Serum immunoglobulin A levels: Diagnostic utility in alcoholic liver disease and association with liver fibrosis in steatotic liver disease

TATSUKI ICHIKAWA[1-3], MIO YAMASHIMA[1], SHINOBU YAMAMICHI[1], MAKIKO KOIKE[3], YUSUKE NAKANO[3], HIROYUKI YAJIMA[1], OSAMU MIYAZAKI[1], TOMONARI IKEDA[1], TAKUMA OKAMURA[1,2], KAZUYOSHI NAGATA[1], KENICHI SAWA[1], KAZUTAKA NIIYA[1] and KAZUHIKO NAKAO[4]

[1]Department of Gastroenterology, Nagasaki Harbor Medical Center, Nagasaki 850-8555, Japan; [2]Department of Comprehensive Community Care Systems, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8501, Japan; [3]Innovation and Translational Research Center, Nagasaki Harbor Medical Center, Nagasaki 850-8555, Japan; [4]Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8501, Japan

Received May 15, 2024; Accepted July 9, 2024

DOI: 10.3892/br.2024.1830

Abstract. The relationship between immunoglobulin A (IgA) levels and chronic liver disease remains poorly understood. The present study evaluated the clinical significance of IgA in 478 new patients who visited the Outpatient Clinic of Nagasaki Harbor Medical Center (Nagasaki, Japan). Serum IgA levels in comparison to liver stiffness (LS), as measured using a FibroScan® device, were evaluated in 358 patients. Furthermore, in 270 patients, the associations between serum IgA levels and body composition were analyzed using computed tomography. The IgA levels of patients in the groups with Child-Pugh classification B and C (CPGBC), alcoholic liver disease (ALD), steatotic liver disease (SLD) or diabetes were higher than the IgA levels of patients in the groups with CPGA, non-ALD, non-SLD or no diabetes, respectively. Logistic regression analysis showed that CPGBC, ALD, high IgG (>1,700 mg/dl), high macrophage galactose-specific lectin-2 binding protein glycosylation isomer (M2BPGi) (>1 cut-off index) and diabetes were contributing factors for high serum IgA level (>410 mg/dl). The ratio of IgA level divided by IgG level was highest in patients with ALD, followed by those with metabolic dysfunction-associated SLD (MASLD) and non-SLD. In SLD, IgA level was associated more with LS than M2BPGi and fibrosis-4 (FIB-4) in multiple regression analysis. In the receiver operating characteristic analysis, IgA level, M2BPG, and FIB-4 had similar area under the curve values for discriminating high LS (>8 kPa) from low LS (≤8 kPa) in SLD. IgA levels were also associated with visceral fat, and this association was only found in women. In conclusion, elevated IgA is an indicator of liver fibrosis that also reflects the presence of diabetes and an increased visceral fat level. Therefore, IgA is considered a useful marker of liver disease severity in the current era of increased SLD.

Correspondence to: Dr Tatsuki Ichikawa, Department of Gastroenterology, Nagasaki Harbor Medical Center, 6-39 Shinchi, Nagasaki 850-8555, Japan
E-mail: ichikawa@nagasaki-u.ac.jp

Abbreviations: Ig, immunoglobulin; CLD, chronic liver disease; LS, liver stiffness; CAP, controlled attenuation parameter; CPG, Child-Pugh group; ALD, alcoholic liver disease; SLD, steatotic liver disease; DM, diabetes mellitus; AFP, α-fetoprotein; FIB-4, fibrosis-4; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; MASLD, metabolic dysfunction-associated steatotic liver disease; ROC, receiver operating characteristic; AUC, area under the curve; NASH, non-alcoholic steatohepatitis; NIT, non-invasive test; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; SM, skeletal muscle; VAT, visceral adipose tissue; BMI, body mass index; Cr, creatinine; eGFR, estimated glomerular filtration rate; CysC, cystatin C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALBI, albumin bilirubin score; CI, confidence interval

Key words: immunoglobulin A, alcoholic liver disease, steatotic liver disease, liver stiffness, visceral adipose tissue

Introduction

Immunoglobulin A (IgA) is a component of the balance between bacterial colonization and containment in the intestines (1,2). The importance of gut microbial metabolites in regulating IgA production has been reported previously (3).

