Original Article

Practical Use of Transient Elastography in Screening for Nonalcoholic Steatohepatitis in a Japanese Population

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Abstract

Background and Aims: Fatty infiltration of liver may induce insulin resistance (IR), and a proportion of patients with nonalcoholic fatty liver disease (NAFLD) is diagnosed with nonalcoholic steatohepatitis. Transient elastography is gaining popularity as a means of non-invasively determining both liver stiffness (fibrosis level) and degree of fatty infiltration, expressed as controlled attenuation parameter (CAP) value. ***Methods:*** The aims of this study were to investigate the association between IR and level of fatty liver, and to identify the group at a greater risk of nonalcoholic steatohepatitis using transient elastography and other noninvasive fibrosis markers. A total of 169 patients without chronic hepatitis B and C were analyzed. ***Results:*** The CAP value was significantly associated with IR (HOMA-IR ≥2.5; AUROC = 0.81), and the optimal cut-off to discriminate IR was 264 dB/m. The liver stiffness measurement and aspartate aminotransferase-to-platelet ratio index values were significantly higher for CAP ≥264 than in CAP <264. The 9 patients among the overall 169 patients (5.3%) and among the 102 NAFLD patients (8.8%) who showed ≥264 dB and ≥7.0 kPa in transient elastography could represent good candidates for liver biopsy. ***Conclusions:*** Evaluation of NAFLD based on CAP values was useful in diagnosing IR. About 9% of NAFLD patients in a Japanese outpatient clinic with a few metabolic complications might be considered good candidates for liver biopsy to confirm nonalcoholic steatohepatitis.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, estimated to affect up to about 30% of the general population.[1] NAFLD consists of simple steatosis, which accounts for the majority of cases, and NASH, which results in liver cirrhosis and hepatocellular carcinoma.[2,3] NAFLD is one of the signs of obesity and is highly correlated with metabolic syndrome, which includes type 2 diabetes mellitus (T2DM) and is characterized by insulin resistance (IR). IR may be induced by fat infiltrating the liver.[4] So far, few studies have shown the relationship between levels of fatty infiltration of the liver and IR or the severity of T2DM.

Transient elastography (TE) (FibroScan®, Echosens, Paris, France) is becoming popular as a routine and convenient technique for the diagnosis of liver diseases.[5,6] This modality can assess hepatic stiffness as a liver stiffness measurement (LSM) and can quantify the degree of steatosis as a controlled attenuation parameter (CAP). Because the method is noninvasive and can be performed in a short time, it is well suited to population-based screening as a first-line method.[7]

The standard method to diagnose NASH among NAFLD is biopsy of the liver. Such histological findings as ballooning of hepatocytes, infiltration of lymphocytes and the presence of fibrosis allow the discrimination of NASH from simple steatosis. Because of the invasiveness of biopsy, alternate serological markers have been studied.[8,9] In addition to LSM and CAP evaluations, many serological markers, including the aspartate aminotransferase (AST)-to-platelet ratio index (APRI),[10] Fib-4 index,[11] NAFLD fibrosis score,[12] and hyaluronic acid (HA) have been evaluated for their utility in detecting liver fibrosis.[9,13] Recently, the assay of Mac-2 binding protein glycosylation isomer (M2BPGi) has proven useful in the evaluation of liver fibrosis levels for various chronic liver diseases, including NASH.[14–17] The values were clearly associated with liver fibrosis levels among NASH patients.[14,16] However, few reports have attempted to verify its usefulness in an outpatient population with only a few metabolic complications.

This study examined the association between CAP values and IR. We have also attempted to estimate the incidence of NASH using TE and other fibrosis markers, including M2BPGi, among outpatients of our hospital.

Methods

Patients

This was a single-center study of patients carried out between October 2016 and March 2018. Demographic, clinical, and biochemical characteristics of the outpatient population are shown in Table 1. A total of 169 patients without excessive alcohol intake or chronic hepatitis B or C, who agreed to be enrolled in this study and to undergo TE and serum fibrosis tests were investigated. Thirty-two (19%) of them were from

Keywords: NAFLD; Insulin resistance; NASH; Transient elastography; CAP; Fibrosis marker.

