Table 1 Clinical characteristics of patients in the estimation and validation groups

Clinical parameter	Estimation group ($n = 177$)			Validation group ($n = 442$)		
	NASH ($n = 98$)	NonNASH ($n = 79$)	P value	NASH ($n = 244$)	NonNASH ($n = 198$)	P value
Age (years)	57.7 ± 15.2	47.8 ± 16.5	0.0001	54.2 ± 15.2	48.2 ± 14.3	<0.0001
Gender (female)	58 (59%)	28 (35%)	0.0024	127 (52%)	77 (39%)	0.0072
BMI (kg/m^2)	28.5 ± 5.0	26.6 ± 4.7	0.0075	28.4 ± 5.0	27.4 ± 4.7	0.0207
Obesity (BMI >25)	74 (76%)	46 (58%)	0.0311	187 (77%)	139 (70%)	0.1548
Dyslipidemia	57 (58%)	39 (49%)	0.2886	173 (71%)	149 (75%)	0.3339
Hypertension (yes)	33 (34%)	12 (15%)	0.0055	100 (41%)	47 (24%)	0.0002
Type 2 diabetes (yes)	50 (51%)	14 (18%)	<0.0001	121 (50%)	69 (35%)	0.0027
Hemoglobin (g/dl)	14.1 ± 1.6 ($n = 97$)	14.8 ± 1.5 ($n = 78$)	0.0029	14.7 ± 2.5 ($n = 239$)	14.7 ± 1.5 ($n = 195$)	0.9758
Platelet count ($\times 10^4$/μl)	21.3 ± 6.4 ($n = 97$)	24.8 ± 8.1 ($n = 78$)	0.0018	21.2 ± 6.7 ($n = 240$)	24.1 ± 5.7 ($n = 194$)	<0.0001
AST (IU/l)	70 ± 30	44 ± 25	<0.0001	88 ± 387	38 ± 22	0.0694
ALT (IU/l)	102 ± 53	79 ± 54	0.0002	111 ± 217	65 ± 43	0.0001
AST/ALT ratio	0.77 ± 0.32	0.63 ± 0.23	0.0022	0.75 ± 0.32	0.65 ± 0.21	0.0001
GGT (IU/l)	105 ± 128	86 ± 66 ($n = 78$)	0.1211	85 ± 73 ($n = 242$)	85 ± 102 ($n = 197$)	0.9852
Cholinesterase (IU/l)	365 ± 83 ($n = 93$)	390 ± 83 ($n = 78$)	0.0317	364 ± 89 ($n = 224$)	387 ± 85 ($n = 176$)	0.0091
Total cholesterol (mg/dl)	206 ± 43 ($n = 97$)	214 ± 42 ($n = 77$)	0.2431	207 ± 43 ($n = 195$)	210 ± 39 ($n = 125$)	0.5121
Triglyceride (mg/dl)	189 ± 106 ($n = 93$)	167 ± 81 ($n = 73$)	0.1365	172 ± 106 ($n = 241$)	173 ± 86	0.9038
Ferritin (ng/ml)	270.7 ± 231	160 ± 158	0.0011	346 ± 989	183 ± 159	0.0221
FPG (mg/dl)	108 ± 45	96 ± 17.0	0.0301	113 ± 63	105 ± 39	0.1081
IRI (μU/ml)	18.5 ± 14.7	9.6 ± 6.3	<0.0001	16.8 ± 12.9	11.9 ± 8.3	<0.0001
Hyaluronic acid (ng/ml)	95 ± 134	29 ± 30	<0.0001	67 ± 74 ($n = 211$)	34 ± 37 ($n = 181$)	<0.0001
Type IV collagen 7S (ng/ml)	5.4 ± 1.7	3.9 ± 0.7	<0.0001	5.2 ± 2.1	3.9 ± 0.8	<0.0001
Histological fibrosis						
0–1	47 (48%)			143 (59%)		
2	29 (30%)			56 (23%)		
3	18 (18%)			30 (12%)		
4	4 (4%)			15 (6%)		

Results are presented as numbers with percentages *in parenthesis* for qualitative data or as means ± SD for quantitative data

BMI Body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin

P values were calculated by t test or χ^2 analysis

(≥55 years), female sex, obesity, Hb ≤14.5 g/dl, presence of T2DM, platelet count ≤22 × 10^4/μl, AST ≥60 IU/l, ALT ≥90 IU/l, AAR ≥0.8, ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male), FPG ≥100 mg/dl, IRI ≥10.0 μU/ml, HA ≥50 ng/ml, and type IV collagen 7S ≥5.0 ng/ml were significant variables. By multivariate analysis, three variables remained significant, including ferritin, IRI, and type IV collagen 7S. Thus, these three variables, ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male), IRI ≥10.0 μU/ml, and type IV collagen 7S ≥5.0 ng/ml, were combined to form the NAFIC score. The score was weighted based on OR obtained from logistic regression analysis (Table 2). Ferritin was given 1 point, IRI 1 point, and type IV collagen 7S 2 points. A score ranging from 0 to 4, defined by the presence of laboratory parameters, was calculated. The score for NASH patients ($n = 98$, 2.36 ± 1.28) was significantly higher than that for nonNASH patients ($n = 79$, 0.66 ± 0.82, $P < 0.0001$). The percentage of NASH in NAFLD with an NAFIC score of 0, 1, 2, 3, and 4 was 14% (6/44), 44% (34/61), 74% (14/19), 100% (28/28), and 92% (23/25), respectively (Fig. 1). The score was significantly higher even in NASH patients without significant fibrosis (stage 0 or 1) ($n = 47$, 1.83 ± 1.15) than in nonNASH patients ($P < 0.0001$). Figure 2 shows the ROC curve for NAFIC score to differentiate NASH from NAFLD. This scoring system had an AUROC of 0.851. At a cutoff value of NAFIC score 2, the sensitivity, specificity, PPV, and NPV were 66, 91, 90, and 67%, respectively. At a cutoff value of NAFIC score 1, the sensitivity, specificity, PPV, and NPV were 94, 48, 31, and 86%, respectively.

Table 2 Results of univariate and multivariate analysis: independent predictors of NASH and assigned score values in the estimation group ($n = 177$)

Variables	Unadjusted (univariate)			Adjusted (multivariate)			Score value
	OR	95%CI	*P* value	OR	95%CI	*P* value	
Age ≥55 years	2.28	1.24–4.18	0.0077				
Gender (female)	2.64	1.43–4.87	0.0019				
Obesity (BMI ≥25)	2.10	1.09–4.04	0.0268				
Hemoglobin ≤14.5 g/dl	1.94	1.06–3.56	0.0312				
Hypertension	2.83	1.35–5.96	0.0060				
Type 2 diabetes	4.84	2.40–9.74	<0.0001				
Platelet count $\leq 22 \times 10^4/\mu l$	2.66	1.43–4.91	0.0019				
AST ≥60 IU/l	5.74	2.81–11.73	<0.0001				
ALT ≥90 IU/l	2.04	1.10–3.77	0.0230				
AST/ALT ratio ≥0.8	1.98	1.18–4.76	0.0153				
Cholinesterase ≤380 IU/l	1.55	0.83–2.90	0.1689				
Ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male)	5.08	2.48–10.37	<0.0001	4.01	1.07–15.02	0.0396	1
FPG ≥100 mg/dl	2.25	1.19–4.26	0.0127				
IRI ≥10 μU/ml	5.33	2.78–10.22	<0.0001	5.59	1.71–18.31	0.0045	1
Hyaluronic acid ≥50 ng/ml	4.94	2.38–10.26	<0.0001				
Type IV collagen 7S ≥5.0 ng/ml	21.20	7.19–62.49	<0.0001	15.54	1.49–162.39	0.0219	2

OR Odds ratio, *CI* confidence interval, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin

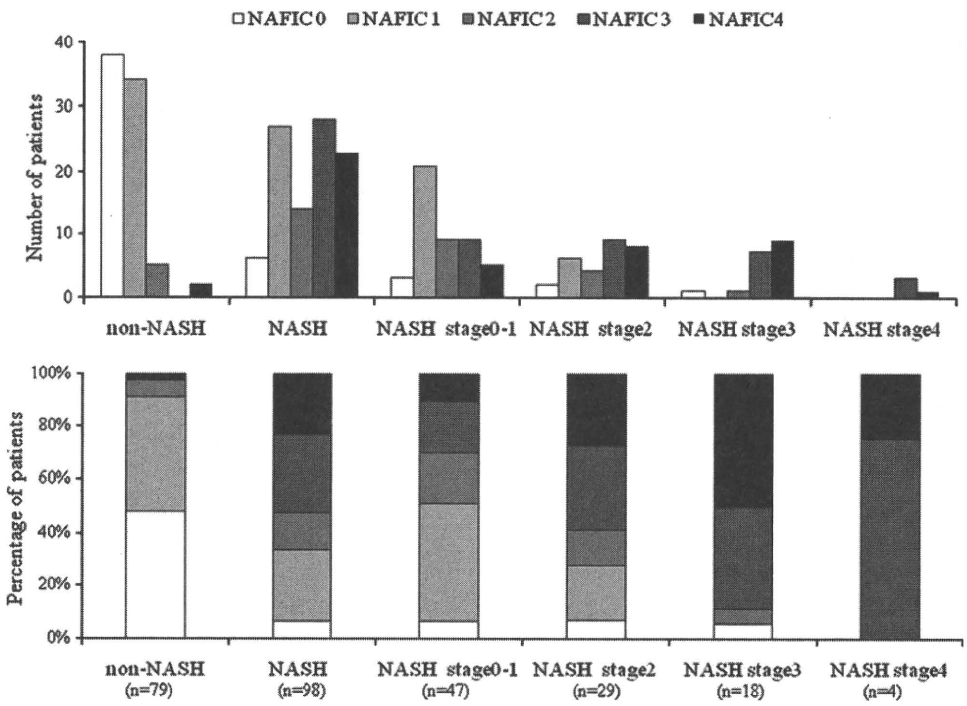

Fig. 1 Distribution of NAFIC scores in patients with NASH and nonNASH in the estimation group ($n = 177$)