The liver is a frontline organ that receives gut-derived products through the portal vein; thus, the liver can be severely affected by disrupted intestinal homeostasis (4). A retrospective analysis reported that advancing cirrhosis, irrespective of the underlying etiology or hepatocellular carcinoma, resulted in progressively increasing serum IgG and IgA levels (5). IgA secretion and Fc receptor γ signaling aggravate hepatic fibrosis in mice and patients with non-alcoholic steatohepatitis (NASH) (6). Additionally, the positive correlation between serum IgA levels and activated Fc receptor γ-positive hepatic myeloid cells, as well as the extent of liver fibrosis, has been

reported (6). Moreover, the association between elevated serum IgA level and advanced liver disease was demonstrated in steatotic liver diseases (SLDs), including alcoholic liver disease (ALD) and metabolic dysfunction-associated SLD (MASLD) (5-8).

As ALD and MASLD have a heavy disease burden on a global basis, the diagnosis of advanced fibrosis in SLD is commonly required in primary medicine (9,10). Additionally, since the twin epidemics of obesity and type 2 diabetes mellitus (T2DM) also increase the incidence of MASLD, non-invasive tests (NITs) have been used to identify patients with non-alcoholic fatty liver disease (NAFLD) and those who are at risk of liver disease progression (11). Patients at risk for MASLD [those with T2DM, obesity or chronically elevated alanine aminotransferase (ALT) levels] have been screened for fibrosis-4 (FIB-4) (11,12). As a FIB-4 level >1.3 is related to a moderate-to-high risk for liver fibrosis, these patients should be assessed using second-line NITs (11,12). Liver stiffness (LS), measured using a FibroScan® device (Echosens), is the most useful second-line tool for assessing liver fibrosis in SLD (13). LS >8 kPa indicates an intermediate or high risk of advanced liver fibrosis (F2-F4 by biopsy) (11-13). Macrophage galactose-specific lectin-2 binding protein glycosylation isomer (M2BPGi) is also associated with advanced liver fibrosis in MASLD (14).

The association between IgA and metabolic syndrome is mediated via gut microbiota (15). Serum IgA may bind to these gut microbial antigens, restrict their toxicity and control gut microbial antigens in the circulation, thereby reducing systemic inflammation (15). Decreased IgG and IgM levels, and increased IgA levels are independently associated with T2DM prevalence in the adult population (16). Poor glycemic management may be associated with elevated serum IgA levels and IgG antibodies in patients with T2DM (17). Furthermore, the onset of T2DM is predicted by visceral fat mass and the ratio of visceral to subcutaneous fat mass evaluated using computed tomography (CT) (18). Visceral fat mass is an important prognostic marker of liver disease and sarcopenia (19).

The present study investigated the significance of serum IgA levels in patients with liver disease who were initially diagnosed in the Department of Gastroenterology in Nagasaki Harbor Medical Center (Nagasaki, Japan). As the association between NITs (LS, FIB-4 and M2BPGi) and IgA levels has not been reported, a focus was placed on such NITs Additionally, the associations between body composition and IgA levels were evaluated in patients who underwent CT.

Materials and methods

Patients. In total, 478 patients first diagnosed with liver disease in Nagasaki Harbor Medical Center between May 2017 and October 2023 were initially included in the present study (Table I; Fig. S1A). The median patient age was 68 years (range, 27-84 years). A total of 249 patients were female and 229 were male. Of them, clinically, 18 patients presented with autoimmune hepatitis, 64 patients presented with ALD and 54 patients presented with the treatment-naïve hepatitis B virus (HBV). Furthermore, 114 patients had a treatment-naïve hepatitis C virus (HCV) infection, 1 had a treatment-naïve HBV and HCV infection, 129 had MASLD and 24 had treatment-naïve primary biliary cholangitis. Another 2 patients had treatment-naïve primary sclerosing cholangitis. The diagnosis of fatty liver was obtained by ultrasound echography. ALD was diagnosed using the new nomenclature (20). Metabolic and alcohol related/associated liver disease Met-ALD (20) was included in the definition of MASLD in this study, whereas SLD included both ALD and MASLD. A further 72 patients had other treatment-naïve liver diseases (e.g., unknown cause or drug-induced liver damage). T2DM was defined as follows: Fasting serum glucose ≥100 mg/dl, 2-h post-load glucose levels ≥140 mg/dl, HbA1c ≥5.7%, diagnosed as T2DM at the first visit or receiving treatment for T2DM (20).

