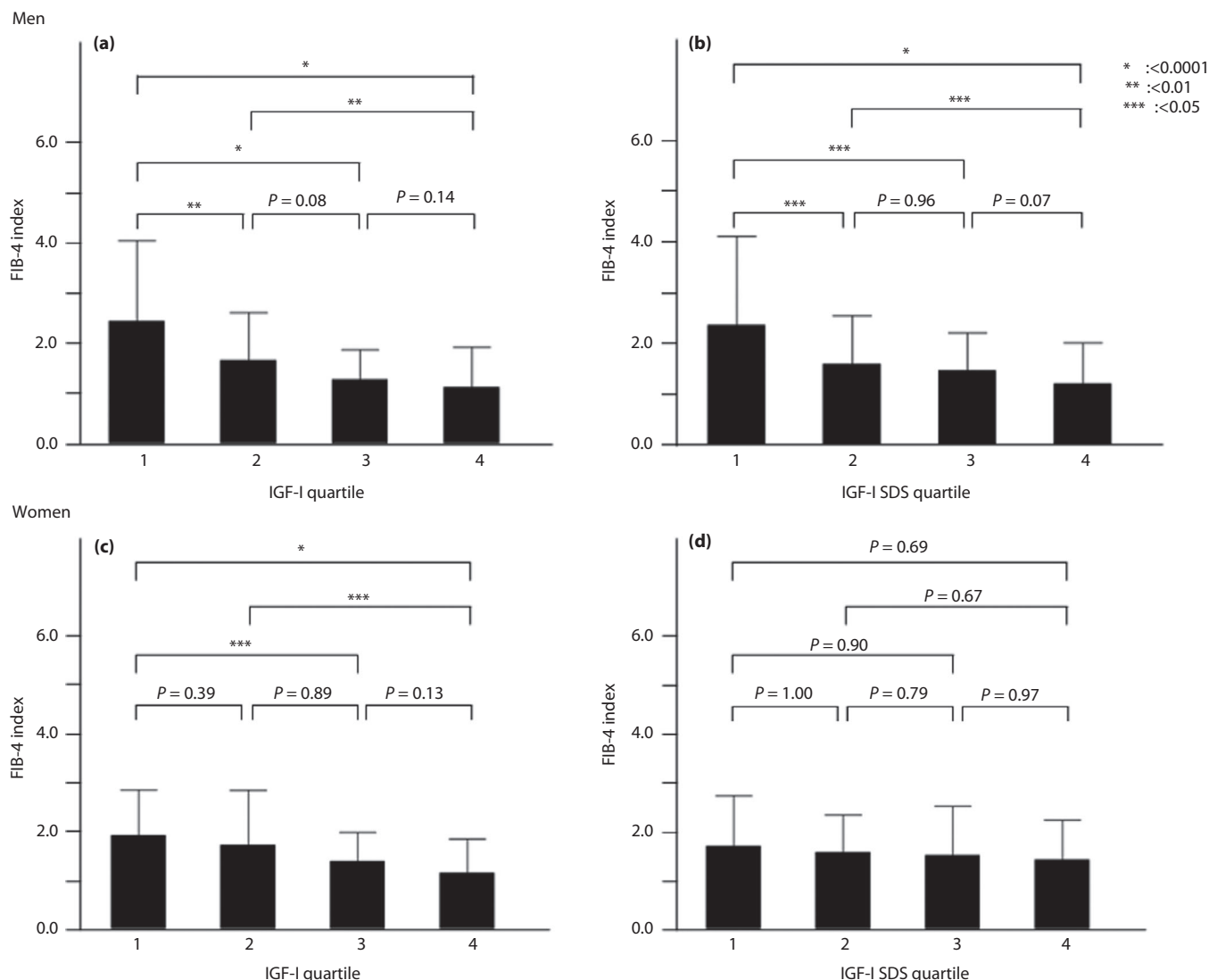
Men

Women

Figure 2 | (a) Analysis of the fibrosis-4 (FIB-4) index grouped by quartiles of insulin-like growth factor-1 (IGF-1) in men. (b) Analysis of FIB-4 index grouped by quartiles of IGF-1 standard deviation scores (SDS) in men. (c) Analysis of FIB-4 index grouped by quartiles of IGF-1 in women. (d) Analysis of FIB-4 index grouped by quartiles of IGF-1 SDS in women.