Validation results

The diagnostic accuracy of the scoring system in separating patients with and without NASH was validated in 442 patients. Table 1 summarizes the clinical, laboratory, and liver biopsy data of the patient population in the validation group. Two hundred and two patients (46%) were female, and 326 (74%) patients were obese. Patients with NASH were significantly older, predominantly female, heavier, hypertensive, and more likely to have T2DM; had lower platelet count and ChE level; and had higher levels of AST, ALT, AAR, ferritin, IRI, HA, and type IV collagen 7S,

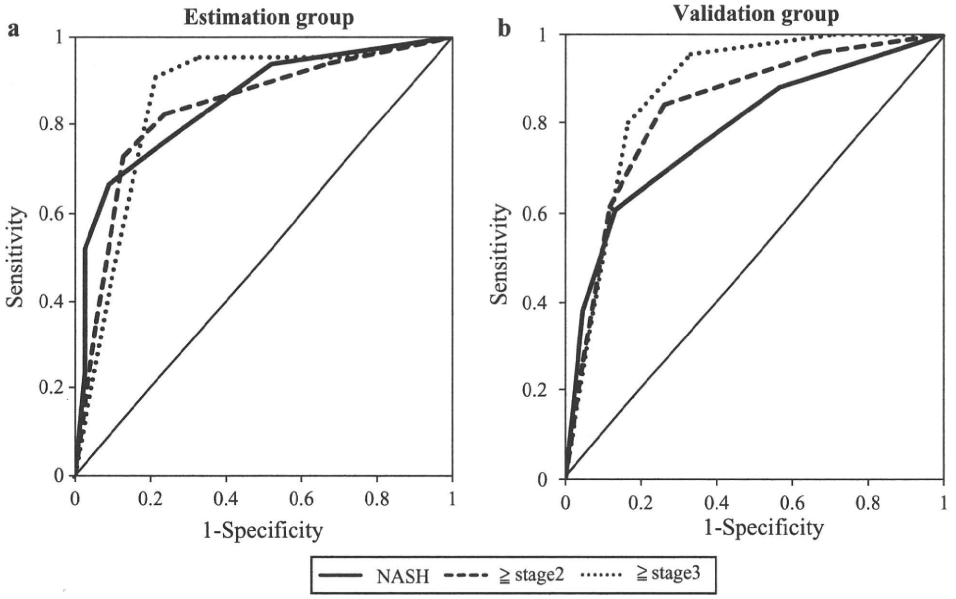

Fig. 2 ROC curves for the NAFIC score in the estimation (**a**) and validation (**b**) groups

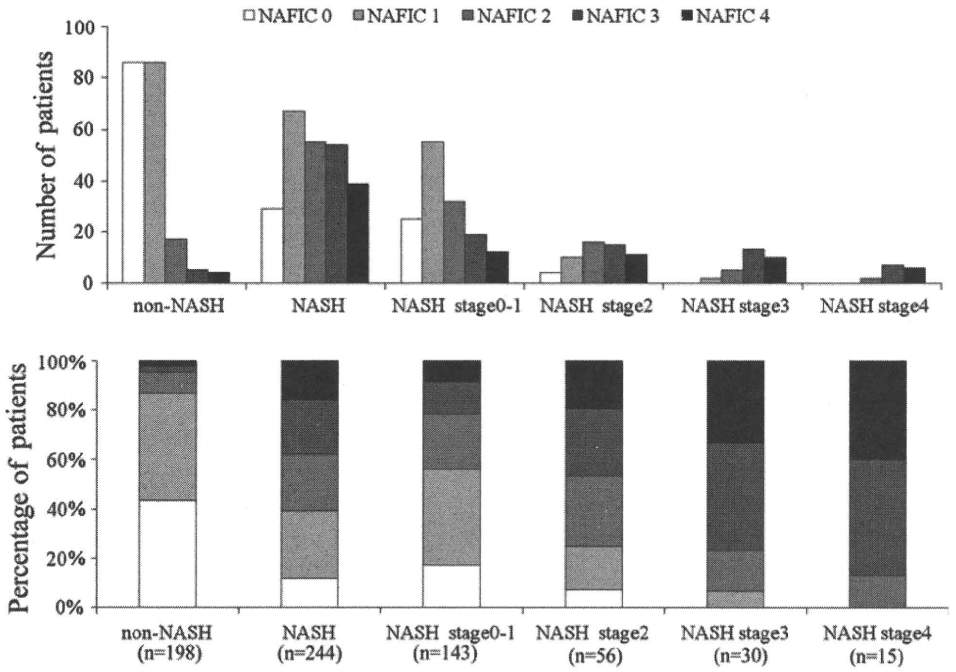

Fig. 3 Distribution of NAFIC score in patients with NASH and nonNASH in the validation group ($n = 442$)

than those with nonNASH NAFLD. The NAFIC score of NASH patients ($n = 244$, 2.03 ± 1.27) was significantly higher than that of nonNASH patients ($n = 198$, 0.76 ± 0.87, $p < 0.0001$). The percentage of NASH in NAFLD with an NAFIC score of 0, 1, 2, 3, and 4 was 25% (29/115), 44% (67/153), 76% (55/72), 92% (54/59), and 91% (39/43), respectively (Fig. 3). The score was significantly higher even in NASH patients without significant fibrosis (stage 0 or 1) ($n = 143$, 1.57 ± 1.17) than non-NASH patients ($p < 0.0001$). The AUROC remained relatively high in the validation set (0.782, Fig. 2; Table 3). At a cutoff value of NAFIC score 2, the sensitivity, specificity, PPV, and NPV were 60, 87, 85 and 64%, respectively. At a cutoff value of NAFIC score 1, the sensitivity, specificity, PPV, and NPV were 88, 43, 66, and 75%, respectively.

Comparing the NAFIC score to several previously established scoring systems

The AUROCs of various scoring systems that have been reported to differentiate NASH from NAFLD, or advanced from mild fibrosis, are shown in Table 3. To differentiate NASH from NAFLD, the AUROC in the validation group

Table 3 AUROC of NAFIC score and various scoring systems previously reported in the estimation and validation groups

Score	Group	AUROC for NASH	AUROC for significant fibrosis	AUROC for advanced fibrosis
NAFIC	Estimation ($n = 177$)	0.851	0.835	0.856
	Validation ($n = 442$)	0.782	0.833	0.874
	Total ($n = 619$)	0.803	0.834	0.869
HAIR [15]	Estimation ($n = 177$)	0.632	0.549	0.448
	Validation ($n = 432$)	0.636	0.620	0.631
	Total ($n = 609$)	0.631	0.593	0.566
Palekar et al. [17]	Estimation ($n = 173$)	0.784	0.794	0.847
	Validation ($n = 390$)	0.711	0.798	0.826
	Total ($n = 563$)	0.733	0.799	0.835
Modified Palekar et al.	Estimation ($n = 173$)	0.780	0.801	0.843
	Validation ($n = 390$)	0.709	0.810	0.830
	Total ($n = 563$)	0.730	0.808	0.837
Gholam et al. [18]	Estimation ($n = 177$)	0.829	0.784	0.713
	Validation ($n = 442$)	0.758	0.787	0.739
	Total ($n = 619$)	0.777	0.786	0.729
BAAT [19]	Estimation ($n = 164$)	0.672	0.533	0.473
	Validation ($n = 440$)	0.633	0.560	0.498
	Total ($n = 604$)	0.647	0.585	0.526
Modified BAAT	Estimation ($n = 164$)	0.741	0.615	0.566
	Validation ($n = 440$)	0.666	0.654	0.576
	Total ($n = 604$)	0.687	0.641	0.573
BARD [20]	Estimation ($n = 164$)	0.646	0.686	0.745
	Validation ($n = 440$)	0.621	0.689	0.731
	Total ($n = 604$)	0.627	0.688	0.734
Modified BARD	Estimation ($n = 164$)	0.647	0.709	0.734
	Validation ($n = 440$)	0.603	0.689	0.730
	Total ($n = 604$)	0.614	0.695	0.730
NAFLD fibrosis score [21]	Estimation ($n = 168$)	0.735	0.843	0.834
	Validation ($n = 420$)	0.663	0.805	0.862
	Total ($n = 588$)	0.685	0.817	0.853
N score (Nippon) [22]	Estimation ($n = 177$)	0.733	0.739	0.728
	Validation ($n = 408$)	0.642	0.715	0.698
	Total ($n = 585$)	0.668	0.720	0.704