Of the 478 patients, 353 patients with liver disease were evaluated with the FibroScan device. The clinical characteristics of these patients are presented in Table SI. LS (kPa) was evaluated using vibration-controlled transient elastography, and liver fat content (dB/m) was evaluated using the controlled attenuation parameter (CAP), both functions of FibroScan. Of the 478 patients, 270 patients with liver disease were evaluated using CT for hepatoma screening. The clinical characteristics of these patients are presented in Table SII. Cross-sectional CT images of the third lumbar vertebrae (L3) were analyzed using Slice-O-Matic software (version 5.0; TomoVision) to determine the skeletal muscle (SM) mass, including the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles. Tissue Hounsfield unit (HU) thresholds were employed as follows: 29 to 150 HU for SM, 190 to 30 for subcutaneous adipose tissue and 150 to 50 for visceral adipose tissue (VAT) (21). The visceral-to-subcutaneous fat ratio (VSR) is an index of VAT divided by SAT.

The medical records of 478 patients were retrospectively reviewed, and all laboratory measurements were obtained from these records. Informed consent was obtained from each patient included in the study, and they were guaranteed the right to leave the study if desired. The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki (22) and was approved by the Human Research Ethics Committee of Nagasaki Harbor Medical Center (approval no. H30-031).

Laboratory measurements. Laboratory data and anthropometric measurements were obtained from each participant during outpatient visits. The body mass index (BMI) of each patient was calculated by dividing their weight (kg) by the square of their height (m). The normal BMI range is 20-25 kg/m^2. Grip strength was measured using a dynamometer (Smedley Dynamo Meter; Tsutsumi Co., Ltd.) with the participants standing in an erect position with both arms at their sides. The normal laboratory ranges used were as follows: Total bilirubin, 0.3-1.2 mg/dl; albumin, 3.8-5.2 g/dl; prothrombin time international normalized ratio, 0.85-1.15; creatinine (Cr) for male patients (M), 0.61-1.04 mg/dl, and for female patients (F), 0.47-0.79 mg/dl; Cr-estimated glomerular filtration rate (eGFR), <90 ml/min/1.73 m^2; cystatin C (CysC) for M, 0.63-0.95 mg/l, and for F, 0.56-0.87 mg/l; CysC-eGFR, <90 ml/min/1.73 m^2; platelets for M, 13.1-26.2x10^4/μl, and for F, 13.0-36.9x10^4/μl; aspartate aminotransferase (AST), 10-40 U/l; ALT, 5-40 U/l; M2BPGi, less than the cut-off index (C.O.I.) value of 1; α-fetoprotein (AFP), <10 ng/ml; protein induced by vitamin K absence or antagonist-II, <40 mAU/ml; IgG,

Table I. Clinical characteristics (n=478).

Characteristic	Value	95% CI	%
Age, years[a]	68	27.4-87	
Sex, n			
Female	249		52.09
Male	229		47.91
Disease, n			
AIH	18		3.77
Alcohol	64		13.39
HBV	54		11.3
HBV + HCV	1		0.21
HCV	114		23.85
MASLD	129		26.99
PBC	24		5.02
PSC	2		0.42
Other	72		15.06
Malignant disease, n			
Breast cancer	15		3.14
Bladder cancer	1		0.21
Biliary cancer	5		1.05
Colorectal cancer	5		0.84
Cholangioma	4		0.84
Hepatoma	35		7.32
Lung cancer	2		0.42
Gastric cancer	4		0.84
Malignant lymphoma	3		0.63
Gynecological cancer	2		0.41
Pancreatic cancer	8		1.67
None	392		82.01
Diabetes, n			
Positive	106		22.18
Negative	372		77.82
Total bilirubin, mg/dl[a]	0.8	0.3-2.86	
Albumin, g/dl[a]	4.1	3.8-4.8	
ALBI[a]	-2.784	-3.329-(-1.4589)	
ALBI grade, n			
1	314		65.69
2	154		32.22
3	10		2.09
PT INR[a]	1.01	0.8-1.391	
CPS[a]	5	5-8	
CP grade A/B/C, n			
A	442		92.47
B	31		6.49
C	5		1.05
MELD[a]	7	5-8	
Cr, mg/dl[a]	0.76	0.48-2.23	
Cr-eGFR, ml/min/1.73 m^{2a}	68.6	20.74-110.9	
CysC, mg/l[a]	1.05	0.6645-3.062	
CysC-eGFR, ml/min/1.73 m^{2a}	65.75	14.25-117.93	
Height, m[a]	1.6	1.4-1.77	
Body weight, kg[a]	59.35	37-94.4	
BMI, kg/m^{2a}	23.37	16.07-34.19	
BMI, n			
Normal	302		63.18
Obesity	176		36.82
Platelets, x$10^4/\mu$l[a]	19.3	6.19-34.06	
AST, U/l[a]	38.5	15.5-290.5	
ALT, U/l[a]	40	8.45-367.9	
FIB-4[a]	2.3128	0.6092-11.4661	
M2BPGi (cut-off index COI) [a]	1.2	0.3-2.3	
AFP, ng/ml[a]	4.6	1.6-122.9	
PIVKA-II, mAU/ml[a]	23	12-7484	
IgG, mg/dl[a]	1438	798.3-1753	
IgG , n			
>1,700 mg/dl	134		28.03
≤1,700 mg/dl	344		71.97
IgM, mg/dl[a]	89	29-137	
IgM by sex, n			
>190 for males/>260 for females, mg/dl	36	7.53	
≤190 for males/≤260 for females, mg/dl	442	92.47	
IgA, mg/dl[a]	282	83.5-376	
IgA, n			
>410 mg/dl	90	18.83	
≤410 mg/dl	388	81.17	