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AUROC, area under the receiver operating characteristic curve; CAP, controlled attenuation parameter; HA, hyaluronic acid; IR, insulin resistance; LSM, liver stiffness measurement; M2BPGi, Mac-2 binding protein glycosylation isomer; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ROC, receiver operating characteristic; T2DM, type 2 diabetes mellitus; TE, transient elastography.

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among those who had an annual thorough medical checkup. Others (81%) were those who were seen sequentially in the clinic during the above-mentioned period.

NAFLD was defined by the presence of fatty liver on ultrasonography and alcohol intake <20 g/day. Based on the clinical diagnosis and medications in the medical charts, ongoing pharmacotherapy for hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, and hyperuricemia were seen in 42.0%, 17.2%, 25.4%, 8.9%, and 5.9%. In addition, 88 (52.1%) patients had obstructive sleep apnea syndrome. CAP measurement software was installed with TE, and values were obtained during LSM assessment.[6] Their values were expressed in dB/m.

This study was carried out in accordance with the ethical standards of the Declaration of Helsinki and was approved by the ethics committee of School of Life Dentistry at Niigata, The Nippon Dental University (ECNG-H-247).

Statistical analysis

Quantitative values were presented as mean±standard deviation. An unpaired *t*-test was used for comparing continuous variables between groups for normally distributed data, and the Welch's *t*-test was applied for data displaying nonnormal distributions. Receiver operating characteristic (ROC) curves were constructed to assess the accuracy of CAP and to identify optimal cut-offs. Optimal cut-offs of CAP to elucidate the IR were chosen to maximize Youden's index. All analyses were carried out using the JMP software version 13.2.1 in statistical analysis SAS software.

Table 1. Clinical backgrounds of the studied population, *n* = 169

Characteristic	Results
M / F	100 (59.2%)/ 69 (40.8%)
Hypertension −/+	98 (58.0%)/ 71 (42.0%)
Type 2 diabetes mellitus −/+	140 (82.8%)/ 29 (17.2%)
Hypercholesterolemia −/+	126 (74.6%)/ 43 (25.4%)
Hypertriglyceridemia −/+	154 (91.1%)/ 15 (8.9%)
Hyperuricemia −/+	159 (94.1%)/ 10 (5.9%)
Obstructive sleep apnea syndrome −/+	81 (47.9%)/ 88 (52.1%)
Age (years)	59.8±13.2
Body mass index, kg/m^2	25.0±4.1
Aspartate aminotransferase, IU/L	26.3±11.0
Alanine aminotransferase, IU/L	32.7±22.8
AST/ALT	0.95±0.33
Gamma-glutamyl transpeptidase, IU/L	40.8±37.6
Triglyceride, mg/dL	137.4±113.3
Low-density lipoprotein cholesterol, mg/dL	122.8±31.5
High-density lipoprotein cholesterol, mg/dL	59.9±16.2
Uric acid, mg/dL	5.36±1.21
Hemoglobin A1C, %	6.17±0.91
Highly sensitive-C-reactive protein, mg/dL	0.1179±0.1907

Results

Table 1 shows the characteristics of the studied population (*n* = 169). NAFLD was diagnosed in 102 (60.4%) patients. Area under the ROC curve (AUROCs) for body mass index and degree of fatty infiltration (expressed as CAP values) for diagnosing insulin resistance (HOMA-IR ≥2.5) were both 0.81, showing a good correlation (Fig. 1). Cut-off values were 25.49 kg/m^2 for body mass index and 264 dB/m for CAP, respectively. Patients were divided into two groups using a cut-off of 264 dB/m, and clinical factors were compared (Table 2). Body mass index, AST, alanine aminotransferase, gamma-glutamyl transpeptidase, triglyceride, uric acid, hemoglobin A1c, and highly sensitive-C-reactive protein were significantly higher in the CAP ≥264 dB/m group, and high-density lipoprotein cholesterol was lower in this group. Regarding liver fibrosis makers, APRI and LSM were significantly higher in the CAP 264 dB/m group (0.432 vs. 0.336 and 5.05 kPa vs. 3.92 kPa, respectively).