was greatest for NAFIC (0.782, Fig. 2), then Gholam's score (0.758), followed by Palekar's score (0.711), modified Palekar's score (0.709), modified BAAT (0.666), NFS (0.663), *N* score (0.642), HAIR (0.636), BAAT (0.633), BARD score (0.621), and modified BARD score (0.603). Based on an evaluation of AUROC, NAFIC score outperformed other scoring systems in the estimation and validation groups to differentiate NASH from NAFLD. To differentiate NASH with significant fibrosis from NAFLD, the AUROC in the total cohort was greatest for NAFIC score (0.834), then NFS (0.817), followed by modified Palekar's score (0.808), Palekar's score (0.799), Gholam's score (0.786), *N* score (0.720), modified BARD (0.695), BARD (0.688), modified BAAT score (0.641), HAIR (0.593), and BAAT score (0.585). To differentiate NASH with advanced fibrosis from NAFLD, the AUROC in the total cohort was greatest for NAFIC score (0.869), then NFS (0.853), followed by modified Palekar's score (0.837), Palekar's score (0.835), BARD (0.734), modified BARD (0.730), Gholam's score (0.729), *N* score (0.704), modified BAAT (0.573), HAIR (0.566), and BAAT score (0.526). Among these prediction models, NAFIC score was superior to others in not only detecting NASH, but also predicting fibrosis stage.

We compared the diagnostic accuracy of NAFIC score to that of NFS in detecting advanced fibrosis (stage 3–4)

Table 4 Accuracy of the NAFIC score and NAFLD fibrosis score (NFS) in predicting advanced fibrosis (stage 3–4) and significant fibrosis (stage 2–4) in the total cohort

	NAFIC score			NAFLD fibrosis score		
	0–1	2	3–4	Low cutoff point (<−1.455)	Indeterminate (−1.455 to 0.676)	High cutoff point (>0.676)
Predicting advanced fibrosis (stage 3–4)						
N (%)	374 (60%)	90 (15%)	155 (25%)	330 (56%)	209 (36%)	49 (8%)
Stage 0–2	371	82	99	325	171	28
Stage 3–4	3	8	56	5	38	21
Se	96%		84%	92%		33%
Sp	67%		82%	62%		95%
PPV	26%		36%	23%		43%
NPV	99%		98%	98%		92%
LR (+)	2.913		4.660	2.427		6.141
LR (−)	0.067		0.200	0.126		0.710
Interpretation	Absence of advanced fibrosis (99% certainty)		Presence of advanced fibrosis (36% certainty)	Absence of advanced fibrosis (98% certainty)		Presence of advanced fibrosis (43% certainty)
	NAFIC score			NAFLD fibrosis score		
	0	1	2–4	Low cutoff point (<−1.455)	Indeterminate (−1.455 to 0.676)	High cutoff point (>0.676)
Predicting significant fibrosis (stage 2–4)						
N (%)	160 (26%)	214 (35%)	245 (40%)	330 (56%)	209 (36%)	49 (8%)
Stage 0–1	153	196	118	305	122	16
Stage 2–4	7	18	127	25	87	33
Se	95%		84%	86%		23%
Sp	33%		74%	69%		96%
PPV	32%		52%	47%		67%
NPV	96%		93%	92%		79%
LR (+)	1.416		3.266	2.657		6.301
LR (−)	0.141		0.070	0.250		0.801
Interpretation	Absence of significant fibrosis (96% certainty)		Presence of significant fibrosis (52% certainty)	Absence of significant fibrosis (92% certainty)		Presence of significant fibrosis (67% certainty)

Se Sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR* likelihood ratio

and significant fibrosis (stage 2–4) (Table 4). To exclude advanced fibrosis using the low cutoff point (NFS < −1.455), 325 of 330 (98%) patients were correctly staged, whereas only 5 (2%) were understaged. The NPV of this cutoff for advanced fibrosis was 98%. Using the low cutoff point (NAFIC ≤1), 371 of 374 (99%) patients were correctly staged, whereas only 3 (1%) were understaged. The NPV of this cutoff for advanced fibrosis was 99%, which was equal to that of NFS. Using the high cutoff point (NFS >0.676), 21 of 49 (43%) patients were correctly staged, whereas 28 (57%) were overstaged. The PPV of this cutoff for advanced fibrosis was 43%. Using the high cutoff point (NAFIC ≥3), 56 of 155 (36%) patients were correctly staged, whereas 99 (64%) were overstaged. The PPV of this cutoff for advanced fibrosis was 36%, which was lower than that of NFS. The percentage of the undetermined range was much lower for the NAFIC score (15%) than for NFS (36%) (Table 4).

When the NFS low cutoff (NFS <−1.455) was applied to predict significant fibrosis, 305 of 330 (92%) patients were correctly staged, whereas 25 of 330 (8%) patients were understaged. The NPV for significant fibrosis was 92%. When their high cutoff (NFS >0.676) was applied to predict significant fibrosis, 33 of 49 (67%) patients were correctly staged, whereas 16 of 49 (33%) patients were overstaged. The PPV for significant fibrosis was 67%. When our low cutoff point (NAFIC = 0) was applied to exclude significant fibrosis, 153 of 160 (96%) patients were correctly staged, whereas only seven (4%) were understaged. The NPV of this cutoff for significant fibrosis was 96%, which

was slightly higher than NFS. Using our high cutoff point (NAFIC ≥2), 127 of 245 (52%) patients were correctly staged, whereas 118 (48%) were overstaged. The PPV of this cutoff for significant fibrosis was 52%, which was lower than that of NFS (67%) (Table 4).

Discussion

In this study, we developed and validated a simple scoring system to differentiate NASH from NAFLD. Our scoring system with the three variables ferritin, IRI, and type IV collagen 7S had an AUROC of 0.851 and 0.782 in the estimation and validation groups, respectively. Elevation of serum ferritin levels, a marker of iron storage, is associated with NASH [24, 25]. We previously reported high frequencies of hyperferritinemia and increased hepatic iron stores in Japanese NASH patients [11]. Yoneda and colleagues [26], our collaborative research group, also have reported that measurement of serum ferritin is useful to distinguish NASH from NAFLD. Their optimal cutoff value was 196 ng/ml, and their results for sensitivity, specificity, PPV, and NPV were 64, 77, 89, and 43%, respectively. Serum ferritin levels have been found to be a significant independent predictor of severe fibrosis in 167 Italian NAFLD subjects [27], but this has not been confirmed by other studies [28]. In Western countries, mildly increased serum ferritin does not necessarily indicate coexisting iron overload. However, it is well known that serum ferritin is closely associated with IR and can be considered a marker for metabolic syndrome [29].

Hyperinsulinemia (IRI ≥10.0 μU/ml) was also selected as an independent predictor of NASH. Hyperinsulinemia and increased IR could have important roles in the pathogenesis of NASH in both Western and Asian countries [30–33]. Hyperinsulinemia in NASH patients is attributable to increased insulin secretion, which compensates for reduced insulin sensitivity, and is not the consequence of decreased hepatic extraction of insulin, which occurs in all forms of CLD at the stage of advanced fibrosis or cirrhosis [30, 31]. The homeostasis model assessment (HOMA) has been validated and widely used for determining the degree of IR, and strongly predicts the development of T2DM [34]. Patients with NASH have a higher HOMA index compared with those with nonNASH NAFLD [30, 35]. Similarly, another study has reported the QUICKI model as being useful for predicting NASH [15]. However, appropriate cutoff values of these models have never been established. In contrast with these parameters that are mathematical transformations of FPG and IRI levels, fasting IRI, which has the advantage of being easily determined without calculations, was only applied to multivariate analysis in our study.