[a]Data are presented as the median. HBV, hepatitis B virus; HCV, hepatitis C virus; MASLD, metabolic dysfunction-associated steatotic liver disease; AIH, autoimmune hepatitis; PBS, primary biliary cholangitis; PSC, primary sclerosing cholangitis; ALBI, albumin bilirubin score; PT INR, prothrombin time international normalized ratio; CP, Child-Pugh; CPS, Child-Pugh Score; MELD, Model for End-Stage Liver Disease; Cr, creatinine; Cr-eGFR, creatinine-estimated glomerular filtration rate; CysC, cystatin C; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; FIB-4, fibrosis-4; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; PIVKA-II, protein induced by vitamin K absence or antagonist-II; Ig, immunoglobulin.

<1,700 mg/dl; IgM for M, <190 mg/dl and for F, <260 mg/dl; and IgA <410 mg/dl (Fig. S1B). The Child-Pugh score (CPS) (23), model of end-stage liver disease (24), albumin-bilirubin score (ALBI) (25), FIB-4 (26) and Fibroscan-AST score (FAST) (27) were calculated as previously reported. A normal FIB-4 score is <1.3 (11,12).

Statistical analysis. Data were analyzed using StatFlex (version 6.0; Artech LLC) and are presented as the median and 95% confidence interval (CI). Laboratory variables were compared using Mann-Whitney U tests (for differences

Table II. Association between IgA levels and clinical factors.

Factor	Median	R value	P-value
Sex, (n=478)			<0.00001
Female	256		
Male	310		
Age, years (n=478)		0.0658	0.15080
ALD, (n=64)			<0.00001
Positive	360.5		
Negative	270		
MASLD, (n=129)			0.87964
Positive	287		
Negative	280		
SLD, (n=193)			0.00037
Positive	304		
Negative	263		
HCC, (n=35)			0.37400
Positive	406.8		
Negative	307.3		
CPG, (n=478)			<0.00001
A	274.5		
BC	431		
ALBI (n=478)		0.4111	<0.00001
FIB-4 (n=478)		0.2638	<0.00001
M2BPGi (COI) (n=478)		0.3676	<0.00001
BMI, kg/cm^2 (n=478)		0.1089	0.01720
DM, (n=106)			0.00052
Positive	330		
Negative	273		
AFP, ng/ml (n=478)		0.2349	<0.00001
PIVKA-II, mAU/ml (n=478)		0.0245	0.59327
Total protein, g/dl (n=478)		0.19	0.00003
Albumin, g/dl (n=478)		0.395	<0.00001
IgG, mg/dl (n=478)		0.2778	<0.00001
IgM, mg/dl (n=478)		0.0582	0.20407

ALD, alcoholic liver disease; SLD, steatotic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; HCC, hepatocellular carcinoma; ALBI, albumin bilirubin score; CPG, Child-Pugh group; BMI, body mass index; COI, cut-off index; AFP, α-fetoprotein; FIB-4, fibrosis-4; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; PIVKA-II, protein induced by vitamin K absence or antagonist-II; Ig, immunoglobulin; DM, diabetes mellitus.

between two groups) and Kruskal-Wallis tests (for differences between three groups). Multiple comparisons among independent groups were conducted using Dunn's post hoc test. A multiple regression analysis was performed, and a standardized partial regression coefficient, β, was employed. Univariate and multivariate analyses were performed using logistic regression. Correlations were evaluated using the Pearson's correlation coefficient (R). The detection level was analyzed using receiver operating characteristic (ROC) curves. P<0.05 was used to indicate a statistically significant difference.