Based on the results of past reports that defined cut-off values for the presence of fibrosis at around 7.0 kPa in NAFLD,[18,19] we tentatively extracted those patients showing values over these thresholds (CAP ≥264, LSM ≥7.0) to detect patients with suspected NASH. Nine patients (5.3% of the total cohort of 169 patients, 8.8% of the NAFLD subgroup of 102 patients) fulfilled these criteria, and the results of other fibrosis tests for these nine patients are shown in Table 3. One to four serum markers of fibrosis showed abnormal values.

Discussion

Obesity and IR are the main causes of metabolic syndrome. NAFLD is closely associated with metabolic syndrome, and T2DM is associated with a higher rate of severe NAFLD.[4] Liver fat is well known to induce IR, so a close association exists between the level of IR and severity of NAFLD.[20] Values of HOMA-IR and CAP of TE reportedly increase, as people with NAFLD proceed from normal glucose tolerance to pre-diabetes mellitus and diabetes mellitus.[21] We studied the relationship between levels of fatty liver and IR, using the CAP evaluation to which many studies have shown a clear correlation between CAP values and levels of steatosis obtained by biopsied samples.[5,22,23] ROC curve analysis between CAP values and body mass index showed a significant correlation (AUROC = 0.81; Fig. 1). We also observed a significant association between IR (HOMA-IR ≥2.5) and CAP values (AUROC = 0.81) and found that the cut-off CAP value to best show IR was 264 dB/m.

Liu *et al.*[24] reported that the median CAP value for a large population was 250 dB/m. This value has been confirmed by several reports as a significant level for showing minimal hepatic steatosis in biopsy samples.[5,25,26] For example, Karlas *et al.*[5] reported that a CAP of 248 dB/m corresponded to histologically confirmed steatosis. Interestingly, the

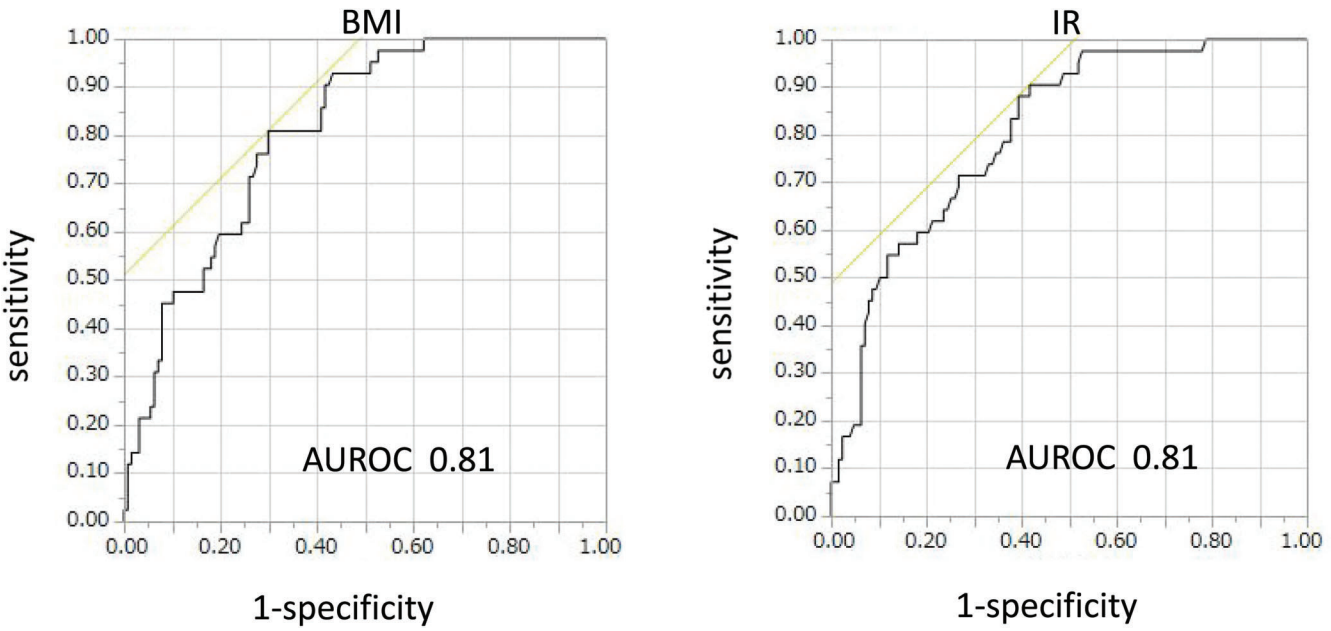

Fig. 1. Diagnostic capabilities of body mass index and controlled attenuation parameter values for assessing insulin resistance. AUROC values were 0.81 for both. AUROC of body mass index and controlled attenuation parameter values for diagnosing insulin resistance (HOMA-IR ≥2.5). Abbreviation: AUROC, area under the receiver operating characteristic curve.