Type IV collagen is one of the extracellular matrices that are produced by hepatic fibroblasts. The 7S domain in the N-terminus of type IV collagen is inserted in tissues and released into the blood by turnover in connective tissues. Therefore, the serum 7S domain level increases in parallel with the amount of fibrosis and in synthesis from stellate cells and myofibroblasts following increased liver fibrosis [36]. In Japan, type IV collagen 7S is now widely used for assessing the extent of hepatic fibrosis in CLD because the test is covered by public health insurance. According to two reports [37, 38], a cutoff point of 5.0 or 4.25 ng/ml provided high NPV to exclude advanced fibrosis in Japanese NAFLD patients. Shimada et al. [39] have demonstrated that a cutoff point of 5.0 ng/ml provided sensitivity and specificity of 41 and 95%, respectively, to detect early-stage NASH. Serum HA levels are elevated during accelerated deposition of collagen in the extracellular space due to upregulation of HA production by activated stellate cells and myofibroblasts, and downregulation of its clearance by sinusoidal endothelial cells. Serum HA appears to be a relatively accurate predictor of advanced fibrosis stage in NAFLD, but less for distinguishing between minor degrees of fibrosis in NASH and nonNASH NAFLD [40, 41]. In our study, serum HA level was not an independent predictor of NASH by multivariate analysis. Moreover, HA increases in systematic inflammatory conditions, which might produce false-positive results. We believe that type IV collagen 7S is superior to HA in predicting the extent of fibrosis in NAFLD patients.

Currently, the NAFLD biomarkers have been evaluated for (1) distinguishing NASH from NAFLD and/or (2) diagnosing advanced fibrosis or cirrhosis. HAIR [15], Palekar's [17], and Gholam's [18] scores were derived for distinguishing NASH from NAFLD, and the others are for detecting significant or advanced fibrosis. The present study clearly demonstrated that NAFIC score was more useful than other scoring systems for detection of NASH and for prediction of fibrosis (Table 3). The HAIR score uses a combination of presence of hypertension, elevated ALT (>40 U/L), and IR, with at least two parameters that indicate NASH with high sensitivity and specificity [15]. None had an IR index >5 in our overall cohort (data not shown), and AUROCs were 0.632 and 0.634 in the estimation and validation groups, respectively. The score does not appear useful in a less obese population because it was described in a group of severely obese patients who were undergoing gastric bypass surgery. In Palekar's study [17], the presence of at least three out of six factors (age ≥50 years, female sex, AST ≥45 IU/l, BMI ≥30 kg/m^2, AAR ≥0.80, and HA ≥55 ng/ml) had a sensitivity and specificity for NASH diagnosis of 74 and 66%, respectively. In our estimation group, the presence of at least three of these factors had a sensitivity and specificity for

NASH diagnosis of 68 and 71%, respectively. In our validation group, the presence of at least three of these factors had a sensitivity and specificity for NASH diagnosis of 63 and 64%, respectively. AUROCs were 0.784 and 0.711 in the estimation and validation groups, respectively. Therefore, Palekar's score was not superior to NAFIC score for predicting NASH (Table 3).

Gholam's score [18], which consists of only two variables (AST and the presence of DM), is very simple and equally useful as NAFIC score for detection of NASH, but it was not superior to our score for predicting severe fibrosis in our cohort. Gholam et al. [18] have constructed other models that consist of ALT and HbA1c to detect the presence of fibrosis. We could not evaluate these models because HbA1c was only measured in a limited number of patients. Angulo et al. [21] have shown that the NFS, which consists of six variables (age, BMI, AAR, IFG/DM, platelet count, and albumin), can reliably predict advanced fibrosis. In ROC analysis, NFS is shown to be useful for prediction of advanced or significant fibrosis. The low cutoff point (NFS <-1.455) showed higher NPV (98%) than that in the estimation (93%) and validation (88%) cohort reported by Angulo et al. The low cutoff point in NAFIC score (≤ 1) had equally high NPV (99%). In contrast, the high cutoff point (NFS >0.676) showed lower PPV (43%) than that in the estimation (98%) and validation (80%) cohort reported by Angulo et al. The high cutoff point in NAFIC score (≥ 3) had lower PPV (36%). By applying the low cutoff score (NFS <-1.455, NAFIC ≤ 1), advanced fibrosis could be excluded with high accuracy. By applying the high cutoff score (NFS >0.676, NAFIC ≥ 3), the presence of advanced fibrosis could not be diagnosed with high accuracy. Consistent with our results, a separate validation study of NFS in 162 Chinese patients found that the NPV for excluding advanced fibrosis was 91%, but the PPV for predicting advanced fibrosis was 0% [42]. It is suggested that this low PPV might be due to lower prevalence of advanced fibrosis in the study of Wong et al. (11%) [42] than in that by Angulo et al. (27%) [21]. Similarly, the prevalence of advanced fibrosis was low (11%) in our study.

In Asian patients, steatohepatitis and other metabolic complications tend to develop at a lower BMI, which is one of the factors in the equation of the NFS. Therefore, NFS and NAFIC score were applicable to exclusion rather than detection of significant or advanced fibrosis. NFS can be easily obtained in clinical practice, but this scoring system can be cumbersome and difficult to apply in every practice. The easily determined NAFIC score is at least equivalent to the more complex NFS. Our results suggest that liver biopsies can be avoided in NAFLD patients with a NAFIC score of 0 or 1 because they are likely to have NAFLD without advanced fibrosis. In contrast, liver biopsies should be recommended in NAFLD patients with an NAFIC score of ≥ 2 to assess the extent of hepatic fibrosis and predict prognosis. The BARD score developed by Harrison et al. is a weighted sum of three easily available variables [BMI ≥ 28 kg/m^2 (1 point), AAR ≥ 0.8 (2 points), and DM (1 point)], and the authors have shown that a score of 2–4 was associated with an OR of 17 for predicting advanced fibrosis [20]. Although the BARD score is simple to calculate, our validation study did not reveal an advantage of this score over others. In our cohort, when a BARD score of ≥ 2 was found, the sensitivity, specificity, PPV, and NPV for detecting advanced fibrosis were 73, 65, 19, and 95%, respectively. According to a study of 122 Japanese NAFLD patients by Fujii and colleagues [43], our collaborative research group, when a BARD score of ≥ 2 was used, the AUROC was 0.73 with an OR of 4.9 for detection of advanced fibrosis. It has been concluded that BARD score is less predictive of advanced fibrosis in Japanese NAFLD patients because they are not as obese as those in Western countries. Disappointingly, modified scores of Palekar's score, BAAT, and BARD could not improve the diagnostic accuracy for NASH or advanced fibrosis. The *N* score (the total number of the following risk factors: female sex, age >60 years, T2DM, and hypertension), which was established on the basis of data collection from 182 Japanese NAFLD patients in multiple centers in Nagasaki [22], is very simple, without the need for detailed laboratory tests. However, it was not superior to other scoring systems in our validation study.

Our study had several limitations. The fact that we excluded diabetic patients treated with exogenous insulin or insulin sensitizers (metformin or pioglitazone) from the analysis was a major limitation. In the future, we must find better scoring systems that are applicable to these patients. Other limitations include the largely retrospective study design and lack of complete data in many subjects. We included patients from different hepatology centers in Japan that have a particular interest in studying NAFLD, and thus, some referral bias could not be ruled out. Patient selection bias could also have existed because liver biopsy might have been considered for NAFLD patients who were likely to have NASH. We acknowledge that pathological diagnosis was mainly determined using liver tissues derived from percutaneous liver biopsy, which is prone to sampling error or interobserver variability [8, 9]. In fact, 11 patients of our total cohort were diagnosed as nonNASH in spite of an NAFIC score of 3 or 4. Although the exact reason was unknown, sampling error could have led to this misdiagnosis. These patients need follow-up care or repeat liver biopsies. It should be emphasized that we had a central pathology review by two hepatopathologists to prevent interobserver variability, although we were not able to quantify the effect on our results of some

intraobserver variability. Because all participants were Japanese, there is a possibility that our results might not be adaptable for NAFLD patients of other races. Due to these limitations, the present results need to be validated in independent populations by other investigators.

In conclusion, NAFIC score can predict NASH in Japanese NAFLD patients with sufficient accuracy and simplicity to be considered for clinical use, thus identifying a very high-risk group in whom liver biopsy would be very likely to detect NASH, as well as a low-risk group in whom liver biopsy can be safely delayed or avoided.

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Conflicts of interest The authors have no conflicts of interest to disclose.

