Histological Course of Nonalcoholic Fatty Liver Disease in Japanese Patients

Tight glycemic control, rather than weight reduction, ameliorates liver fibrosis

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OBJECTIVE — The goal of this study was to examine whether metabolic abnormalities are responsible for the histological changes observed in Japanese patients with nonalcoholic fatty liver disease (NAFLD) who have undergone serial liver biopsies.

RESEARCH DESIGN AND METHODS — In total, 39 patients had undergone consecutive liver biopsies. Changes in their clinical data were analyzed, and biopsy specimens were scored histologically for stage.

RESULTS — The median follow-up time was 2.4 years (range 1.0–8.5). Liver fibrosis had improved in 12 patients (30.7%), progressed in 11 patients (28.2%), and remained unchanged in 16 patients (41%). In a Cox proportional hazard model, decrease in A1C and use of insulin were associated with improvement of liver fibrosis independent of age, sex, and BMI. However, ΔA1C was more strongly associated with the improvement of liver fibrosis than use of insulin after adjustment for each other (χ^2; 7.97 vs. 4.58, respectively).

CONCLUSIONS — Tight glycemic control may prevent histological progression in Japanese patients with NAFLD.

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Accumulating trans-sectional evidence suggests that the presence of multiple metabolic disorders, including obesity, diabetes, dyslipidemia, hypertension, and ultimately metabolic syndrome, are associated with nonalcoholic fatty liver disease (NAFLD) (1). However, it remains unclear which metabolic abnormalities are responsible for the pathological progression of NAFLD, especially in Japanese patients, who generally are not severely obese compared with Western patients.

We retrospectively compared clinical features with the histological changes in the livers of Japanese patients with NAFLD who had undergone serial liver biopsies.

RESEARCH DESIGN AND METHODS

— We recruited 195 patients with clinically suspected NAFLD who had undergone liver biopsies at Kanazawa University Hospital from 1997 through 2008. For details about the study subjects and the exclusion criteria, see supplementary Fig. 1 in the online appendix, available at http://care.diabetesjournals.org/cgi/content/full/dc09-0148/DC1. Of 178 patients diagnosed histologically as having NAFLD, 39 had undergone serial liver biopsies.

Data collection

Clinical information, including age, sex, body measurements, and prevalence of metabolic abnormalities, was obtained for each patient. Venous blood samples drawn for laboratory testing before the liver biopsies were obtained. All subjects had been administered a 75-g oral glucose tolerance test at baseline and at follow-up.

Liver biopsies

Biopsies were obtained after a thorough clinical evaluation and receipt of signed informed consent from each patient. All biopsies were analyzed twice and at separate times randomly by a single pathologist who was blinded to the clinical information and the order in which the biopsies were obtained. The biopsied tissues were scored for steatosis, stage, and grade as described (2), according to the standard criteria for grading and staging of nonalcoholic steatohepatitis proposed by Brunt et al. (3).

For additional details on subjects, data collection methods, liver pathology, and statistical analyses, see supplementary Methods in the online appendix.

RESULTS

— The basal clinical and biochemical data from 39 patients with NAFLD are described in supplementary Table 1. Prevalence of type 2 diabetes, hypertension, and dyslipidemia were 77, 36, and 64%, respectively. The median follow-up period was 2.4 years (range 1.0–8.5). Medications for diabetes and medication changes during the follow-up period are described in supplementary Table 2. Seventeen patients treated with oral diabetic agents were switched to insulin therapy after the initial biopsy. No patients initiated pioglitazone during follow-up.