Table 4 | Differences in the fibrosis-4 index 3 years after hospitalization between two groups divided by insulin-like growth factor-1 cut-off levels

IGF-1 (ng/mL)	Low IGF-1 level group (<96.0)	High IGF-1 level group (≥96.0)	P-value
n	59	101	
⊿FIB-4	0.27 ± 1.31	−0.03 ± 0.06	0.049

The ⊿ fibrosis-4 (FIB-4) was calculated as follows: FIB-4 index (3 years) − FIB-4 index (original).

collected the serum samples of 92 NAFLD patients, and showed IGF-1 levels were significantly decreased in patients with moderate-to-severe fibrosis (stage 2–3) compared with patients with no or mild fibrosis (stage 0–1). However, these findings were not determined with IGF-1 levels adjusted by age. In contrast, Sumida *et al.*[26] investigated the SDS of IGF-1 by age and sex in 199 NAFLD patients, and showed that the IGF-1 SDS decreased significantly with increasing lobular inflammation and fibrosis, and showed in the multivariate logistic regression analysis the significant association between the IGF-1 SDS values and the severity of NAFLD (NASH vs NAFL or fibrosis stage 0–2 vs 3–4) after adjusting for age, sex and insulin resistance. Additionally, Dichtel *et al.*[32] examined 21 controls with no steatosis, lobular inflammation or fibrosis and 121 NAFLD patients, and showed that IGF-1 was lower in individuals with lobular inflammation, hepatocyte ballooning, higher fibrosis stages (stage 2–4 vs 0–1) and NASH; all factors examined

remained significant after controlling for age, BMI and the diagnosis of diabetes. However, these studies did not examine the relationship between IGF-1 and liver fibrosis among type 2 diabetes mellitus, which are high-risk groups of advanced liver disease; hence, this was our primary outcome measure. Previous reports showed that glucose intolerance is associated with low levels of IGF-1, and insulin secretion is important for the maintenance of IGF-1 level[11–13]. Therefore, progression of liver fibrosis in diabetes mellitus patients might be affected by both diabetes itself and low levels of IGF-1, and proceed further.

The relationships between IGF-1 and liver fibrosis markers in type 2 diabetes mellitus patients without obvious alcoholic consumption in the present study might be associated with several potential mechanisms. The IGF-1 level regulates mitochondrial functions and oxidative stress, and corrects inflammation molecules. Perez et al.[33] showed that IGF-1 administration exerted a mitochondrial protection (which resulted in the reduced apoptosis and increased adenosine triphosphate production), as well as improved liver dysfunction and fibrosis in a rat cirrhotic model. Sanz et al.[34] reported that the overexpression of IGF-1 in hepatic stellate cells, restricted hepatic stellate cells activation, attenuated fibrosis and accelerated liver regeneration in a cirrhotic model. Furthermore, IGF-1 stimulated the production of hepatocyte growth factor, which is a mitogen for hepatocytes, and suppressed the fibrosis in a cirrhotic model[35]. Therefore, the low IGF-1 levels might be a risk factor for the progression of liver fibrosis.

In contrast, antidiabetic agents affect the pathophysiology of NASH. Pioglitazone, GLP-1RA and SGLT2i are expected to improve inflammation and fibrosis of NASH[35–38]. However, in the present study, the relationship between liver fibrosis markers and antidiabetic agents, such as GLP-1RA and SGLT2i, were not significant. Similarly, antidiabetic agents did not affect the IGF-1 level. In a small sample size and short duration study, glimepiride treatment for 6 weeks for patients with type 1 diabetes with multiple insulin injection therapy increased serum levels of IGF-1[39]. An experimental study also showed that sitagliptin significantly increased the plasma IGF-1 level in rabbits[40]. The reasons for why there are differences between the present results and previous studies might be because the number of patients treated with GLP-1RA and SGLT2i was small.