proximity of our cut-off index for CAP to reveal IR (264 dB/m) to this value supports the close association between level of hepatic steatosis and presence of IR in our mostly (82.8%) nondiabetic population.

Chon *et al.*[21] also reported that median CAP values in subjects with T2DM was 265 dB/m, so the presence of small diabetic population in our study (17.2%) may not have a great effect on our overall data. NAFLD is reportedly associated with

Table 2. Comparison of biochemical and fibrosis tests between individuals divided by controlled attenuation parameter value that showed insulin resistance (HOMA-IR ≥2.5)

Controlled attenuation parameter, dB/m	<264, *n* = 78	≧264, *n* = 91	*p*-value
Body mass index, kg/m^2	22.3±3.0	27.3±3.6	<0.001
Aspartate aminotransferase, IU/L	22.3±5.2	29.7±13.4	<0.001
Alanine aminotransferase, IU/L	21.7±8.3	42.1±26.8	<0.001
AST/ALT	1.10±0.29	0.82±0.30	<0.001
Gamma-glutamyl transpeptidase, IU/L	30.3±31.2	49.8±40.4	<0.001
Triglyceride, mg/dL	111.1±12.6	160.0±11.6	<0.01
Low-density lipoprotein cholesterol, mg/dL	120.0±29.6	125.3±32.9	0.267
High-density lipoprotein cholesterol, mg/dL	66.4±18.1	54.3±11.8	<0.001
Uric acid, mg/dL	5.10±1.17	5.58±1.21	<0.05
Hemoglobin A1c, %	5.90±0.64	6.39±1.04	<0.001
Highly-sensitive-C-reactive protein, mg/dL	0.0729±0.1560	0.1526±0.2079	<0.01
Nonalcoholic fatty liver disease fibrosis score	2.280±0.994	2.161±1.369	0.533
Aspartate aminotransferase-to-platelet ratio index	0.336±0.125	0.432±0.230	<0.001
Fibrosis-4 score	1.45±0.08	1.23±0.07	<0.05
Hyaluronic acid, ng/mL	52.83±48.66	46.93±63.66	0.567
Type IV collagen·7S, ng/mL	3.36±0.78	3.38±0.86	0.860
Mac-2 binding protein glycosylation isomer	0.496±0.261	0.559±0.419	0.236
Liver stiffness measurement, kPa	3.92±1.21	5.05±2.71	<0.001
Interquartile range, %	15.04±6.94	14.39±6.40	0.527

Table 3. Fibrosis markers of 9 patients which showed more than 263 dB/m and 7.0 kPa by transient elastography

Patient #	1	2	3	4	5	6	7	8	9
Age in years	40	68	77	51	63	64	59	55	50
Sex	M	M	F	M	F	F	F	F	M
Body mass index, kg/m^2	37.0	29.7	24.8	25.8	24.0	31.5	34.7	29.1	39.6
SAS +/−	+	−	+	−	−	+	−	+	−
HOMA-IR	11.4	8.5	11.3	8.6	3.1	4.6	5.1	2.0	7.3
Nonalcoholic fatty liver disease fibrosis score	0.61*	0.06*	1.33	1.71	2.01	1.07	ND	1.99	0.52*
Aspartate aminotransferase-to-platelet ratio index	0.63	1.29	1.33	0.82	0.15	0.51	0.57	1.16	0.40
Fibrosis-4 score	0.93	2.22	4.79*	1.15	0.71	1.33	1.55	1.89	0.82
Hyaluronic acid, ng/mL	110.6*	488.9*	116.8*	107.1*	109.3*	63.6*	179.0*	14.0	41.0
Type IV collagen · 7S, ng/mL	3.4	7.4*	5.3	4.2	2.8	3.2	4.3	5.0	4.6
Mac-2 binding protein glycosylation isomer	0.48	1.08*	1.25*	0.45	0.34	0.48	0.81*	3.99*	0.55
Liver stiffness measurement, kPa	21.8	15.1	14.3	8.8	8.7	8.6	7.7	7.3	7.1
Controlled attenuation parameter, dB/m	396	299	266	346	266	349	280	366	396