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Table 1—*Baseline and follow-up clinical features and gradients of laboratory markers associated with changes in liver fibrosis in 39 patients with NAFLD*

	Baseline				Follow-up			
	Improved	Stable	Progressed	*P*	Improved	Stable	Progressed	*P*
n	12	16	11		12	16	11	—
Simple fatty liver:nonalcoholic steatohepatitis (*n*)	3:9	9:7	10:1		10:2	9:7	6:5	
Age (years)	51.5 (29–66)	48.5 (20–79)	51.5 (29–66)	0.97				
Sex (M:F)	5:7	12:4	5:7	0.17				
BMI (kg/m^2)	27.5 (23.2–34.1)	27.7 (22.5–44.4)	30.9 (23.4–37.7)	0.74	26.9 (22.8–31.2)	29.1 (24.3–44.8)	30.7 (24.1–36.3)	0.13
Aspartate transaminase (IU/l)	70 (11–106)	29 (14–86)	32 (13–83)	0.05	23 (11–28)	26 (15–71)	24 (14–164)	0.20
Alanine transaminase (IU/l)	71 (10–209)	48 (23–81)	40 (11–162)	0.13	21 (11–53)	36 (21–66)	31 (12–202)	0.10
Fasting plasma glucose (mg/dl)	133 (96–207)	143 (87–414)	111 (76–167)	0.20	103 (93–220)	121 (83–198)	116 (88–199)	0.51
A1C (%)	8.2 (4.7–11.6)	8.0 (4.9–13.6)	6.2 (5.1–9.5)	0.27	6.0 (5.0–9.0)	6.2 (5.0–10.0)	7.0 (6.0–11.0)	0.10
HOMA-IR	3.9 (0.7–5.5)	3.4 (1.9–7.7)	3.9 (1.6–11.1)	0.91	3.1 (1.5–8.5)	3.4 (1.9–7.7)	3.9 (1.6–11.1)	0.76
QUICKI	0.32 (0.29–0.40)	0.31 (0.27–0.34)	0.31 (0.29–0.35)	0.32	0.33 (0.28–0.37)	0.32 (0.30–0.35)	0.31 (0.29–0.34)	0.82
Muscle insulin resistance	2.1 (1.5–4.0)	1.7 (0.3–3.3)	3.0 (2.1–4.4)	0.20	2.0 (1.3–5.9)	2.4 (1.6–4.5)	1.9 (1.3–4.5)	0.80
Hepatic insulin resistance ($\times 10^6$)	5.3 (2.3–10.2)	5.0 (2.3–10.0)	3.7 (1.4–10.6)	0.66	3.9 (1.4–9.8)	4.3 (1.9–15.9)	4.5 (2.3–8.8)	0.75
Total cholesterol (mg/dl)	191 (128–276)	187 (129–252)	206 (163–244)	0.57	192 (114–224)	195 (136–273)	194 (162–234)	0.74
Triglycerides (mg/dl)	111 (28–224)	114 (36–204)	96 (36–521)	0.87	104 (22–241)	115 (57–241)	131 (36–173)	0.68
HDL cholesterol (mg/dl)	47 (35–82)	51 (31–73)	48 (20–74)	0.68	53 (40–71)	52 (39–64)	52 (36–79)	0.92
Platelets ($\times 10^4/\mu$l)	21.1 (9.4–30.8)	23.0 (7.0–38.2)	24.3 (20.2–41.2)	0.29	23.3 (14.5–27.6)	21.5 (6.3–31.8)	24.0 (15.2–32.6)	0.60
Ferritin (μg/dl)	185 (13–452)	397 (190–604)	46 (10–347)	0.14	74 (16–211)	162 (110–614)	62 (10–171)	0.05
hs-CRP	0.40 (0.08–7.53)	0.14 (0.02–0.61)	0.06 (0.00–0.30)	0.23	0.09 (0.04–0.23)	0.10 (0.00–0.24)	0.09 (0.00–0.89)	0.89
Type IV collagen 7S (ng/dl)	5.1 (2.7–10.0)	4.1 (3.1–7.2)	3.7 (3.3–4.5)	0.27	3.5 (2.3–3.9)	8.3 (3.2–14.0)	4.0 (3.2–5.0)	0.21
HA (ng/dl)	20.6 (0.0–144.7)	25.5 (11.5–299)	30.4 (0.0–61.7)	0.66	32.8 (0.0–117.2)	24.5 (0.0–570)	24.3 (0.0–140.3)	0.63
P-III-P (U/ml)	0.6 (0.5–1.2)	0.6 (0.4–45.0)	0.5 (0.4–0.6)	0.07	0.6 (0.3–0.8)	0.5 (0.5–233.0)	0.6 (0.4–1.0)	0.96
Diabetes (%)	82	69	64	0.59	82	75	64	0.56
Dyslipidemia (%)	73	63	73	0.95	73	63	73	0.86
Hypertension (%)	64	18	36	0.03	64	18	36	0.10
Metabolic syndrome (%)	73	38	27	0.18	67	50	45	0.43
ΔA1C					−1.9 (−6.0 to 0.4)	−1.2 (−6.1 to 4.4)	0.3 (−1.8 to 7.1)	0.02
ΔBody weight					−4.7 (−10.6 to 10.2)	2.2 (−9.4 to 13.4)	−0.9 (−12.7 to 9.6)	0.04
ΔHOMA-IR					−1.3 (−4.4 to 1.2)	−0.3 (−4.3 to 3.3)	−0.7 (−6.1 to 1.8)	0.81