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非アルコール性脂肪性肝疾患の治療

Treatment for nonalcoholic fatty liver disease

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診断・検査・治療

非アルコール性脂肪性肝疾患の治療

Treatment for nonalcoholic fatty liver disease

特集

肝炎診療の新たな展開

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Key words　NAFLD　酸化ストレス　インスリン抵抗性　ビタミンE　ピオグリタゾン

非アルコール性脂肪性肝疾患(NAFLD)には予後良好な単純性脂肪肝(SS)と，炎症や線維化を伴い肝硬変・肝癌に進展する予後不良な非アルコール性脂肪肝炎(NASH)がある．NAFLDの多くは肥満・糖尿病・脂質異常症・高血圧などいわゆる生活習慣病を背景に発症し，NASHの成立には2段階の因子が関与していると言われ(two hit theory)，第1段階の肥満・糖尿病・脂質異常症・高血圧などの生活習慣病(1st hit)によりSSが発症し，そこに内臓脂肪細胞から分泌されるTNFαなどのadipocytokine，脂質過酸化，鉄蓄積などの因子が加わり(2nd hit) NASHが発症するといわれている[1]．したがって，NAFLDの治療の基本は1st hit，2nd hitの各因子を取り除くことであるが(**図1**)，SS，NASHともにその病態形成や進展には酸化ストレスとインスリン抵抗性が深く関与している(**図2**)．したがって，薬物療法としては抗酸化剤やインスリン抵抗性改善薬が有効と考え，前者ではビタミンE(Vit. E)やC(Vita. C)，ウルソデオキシコール酸(UDCA)，後者ではメトホルミン，ピオグリタゾンなど種々の薬剤治療が試みられてきた．

一般的治療と瀉血

肥満・内臓脂肪蓄積のあるSS，NASHではダイエット・運動による体重減少で肝機能が改善する例が多いが，急激な体重減少は好ましくない．肥満・糖尿病患者では心血管系の合併症を有する患者が多いことから，最初はウォーキングから初め，最終的には1日1万歩を目標とする．その後問題なければジョギングも可能であるが，BMI 35以上の超肥満者ではジョギングは避けるべきである．摂取カロリーは肥満度に応じて標準体重25～35Kcal/kg・日を原則とし，摂取蛋白量は1.0～1.5g/kg・日とし，脂肪は摂取カロリーの20%以下とする．

欧米では，BMI 35以上の肥満を伴うNAFLDが多く，食事療法と運動療法による体重減少は週1.6kg未満を目標にされているが[2]，超肥満者の少ない本邦では週1.0～1.2kg以下の体重減少を目標とすることが好ましい．体重が5%以上減少すれば肝機能は明らかに改善する[2]．

大阪府済生会吹田病院(吹田市)　院長

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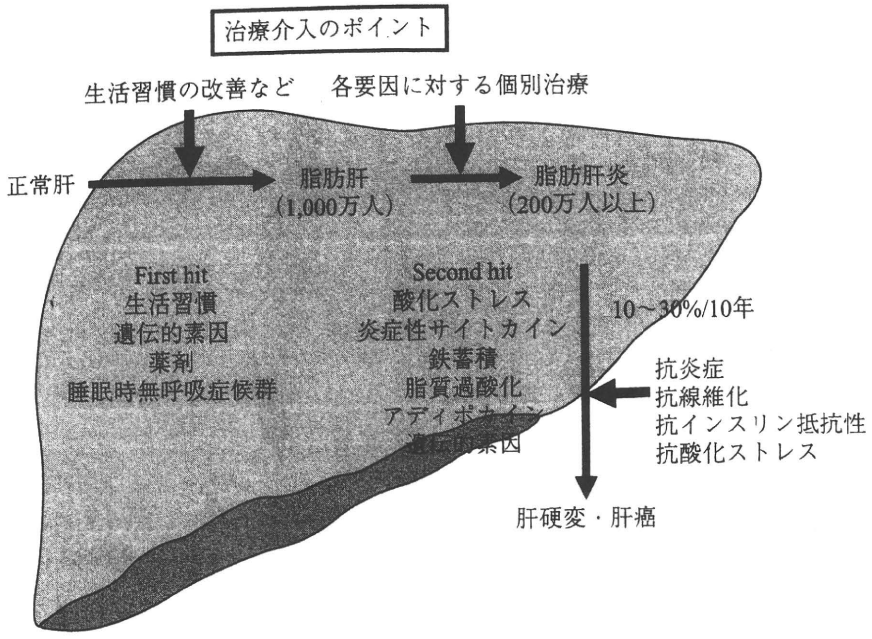

図1 非アルコール性脂肪性肝障害(NAFLD)と非アルコール性脂肪肝炎(NASH)の発症進展(two hits theory)と治療介入

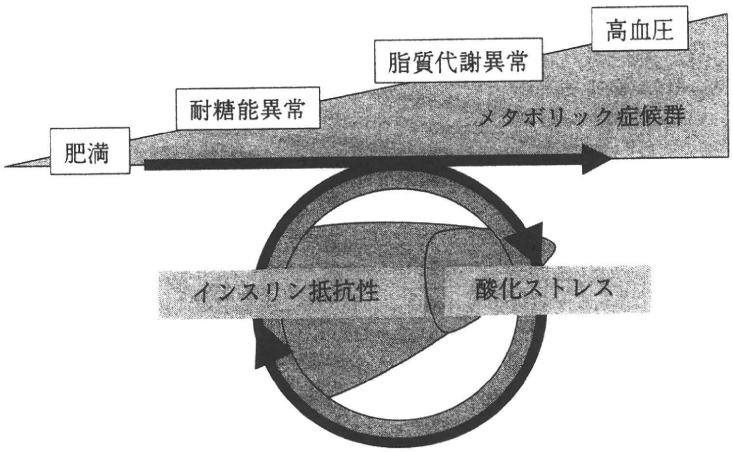

図2 酸化ストレスとインスリン抵抗性の悪循環

SS，NASH ともに肝臓への鉄過剰蓄積の見られる例(多くが血清フェリチン高値)が4割前後ある．過剰な鉄は毒性の強いヒドロキシラジカルを産生し，肝細胞障害・核 DNA 障害をもたらし，病気の進展・発癌の危険因子となるため，ALT 高値で血清フェチリン高値例では最初月2回，その後月1回の割合で1回350～400ml の瀉血を行い，血清フェリチンを20ng/dl 以下に保つようにする．これにより，ALT 値は有意に低下するが，NAFLD への瀉血療法は保険適用外である．血清フェリチン高値例では，維持療法として鉄含量の多い食事を避けることが大切である．

薬物療法

糖尿病，高血圧や脂質異常症を伴う NAFLD では，これらに対する薬物療法で肝機能異常と肝組織所見は改善し，NASH でも初期の例ではこれら背景因子への治療のみで改善する例が多い．

しかし，背景因子への治療で改善しない場合や進展した NASH では，より積極的な薬物療法が必要で，薬物療法に際しては個々の症例の病態を考慮し，抗酸化剤，インスリン抵抗性改善薬や抗線維化剤を選択する．しかし，NASH は heterogeneous な疾患であり，統一した薬物療法治療法

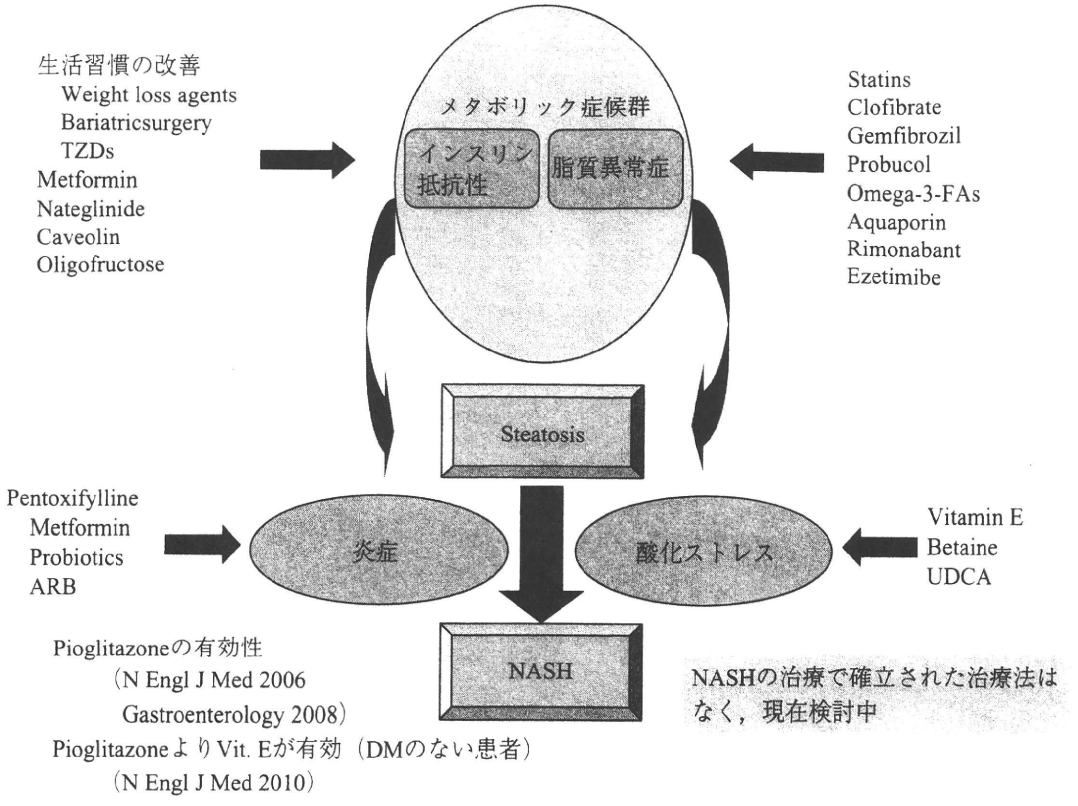

図3 NASHの治療

はない(図3).