Table III. Association among body composition, muscle markers and IgA levels.

	IgA	
Factor	R-value	P-value
SM, cm^2	0.162	0.00748
IMAT, cm^2	0.028	0.65182
VAT, cm^2	0.190	0.00178
SAT, cm^2	0.026	0.67311
VSR	0.258	0.00002
MA, HU	0.021	0.73220
SMI, cm^2/m^2	0.112	0.06579
Grip strength, kg	0.045	0.46230
BMI, kg/m^2	0.130	0.03278

SM, skeletal muscle; IMAT, internal muscle adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, visceral-to-subcutaneous fat ratio; MA, muscle attenuation; SMI, SM index; BMI, body mass index.

Results

First, the associations between IgA levels and clinical factors were evaluated (Table II). If the clinical factors were continuous data, the correlation between the serum IgA titer and clinical factors was evaluated. If the clinical factors were grouped, a Mann-Whitney U analysis was performed. The results of the analysis showed that sex, ALD, SLD, CPG, ALBI, FIB-4, M2BPGi, BMI, T2DM, AFP, total protein, albumin and IgG levels were significantly associated with IgA levels (Table II). Of these factors, continuous data were then evaluated by multiple regression analysis for serum IgA levels (Fig. 1A), demonstrating that ALBI, AFP, CPS, IgG and BMI were significantly associated with serum IgA levels. The R values (P-values) in relation to IgA and LS were 0.4609 (<0.00001) and 0.5997 (<0.00001) in MASLD and ALD, respectively. Factors contributing to high serum IgA levels (high IgA; >410 mg/dl) were analyzed using logistic regression analysis. After including CPGBC, ALD, IgG 1,700 mg/dl (higher than normal range), M2BPGiH (higher than normal range), T2DM, sex, AFP 10 ng/ml (higher than normal range), BMI (>25 kg/m^2) and FIB 2.67 [>2.67 (28)] in the analysis, it was found that CPGBC, ALD, high IgG, high M2BPGiH and T2DM were contributing factors for high IgA levels (Fig. 1B). In the multivariate logistic model, SLD did not contribute to high IgA levels when ALD (Fig. 1B) was changed to SLD (odds ratio, 1.708; 95% CI, 0.962-3.031). The characteristics of patients with ALD were compared with those of patients with MASLD and non-SLD. In patients with ALD, serum IgG levels were lower compared with those in patients with non-SLD, but not compared with those in patients with MASLD (Fig. 2A). Serum IgA levels in patients with ALD were higher than those in patients with MASLD