Data are medians (range) or %. A Kruskal-Wallis test and a χ^2 test were used to compare the continuous and categorical variables among three groups. HA, hyaluronic acid; hs-CRP, high-sensitivity C-reactive protein; P-III-P, procollagen III peptide.

Liver fibrosis improved in 12 patients (30.7%), progressed in 11 patients (28.2%), and remained unchanged in 16 patients (41%). As shown in Table 1, fasting plasma glucose, A1C, insulin resistance indicators, and prevalence of metabolic disorders were not significantly different among the three liver fibrosis groups. In the Cox proportional hazard model (supplementary Table 3), although some of the confidence intervals were very wide because of the small sample size, improvement of liver fibrosis was significantly associated with changes in A1C between the initial and final liver biopsies (ΔA1C) ($P = 0.005$) and use of insulin for the treatment of diabetes ($P = 0.019$). Both ΔA1C and use of insulin were independently associated with the improvement of liver fibrosis after adjusted for each other. However, ΔA1C was more strongly associated with the improvement of liver fibrosis than use of insulin (χ^2; 7.97 vs. 4.58, respectively; supplementary Table 3).

CONCLUSIONS — In the present study, we showed that a change in glycemic control (ΔA1C), but not changes in insulin resistance indicators, was an independent predictor of the progression of liver fibrosis in Japanese patients with NAFLD. This is the first report identifying a change in A1C as a predictor of the histological course in the liver of patients with NAFLD. Two of five previous longitudinal studies have identified obesity, higher BMI, and homeostasis model assessment of insulin resistance (HOMA-IR) as predictors of liver fibrosis progression in Western populations (4,5). The difference between those results and the results of the present study may be due in part to differences in the assessed severity of obesity and insulin resistance between the populations. We previously demonstrated that diabetes is an independent risk factor for the progression of liver fibrosis in hepatitis C (6) and that diabetes accelerates the pathology of nonalcoholic steatohepatitis in the type 2 diabetic rat model OLETF (7).

Liver fibrosis is closely associated with two regulators of fibrosis: transforming growth factor (TGF)-β (8,9) and plasminogen activator inhibitor type 1 (PAI-1) (8,10). High glucose levels induce the expression of TGF-β (11) and PAI-1 (12). We previously reported that the expression of TGF family member genes, PAI-1, and genes involved in fibrogenesis are upregulated in the livers of patients with type 2 diabetes (13,14), suggesting that a diabetic state increases the risk for liver fibrosis.

In the present study, only ΔA1C was associated with the progression of liver fibrosis, but not liver inflammation (data not shown). We speculate that the reduction of A1C inhibits the expression of master genes such as *TGF-β* and *PAI-1* that are involved in the regulation of fibrogenesis, rather than genes involved in liver inflammation, and thereby improves liver fibrosis in NAFLD.

The major limitation of this study was small population size. We could not evaluate the changes of liver histology according to the difference in detail characteristics such as treatment of diabetes. Lower statistical power of this study should be consider when we evaluate the results.

In conclusion, our study suggested that ΔA1C could predict liver fibrosis progression in Japanese patients with NAFLD, and tight glycemic control may ameliorate liver fibrosis. Future large-scale prospective studies are needed to confirm our results.

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