1. 糖尿病合併，非合併NASHに対するpioglitazone投与

PPARγリガンドであるthiazolidinediones(TZDs)系誘導体は，インスリン抵抗性改善作用を有するために糖尿病治療に有効であり，なかでもピオグリタゾンは，①脂肪組織からの肝臓への脂肪酸の動員を抑制する，②AMP kinaseの活性化を介して脂肪酸β酸化を抑制する．さらに，TNFαの血中レベルを下げることも指摘され，実験的には星細胞の活性化を抑制して線維化を改善する.

糖代謝異常を伴う55名のNASH症例への検討では，ピオグリタゾン投与群ではコントロール群に比して糖代謝，ALT値，肝組織所見(脂肪肝，肝細胞風船様変性，炎症)の有意な改善が見られた[3]．糖尿病を合併していないNASH74例への検討では，ピオグリタゾン投与群ではコントロール群に比して有意な体重増加(−0.55 vs +2.77kg；p=0.04)が見られたが，空腹時血糖値(+0.4 vs −0.1mmol/L；p=0.02)，HbA1c値(+0.16 vs −0.18 %；p=0.006)，インスリンCペプチド値(+42 vs −78pmol/L；p=0.02)，ALT値(−10.9 vs −36.2u/L；p=0.002)，γGTP値(−9.4 vs −41.2u/L；p=0.002)，ferritin値(−11.3 vs −90.5μg/L；p=0.01)は有意に改善し，肝組織所見においても肝細胞障害(p=0.005)，Mallory-Denk体(p=0.004)，線維化(p=0.05)の改善が見られている[4]．その後，糖尿病のないNASHへのピオグリタゾン，Vit. E，プラセボでの96週間投与の比較試験では，Vit.E，ピオグリタゾン群ともに血清ALT低下，脂肪肝と小葉内炎症の改善が見られたが，これらの改善はVit.E投与群でのみプラセボ群に比して有意な改善が見られ，糖尿病を合併していないNASHの治療にはVit.Eがより有用と結論している[5]．しかし，Vit.E群でも肝組織の改善は43%(ピオグリタゾン群34%)に見られたのみであり，NASHの原因や病態はかなりheterogeneousであること

から，この結果からVit.Eが治療の第一選択と言えるわけではない．

TZDsではピオグリタゾン以外にトログリタゾン，ロシグリタゾン[6)7)]でも治験が行われたが，前者は副作用のために中止になった．投与に際しては食事療法・運動療法を併用しない限りTZD単独で治療効果は得られないことを心得ておく必要がある[7)]．

2. metformin

本剤は肝臓での糖新生抑制，グルコース吸収阻害，グルコースの取り込みと利用促進によるインスリン抵抗性改善作用を有する糖尿病治療薬で，AMP-activated protein kinase活性化作用を有するが，その詳細な作用機序はまだ明らかでない．NASH患者に投与すると，ALT値の有意な低下と画像検査での脂肪肝の改善が報告されている[8)]．症例数が少なくさらに検討を要するが，副作用がほとんどないことから非糖尿病患者にも推奨する向きもあるが[9)]，現在までのNASHへの本剤投与の結果からは肝組織の有意な改善は得られていない[2)]．

3. orlistat

本剤は，胃と膵臓からのlipaseの分泌を阻害することで，中性脂肪の吸収を30％くらい抑制する．米国でBMI 27以上のNASH 50例を1,400 Kcal/日プラスvitamin E 800IU/日群(コントロール群)と，それに本剤360mg/日を加えた群(オルリスタット群)36週投与のRCTを施行したが，体重減少，肝機能改善などに有意な差はなかったと報告された[10)]．

4. 他の高脂血症改善剤

HMG-CoA還元酵素阻害剤やezetimibeなど，コレステロール合成阻害剤やコレステロール吸収阻害剤が，NAFLDにおける肝機能や脂肪肝の改善をもたらすとの報告がある．われわれも最近ezetimibe 48週間投与が肝機能のみならず，組織も改善することを報告した[11)]．しかし，これらの薬剤に関しては多数例でのRCTでの検討が必要である．

5. 瀉　　血

NASHでは30～40％の例に肝臓への鉄の過剰蓄積がみられ，このような例では血清ferritinが高値である．鉄は，毒性の強いヒドロキシラジカルを産生し肝細胞傷害や核DNA障害を引き起こすため，このような例には瀉血や鉄制限食が有効であることが報告されているが，大規模臨床研究やRCTは施行されていない．

6. 抗線維化療法

rennin-angiotensin系は肝星細胞を活性化し肝線維化を促進する．したがって，降圧剤であるangiotensin Ⅱ type 1 receptor阻害剤(ARB)やangiotensin converting enzyme(ACE)阻害剤がNASHの肝線維化抑制作用を有すると想定される．少数例での検討であるが，NASHにARB(losartan 50mg/日)を48週間投与し，ALT値の低下とともに線維化マーカーや血中TGFβの有意な低下が得られている[12)]．RCTで有意な肝線維化抑制が示されているのはtelmisartanのみである[13)]．線維化や炎症の抑制作用詳しい機序に関してはmethionine-choline-deficient ratのNASHモデルで検討されている[14)]．

7. 外科的治療

BMIが40を超すような超肥満者が多い米国などでは，foregut bariatric surgeryといわれるrouz-en-Y胃バイパス術，胃形成術，胃結紮術などを行い，多量の食物摂取を不可能にする手術が広く行われて，肥満を伴うNAFLD患者に成果をあげている．わが国ではまだごく一部の施設で行われているに過ぎない．

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Original Article

Lower circulating levels of dehydroepiandrosterone, independent of insulin resistance, is an important determinant of severity of non-alcoholic steatohepatitis in Japanese patients

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Aim: The biological basis of variability in histological progression of non-alcoholic fatty liver disease (NAFLD) remains unknown. Dehydroepiandrosterone (DHEA), the most abundant steroid hormone, has been shown to influence sensitivity to reactive oxygen species, insulin sensitivity and expression of peroxisome proliferator-activated receptor-α. Our aim was to determine whether more histologically advanced NAFLD is associated with low circulating levels of DHEA in Japanese patients.

Methods: Serum samples were obtained in 133 Japanese patients with biopsy-proven NAFLD and in 399 sex- and age-matched healthy people undergoing health checkups. Serum levels of sulfated DHEA (DHEA-S) were measured by chemiluminescent enzyme immunoassay.

Results: Serum DHEA-S levels in NAFLD patients were similar to those in the control group. Of 133 patients, 90 patients were diagnosed as non-alcoholic steatohepatitis (NASH): 73 patients had stage 0–2, and 17 had stage 3 or 4. Patients with advanced NAFLD (NASH with fibrosis stage 3 or 4) had lower plasma levels of DHEA-S than patients with mild NAFLD (simple steatosis or NASH with fibrosis stage 0–2). The area under the receiver operating characteristic curve for DHEA in separating patients with and without advanced fibrosis was 0.788. A "dose effect" of lower DHEA-S and incremental fibrosis stage was observed with a mean DHEA-S of 170.4 ± 129.2, 137.6 ± 110.5, 96.2 ± 79.3, 61.2 ± 46.3 and 30.0 ± 32.0 μg/dL for fibrosis stages 0, 1, 2, 3, and 4, respectively. The association between DHEA-S and severity of NAFLD persisted after adjusting for age, sex and insulin resistance.

Conclusion: Low circulating DHEA-S might have a role in the development of advanced NASH.

Key words: fibrosis, dehydroepiandrosterone, insulin resistance, non-alcoholic fatty liver disease.

INTRODUCTION

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) is the most common chronic liver disease in many developed countries and results in a serious public health problem worldwide. NAFLD includes a wide spectrum of liver diseases, ranging from simple steatosis (SS), which is usually a benign and non-progressive condition, to non-alcoholic steatohepatitis (NASH), which may progress to liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in the absence of significant alcohol consumption.[1–3] In Japan, current best estimates make the prevalence of NAFLD approximately 20% and of NASH 2–3% in the general population.[4,5] Although several factors have been associated with more advanced NAFLD, the biological basis of the histological diversity of severity of NAFLD (i.e. why some patients develop

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SS and others develop NASH with advanced fibrosis) remains unknown. More advanced NAFLD is characterized by insulin resistance,[6,7] oxidative stress[8,9] and advanced fibrosis.

Endocrine hormones control cell metabolism and the distribution of body fat and therefore may contribute to the development of NAFLD or NASH. It has been postulated that dehydroepiandrosterone (DHEA) and its sulfate ester, dehydroepiandrosterone sulfate (DHEA-S), the major secretory products of the human adrenal gland, may be discriminators of life expectancy and aging.[10] DHEA-S concentration is independently and inversely related to death from any cause and death from cardiovascular disease in men over the age of 50 years.[11] DHEA is a potential mediator of reactive oxygen species scavenger synthesis[12] and has also been reported to augment insulin sensitivity[13–16] and peroxisome proliferator activation.[17,18] Recently, Charlton *et al.* observed that levels of DHEA are significantly lower in patients with histologically advanced NASH, as compared with patients with mild NASH or SS.[19] DHEA levels exert a good sensitivity and specificity in discriminating patients with more advanced histological disease, as shown by receiver–operator curve (ROC) analysis.