Standard values: Nonalcoholic fatty liver disease fibrosis score >0.676;[12] APRI <1.85;[10] Fibrosis-4 <2.67;[11] hyaluronic acid <50 ng/mL; Type IV collagen · 7S <6 ng/mL; M2BPGi <0.70.[14]

*Values that are not within these standards.

an increased frequency of T2DM[27] and the abnormal CAP values may be involved in the initiation of IR. In our study, HOMA-IR gradually increased from 1.16±0.62 in <248 dB/mL that was reported to be <S1, 2.78±5.03 in 248 to 267 to be S1, to 2.89±2.16 in >280 dB to be S3.[5]

The reported incidence of NASH among NAFLD has been variable (2–20%) because of differences in diagnosis methods and patients cohorts.[2,28] In general, the incidence of NASH becomes higher as steatosis levels increase and is highly associated with the presence of diabetes mellitus.[4,29,30] We compared background and clinical factors between groups separated by a CAP threshold of 264 dB/m, which allowed significant differentiation of IR in our study population. As a result, body mass index, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, triglyceride, uric acid, hemoglobin A1c, and highly sensitive-C-reactive protein were significantly higher in the group with CAP ≥264 dB/m, and high-density lipoprotein cholesterol was lower. Regarding fibrosis markers, LSM values and APRI were significantly higher in CAP ≥264 dB/m. However, no significant differences were seen in other serological markers, including HA, type IV collagen 7S, and M2BPGi.

LSM has been reported as useful for evaluating fibrosis levels of chronic liver diseases, including NASH.[31] High LSM values in the NAFLD patients with CAP ≥264 dB/m might indicate higher levels of fibrosis in this population, relating to the possible presence of NASH. However, the other serum fibrosis markers did not show significant differences between groups. A recent report showed the possibility of overestimating fibrosis among individuals with high CAP values, especially among those with low fibrosis stages.[32] In addition, high body mass index may falsely increase LSM values in obese individuals.[33] Evaluation of liver fibrosis with TE in this population (almost healthy individuals with at best only a few metabolic complications), should thus be considered carefully.

Nine patients (8.8% of the 102 patients with NAFLD) showed values of ≥264 dB/m and ≥7.0 kPa from TE, leading to suspicion of NASH. Abnormal HA was the most frequent finding (7 of 9 patients) among the fibrosis markers, followed by M2BPGi (4 of 9), NAFLD fibrosis score (3 of 9), and Fib-4 (1 of 9) (Table 3). Because serum HA can be elevated after exercise or by the presence of joint disease, these values should be interpreted with caution. Mizuno *et al.*[34] reported that type IV collagen 7S was more useful than HA in detecting early fibrosis of NASH. The diagnostic significance of M2BPGi in diagnosing NASH seemed to be confirmed by our cut-off index for M2BPGi to reveal early fibrosis of around 0.7.[14,35] Referring to this value, 4 patients (3.9% of the 102 NAFLD patients) fulfilled the criteria for LSM ≥7 kPa and M2BPGi >0.70. Liver biopsy could be considered particularly in these 4 patients (3.9%).

This study has some limitations. First, this was a cross-sectional study that analyzed patients who visited our hospital during a certain period, so selection bias may have been present. Second, this study only examined the incidence of patients with a high index of suspicion for NASH among an almost-normal population without confirming the diagnosis by liver biopsy, and further studies are needed to validate these findings.

In conclusion, a significant correlation was seen between CAP values by TE and the presence of IR, showing that fat infiltration in the liver may be a trigger for inducing IR. About 9% of the Japanese population with NAFLD with a few metabolic complications could be suitable targets for liver biopsy to confirm NASH, including 3.9% with M2BPGi >0.70. In addition to TE, use of other serological tests including M2BPGi may strengthen the decision making process for biopsy.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Collection and summarization of the data (HH), critical discussions regarding the data (KW and KH), writing of the paper (SO).

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