Sex differences were observed in the analysis of IGF-1 SDS. In women, the levels of IGF-1 with liver fibrosis were significantly lower than that of without liver fibrosis. However, the levels of IGF-1 SDS were not significant between those with liver fibrosis and without liver fibrosis. These results might be affected by estrogen. In women, as they grow older, estrogen decreases as well as IGF- I. The decrease of estrogen is associated with the progression and severity of NAFLD[41,42]. Therefore, the decrease of not only IGF- I, but also estrogen, might affect the present results.

The present study had a few limitations. First, we did not carry out a liver biopsy, which is a gold standard examination for liver fibrosis. However liver biopsy is an invasive examination for patients and expensive, and a several studies used surrogate markers for predicting the degree of hepatic fibrosis instead of liver biopsy[43,44]. Second, as patients were selected from a single center, selection bias might have occurred. Third, IV-7S data were not available for all participants. However, it is important to add the fibrosis marker in addition to the FIB-4 index to prove that the relationship between IGF-1 and fibrosis is more certain; the present study showed a relationship between IV-7S and IGF-1. Fourth, we did not measure the estrogen level in women. Therefore, we could not research the effect of estrogen to liver fibrosis. Finally, this was a cross-sectional study, and causal relationships between IGF-1 levels and liver fibrosis could not be established in this analysis. Therefore, further prospective studies are required.

Despite the above-mentioned limitations, the present study provided several notable results. In particular, our findings showed that low serum IGF-1 levels are a possible risk factor for liver fibrosis in type 2 diabetes mellitus patients. This result might help clinicians to identify type 2 diabetes mellitus patients with advanced NASH by measuring serum IGF-1 levels. Therefore, we might need to pay attention to IGF-1 in type 2 diabetes mellitus patients.

DISCLOSURE
The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Association between liver fibrosis markers and each medication.

Table S2 | Association of insulin-like growth factor-1, insulin-like growth factor-1 standard deviation score and each medication.

Table S3 | Results of the multiple linear regression analysis of the fibrosis-4 index and insulin-like growth factor-1.

Table S4 | Results of the multiple linear regression analysis of fibrosis-4 index and insulin-like growth factor-1 standard deviation score.

Table S5 | Results of the multiple linear regression analysis of 7S domain of type IV collagen and insulin-like growth factor-1 ($n = 176$).

Table S6 | Results of the multiple linear regression analysis of 7S domain of type IV collagen and insulin-like growth factor-1 standard deviation score ($n = 176$).

Table S7 | Analysis of insulin-like growth factor-1 levels and insulin-like growth factor-1 standard deviation score in patients with and without advanced liver fibrosis of different sexes.

Table S8 | Accuracy of insulin-like growth factor-1 for each fibrosis marker.

Figure S1 | (a) The correlation between insulin-like growth factor-1 (IGF-1) standard deviation score (SDS) and fibrosis-4 (FIB-4) index in men ($n = 248$). IGF-1 SDS was significantly correlated with the FIB-4 index (r −0.33, $P < 0.0001$). (b) The correlation between IGF-1 SDS and 7S domain of type IV collagen (IV-7S) in men ($n = 140$). IGF-1 SDS was significantly correlated with IV-7S (r −0.43, $P < 0.0001$). (c) Correlation between IGF-1 SDS and FIB-4 index in women ($n = 167$). IGF-1 SDS was not significantly correlated with the FIB-4 index (r −0.12, $P = 0.13$). (d) The correlation between IGF-1 SDS and IV-7S in women ($n = 36$). IGF-1 SDS was significantly correlated with IV-7S (r −0.41, $P = 0.01$).