To validate their results, we determined circulating DHEA levels in Japanese patients with biopsy-proven NAFLD.

METHODS

Patients

A TOTAL OF 133 patients with well-characterized and liver biopsy-confirmed NAFLD were included in this study. They were consecutively biopsied patients seen at the Center for Digestive and Liver Diseases, Nara City Hospital during 2007–2009. The diagnosis of NAFLD was based on the following criteria: (i) persistent elevations of transaminase activities for more than 6 months; (ii) liver biopsy showing steatosis in at least 5% of hepatocytes;[20] and (iii) appropriate exclusion of liver diseases of other etiology including viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis (PBC), biliary obstruction, hemochromatosis, Wilson's disease, and α-1-antitrypsin-deficiency-associated liver disease. Patients consuming more than 20 g alcohol/day and patients with evidence of decompensated LC or HCC were excluded from the present study. Written informed consent was obtained from all patients at the time of their liver biopsy, and the study was conducted in conformance with the Helsinki Declaration. In addition, 399 sex- and age-matched healthy people participating in health checkups who showed normal levels of alanine aminotransferase (ALT) levels (≤30 IU/L) were also enrolled as the control group.

Clinical laboratory parameters

Venous blood samples were taken in the morning after a 12-h overnight fast. The laboratory evaluation in all patients included a blood cell count and the measurement of aspartate aminotransferase (AST), ALT, γ-glutamyltransferase (γGT), cholinesterase (ChE), total cholesterol, triglyceride, albumin, fasting plasma glucose (FPG), immunoreactive insulin (IRI), free fatty acid (FFA), ferritin levels, hyaluronic acid and type IV collagen 7S. These parameters were measured using the standard techniques of clinical chemistry laboratories. Body mass index (BMI) was calculated using the following formula: weight in kilograms / (height in meters).[2] Obesity was defined as a BMI greater than 25, according to the criteria of the Japan Society for the Study of Obesity.[21] Patients were assigned a diagnosis of diabetes mellitus (DM) if a documented use of oral hypoglycemic medication, a random glucose level in excess of 200 mg/dL or an FPG greater than 126 mg/dL was present.[22] Dyslipidemia was diagnosed if the cholesterol level was higher than 220 mg/dL and/or the triglyceride level was over 160 mg/dL. Hypertension was diagnosed if the patient was on antihypertensive medication and/or had a resting recumbent blood pressure of greater or equal to 140/90 mmHg on at least two occasions.

Sulfated DHEA concentrations were measured by chemiluminescent enzyme immunoassay (CLEIA). Serum DHEA-S levels of the control group were determined in the Anti-Aging Medical Research Center, Graduate School of Life and Medical Science, Doshisha University, Kyoto, Japan. The Homeostatic Model of Assessment of Insulin Resistance (HOMA-IR) was calculated on the basis of fasting values of plasma glucose and insulin according to the HOMA model formula: HOMA-IR = IRI (μU/mL) × FPG (mg/dL) / 405.[23] Quantitative insulin sensitivity check index (QUICKI) = 1 / (log fasting IRI [μU/mL] + log FPG [mg/dL]).[24]

Histological evaluation

All patients enrolled in this study underwent a percutaneous liver biopsy under ultrasonic guidance. The liver specimens were embedded in paraffin and stained with hematoxylin–eosin, Masson trichrome and reticulin

Table 1 Characteristics of NAFLD patients and control group

Parameters	NAFLD	Control	*P*-value
n	133	399	
Sex (female)	70 (53%)	210 (53%)	Matched
Age (year)	55.2 (15.4)	55.6 (12.1)	0.7990
BMI (kg/m^2)	27.9 (4.9)	23.4 (3.4)	<0.0001
Obesity (BMI > 25 kg/m^2)	98 (74%)	109 (27%)	<0.0001
AST (IU/L)	58.0 (33.0)	21.7 (4.9)	<0.0001
ALT (IU/L)	85.6 (51.7)	19.4 (5.4)	<0.0001
γGT (IU/L)	82.8 (73.0)	33.1 (28.8)	<0.0001
Cholesterol (mg/dL)	207.9 (41.2)	215.6 (34.9)	0.0572
Triglyceride (mg/dL)	179.1 (96.3)	109.0 (87.8)	<0.0001
HDL-C (mg/dL)	52.0 (24.7)	63.8 (16.6)	<0.0001
FPG (mg/dL)	103.5 (38.9)	97.5 (15.7)	0.0131
IRI (μU/mL)	14.70 (9.46)	5.57 (4.17)	<0.0001
HOMA-IR	3.93 (3.83)	1.37 (1.09)	<0.0001
QUICKI	0.33 (0.03)	0.38 (0.04)	<0.0001
DHEA-S (μg/dL)	128.7 (111.2)	113.6 (91.8)	0.1578

P-values were calculated by Student's *t*-test or χ^2-test analysis.
Results are presented as numbers with percentages in parenthesis for qualitative data or as means with standard deviation in parenthesis for quantitative data.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; IRI, immunoreactive insulin; NAFLD, non-alcoholic fatty liver disease; QUICKI, Quantitative insulin sensitivity check index; γGT, gamma glutamyl transpeptidase.

silver stain. A pathologist (S. I.) who was blinded to the clinical data reviewed the liver biopsy specimens. Adequate liver biopsy sample was defined as biopsy specimen length greater than 1.5 cm and/or having more than six portal tracts. NASH was defined as steatosis with lobular inflammation and ballooning degeneration with or without Mallory–Denk body or fibrosis.[2,3] Patients whose liver biopsy specimens showed steatosis, or steatosis with non-specific inflammation, were identified as the SS cohort.[2,3] The severity of hepatic fibrosis (stage) was defined as follows: stage 1, zone 3 perisinusoidal fibrosis; stage 2, zone 3 perisinusoidal fibrosis with portal fibrosis; stage 3, zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis; and stage 4, cirrhosis.[25] Scoring of steatosis included both microvesicular and macrovesicular steatosis and was based on the percentage area of the parenchyma that was fatty. Mild was considered less than 33%, moderate 33–65% and advanced if greater than 66% was observed.[20]

Statistical analysis

Results are presented as the means and standard deviation (SD) for quantitative data or as numbers with percentages in parentheses for qualitative data. Statistical differences in quantitative data were determined using the Student's *t*-test (Table 1). Statistical differences among three groups for quantitative data were determined by one-way ANOVA with Scheffe's post-hoc test (Table 3). Fisher's exact probability test or χ^2-test analysis was used for qualitative data (Tables 1,3). Correlation coefficients were calculated by using Spearman's rank correlation analysis (Table 2). Multivariate analysis was performed by logistic regression analysis to identify variables independently associated with advanced stage of NASH (Table 4). To assess the accuracy of clinical scoring system in differentiating NASH from SS or advanced NAFLD from mild NAFLD, we calculated the sensitivity and the specificity for each value of each test and then constructed ROC by plotting the Se against the reverse Sp (1 – Sp) at each value (Fig. 1). The diagnostic performance of scoring systems was assessed by analysis of ROC. The most commonly used index of accuracy is the area under the ROC (AUC), with values close to 1.0 indicating high diagnostic accuracy (Table 4). The Youden index was used to identify the optimal cut-off points. Differences were considered statistically significant at all $P < 0.05$.

Table 2 Correlation between serum DHEA-S and clinical parameters in 133 patients with biopsy-proven NAFLD

Variables	Correlation coefficient	*P*-value
Age	−0.6982	<0.0001
Hemoglobin	0.4859	<0.0001
Platelet	0.3475	<0.0001
AST	−0.1988	0.0218
ALT	0.1733	0.0460
AST : ALT ratio	−0.5847	<0.0001
γGT	−0.0580	0.5092
Cholinesterase	0.3827	<0.0001
Albumin	0.4165	<0.0001
Prothrombin time	0.0767	0.4029
Cholesterol	0.1525	0.0820
Triglyceride	0.2037	0.0206
HDL-C	−0.2016	0.0033
FPG	−0.1386	0.1158
IRI	−0.0208	0.8138
HOMA-IR	−0.0545	0.5379
QUICKI	0.0545	0.5379
Free fatty acid ($n = 121$)	−0.1023	0.2644
Ferritin	0.0037	0.9666
Hyaluronic acid	−0.6408	<0.0001
Type IV collagen 7 s	−0.4477	<0.0001

P-values are based on Spearman's non-parametric correlation analysis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; DHEA-S, dehydroepiandrosterone sulfate; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; IRI, immunoreactive insulin; NAFLD, non-alcoholic fatty liver disease; QUICKI, Quantitative insulin sensitivity check index; γGT, gamma glutamyl transpeptidase.

RESULTS

Patient demographics

TABLE 1 SUMMARIZES the clinical, laboratory and liver biopsy data of the patient population and the control group. NAFLD patients were predominantly obese, had higher levels of transaminase activities, γGT, triglyceride, FPG, IRI and insulin resistance, and had lower levels of high-density lipoprotein cholesterol (HDL-C). Serum levels of DHEA-S in NAFLD patients were not different from those in sex- and age-matched controls. In both groups, there were significant sex differences in serum levels of DHEA-S (control group, male 154.4 ± 102.1 vs female 76.8 ± 59.6 μg/dL, $P < 0.0001$; NAFLD group, male 186.7 ± 129. 2 vs female 76.5 ± 53.1 μg/dL, $P < 0.0001$).

Of 133 NAFLD patients involved in this study, 90 patients (68%) were histologically diagnosed as NASH, and 43 patients (32%) were SS. NASH patients were significantly older, predominantly female, hypertensive, more likely to have DM, had lower levels of hemoglobin (Hb), platelet count, albumin, cholinesterase and QUICKI, and had higher levels of AST, ALT, IRI, hyaluronic acid, type IV collagen 7S and HOMA-IR. Patients with NASH had lower levels of DHEA-S (108.8 ± 96.1 μg/dL) than those with SS (170.4 ± 129.2 μg/dL, $P = 0.003$). The AUC for DHEA in separating patients with and without NASH was 0.678 (Fig. 1a). The sensitivity of a DHEA-S-value of 99 μg/dL or less for the presence of NASH was 62.2% (56/90) and specificity was 67.4% (29/43).

Correlation between DHEA-S and other clinical variables in NAFLD patients

Levels of DHEA-S were positively correlated with Hb, platelet, ALT, cholinesterase, albumin and triglyceride, and negatively correlated with age, AST, AST/ALT ratio, ALP, HDL-C, hyaluronic acid and type IV collagen 7S. They had no associations with markers of insulin resistance such as HOMA-IR and QUICKI (Table 2). Serum DHEA-S levels were not different between patients with HOMA-IR of more than 2.5 ($n = 73$, 125.6 ± 116.0 μg/dL) and with HOMA-IR of less than 2.5 ($n = 57$, 134.4 ± 107.9 μg/dL, $P = 0.660$). Similarly, serum DHEA-S levels were not different between patients with QUICKI of more than 0.3 ($n = 102$, 123.7 ± 114.4 μg/dL) and with QUICKI of less than 0.3 ($n = 26$, 114.8 ± 108.2 μg/dL, $P = 0.448$).

Comparison between participants with simple steatosis, and mild and advanced NASH

Patients with NAFLD were divided into three groups, including SS, mild NASH (NASH with fibrosis stage 0–2) and advanced NASH (NASH with fibrosis stage 3–4). Female sex was more prevalent in patients with advanced NASH than in those with SS and mild NASH. Participants in the SS group were younger than participants with mild and advanced NASH. The prevalence of obesity and lifestyle-related diseases did not differ among three groups. Platelet count decreased in accordance with the incremental fibrosis of NAFLD. The AST/ALT ratio, fibrosis markers (hyaluronic acid, type IV collagen 7S) and insulin resistance were elevated in the advanced stage of NAFLD. Participants with advanced NASH had significantly lower levels of DHEA-S compared with participants with SS, and tended to have low

Table 3 Comparison between participants with simple steatosis, and mild and advanced NASH

Parameters	Simple steatosis (SS)	Mild NASH (mNASH)	Advanced NASH (aNASH)	*P*-value
n	43	73	17	
Sex (female)	16 (37%)	40 (55%)	14 (82%)	0.0020
Age (year)	48.7 (15.9)[a]	56.8 (14.9)	65.1 (7.9)	[a]0.0007 vs aNASH, 0.0169 vs. mNASH
BMI (kg/m^2)	27.0 (4.8)	28.5 (5.0)	27.2 (4.0)	NS
obesity (BMI > 25 kg/m^2)	30 (70%)	56 (77%)	12 (71%)	NS
Diabetes	10 (23%)	38 (52%)	9 (53%)	NS
Hypertension	8 (19%)	30 (41%)	5 (29%)	NS
Dyslipidemia	12 (43%)	26 (36%)	9 (53%)	NS
Hemoglobin (g/dL)	14.8 (1.5)	14.1 (1.4)[b]	13.9 (1.3)	[b]0.046 vs SS
Platelet (10^4/μL)	24.4 (5.5)	22.2 (6.3)	17.7 (5.6)[c]	[c]0.0007 vs SS, 0.0210 vs mNASH
AST (IU/L)	40.8 (24.0)[d]	64.1 (31.8)	75.6 (40.5)	[d]0.0001 vs mNASH, aNASH
ALT (IU/L)	72.2 (56.5)	93.8 (48.0)	84.4 (50.2)	NS
AST/ALT ratio	0.64 (0.20)	0.75 (0.31)	0.96 (0.28)[e]	[e]0.0003 vs SS, 0.0160 vs mNASH
γGT (IU/L)	75.6 (66.9)	84.4 (81.4)	93.4 (45.8)	NS
Cholinesterase (IU/L)	397.8 (69.2)	378.9 (67.6)[f]	324.9 (101.3)	[f]0.0039 vs SS, 0.0283 vs aNASH
Albumin(g/dL)	4.51 (0.32)	4.37 (0.30)	4.29 (0.38)	NS
Cholesterol (mg/dL)	214.5 (43.7)	208.3 (39.6)	189.9 (38.5)	NS
Triglyceride (mg/dL)	172.9 (83.6)	189.1 (105.0)	153.4 (86.7)	NS
HDL-C (mg/dL)	50.8 (14.7)	52.8 (31.5)	51.8 (10.7)	NS
Prothrombin time (%)	106.9 (16.3)	99.0 (17.8)	90.3 (17.7)[g]	[g]0.0061 vs SS
Ferritin (ng/mL)	179.0 (182.6)	241.0 (182.1)	278.1 (246.2)	NS
Hyaluronic acid (ng/mL)	26.1 (21.8)	69.8 (104.7)	169.1 (172.4)[h]	[h]<0.0001 vs SS, 0.0014 vs mNASH
Type IV collagen 7 s (ng/mL)	3.67 (0.61)	4.99 (1.51)[i]	6.86 (1.68)[j]	[i]<0.0001 vs SS, [j]<0.0001 vs SS, mNASH
FPG (mg/dL)	97.8 (17.8)	107.8 (49.2)	99.1 (24.7)	NS
IRI (μU/mL)	9.0 (5.6)[k]	16.6 (8.7)	21.0 (12.9)	[k]*P* < 0.0001 vs aNASH, 0.001 vs mNASH
HOMA-IR	2.15 (1.34)[l]	4.68 (4.51)	5.21 (3.45)	[l]0.0023 vs mNASH, 0.0160 vs aNASH
QUICKI	0.35 (0.03)[m]	0.32 (0.03)	0.31 (0.03)	[m]<0.0001 vs mNASH, 0.001 vs aNASH
Free fatty acid (mEq/L)	0.56 (0.18)	0.58 (0.20)	0.58 (0.16)	NS
DHEA-S (μg/dL)	170.4 (129.2)	121.2 (100.7)	55.7 (45.0)[n]	[n]0.0012 vs SS

Results are presented as numbers with percentages in parenthesis for qualitative data or as means with standard deviation in parenthesis for quantitative data.

P-values were calculated by Scheffe's method or χ^2-test analysis.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; IRI, immuno-reactive insulin; QUICKI, quantitative insulin sensitivity check index; γGT, γ-glutamyl transpeptidase.

levels of DHEA-S compared with mild NASH (Table 3). Though patients with SS had significantly higher levels of serum DHEA-S (170.4 ± 129.2 μg/dL) than the control group (113.6 ± 91.8 μg/dL, $P < 0.001$), the former was younger (48.7 ± 15.9 years) than the latter (55.6 ± 12.1 years, $P < 0.001$) (Tables 1,3). Thus, we selected 129 sex- and age-matched healthy people out of the control group to clarify whether the real difference exists. Serum DHEA-S levels of 129 sex- and age-matched healthy people (145.0 ± 114.8 μg/dL) were not different from patients with SS ($P = 0.225$).

Participants with advanced NAFLD (NASH with stage 3–4 fibrosis) had significantly lower levels of DHEA-S compared with participants with mild NAFLD (SS and NASH with stage 0–2 fibrosis) (55.7 ± 45.0 vs 139.4 ± 114.1 μg/dL, $P = 0.003$). None of the younger patients (<40 years, $n = 21$) had advanced NASH. We compared DHEA-S levels in patients with more advanced NASH, aged 40–65 years (mean 57.9 ± 4.2 years), with patients with mild NAFLD, aged 40–65 years (mean 53.8 ± 7.5 years, $P = 0.139$). DHEA-S levels tended to be lower in patients with advanced NASH than in patients with mild NAFLD (76.1 ± 55.9 vs 117.1 ± 66.2 μg/dL, $P = 0.098$) without reaching significant difference. Next, we compared DHEA-S levels in patients with more advanced NASH,