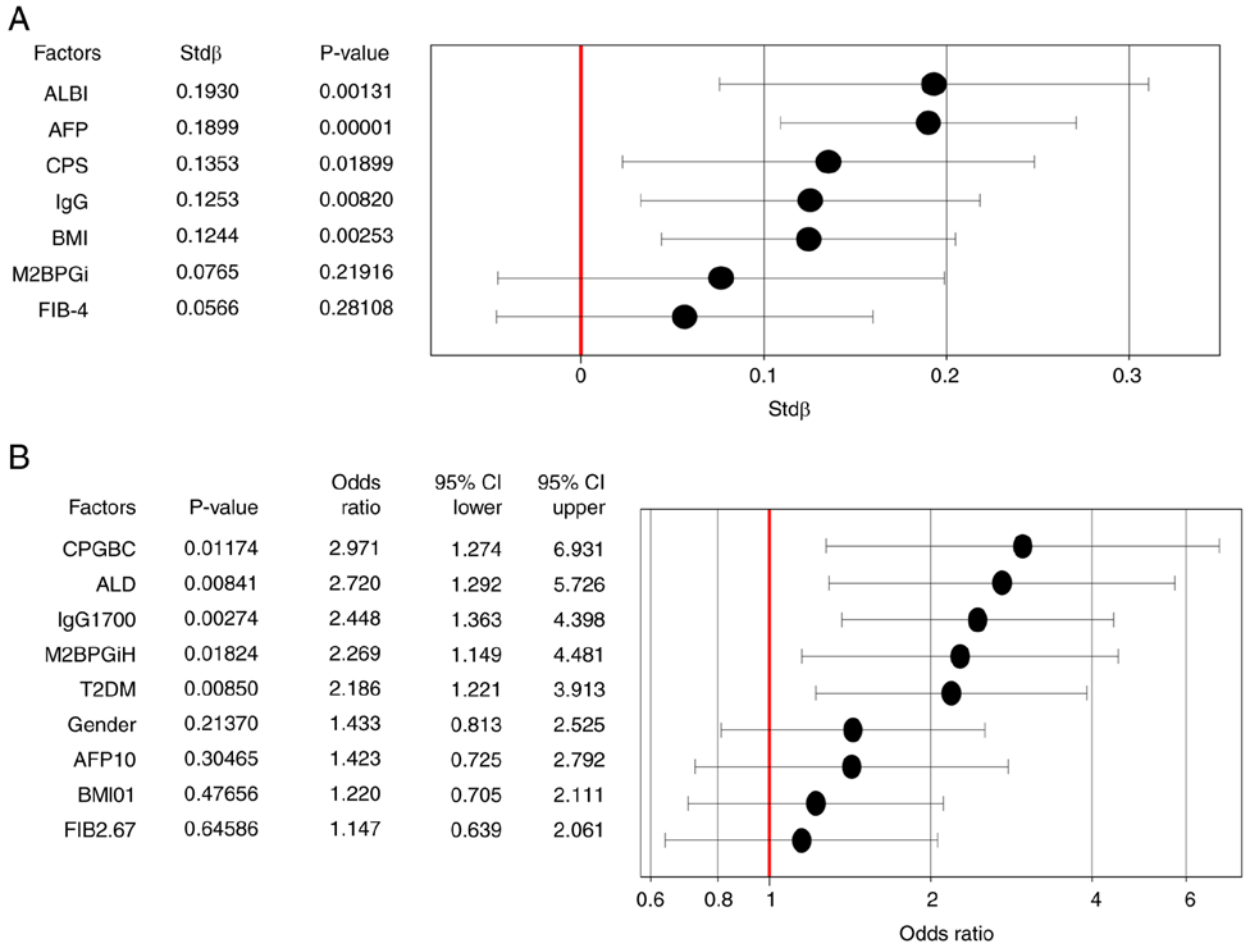


Figure 1. Association between serum IgA level and clinical factors. (A) Multiple regression analysis of serum IgA levels. The explanatory variable is along the y-axis. Stdβ is the standard partial regression coefficient. The stdβ and 95% CI values are indicated on the x-axis. (B) Logistic multiple regression analysis of high serum IgA levels. A level >410 mg/dl was considered a high serum IgA level. The explanatory variable is along the y-axis. Odds ratios and 95% CI are indicated on the x-axis. CI, confidence interval; ALBI, albumin bilirubin score; AFP, α-fetoprotein; CPS, Child-Pugh score; Ig, immunoglobulin G; BMI, body mass index; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer; FIB-4, fibrosis-4.

and non-SLD (Fig. 2B). The IgA/G ratio (serum IgA divided by IgG) was higher in the patients with ALD than that in the patients with MASLD and non-SLD (Fig. 2C). An attempt was made to determine the difference between ALD and non-ALD using serum IgG and IgA levels and IgA/G ratio by ROC analysis (Fig. 2D). The cutoff value was set at the point where sensitivity and specificity are equal. The cut-off point for IgG was 1,358.1 mg/dl (sensitivity, 0.5625), that for IgA was 305.7 mg/dl (sensitivity, 0.614) and the IgA/G ratio was 0.2 (sensitivity, 0.6715). The IgA/G ratio was therefore more valuable than IgG and IgA levels in distinguishing patients with ALD from those with non-ALD.

Next, the associations between IgA levels and LS were evaluated (Table SI; Fig. 3). LS was compared with NITs (M2BPGi and FIB-4), IgG and IgA levels, and IgA/G ratio. Multivariate regression analysis revealed that, in the entire cohort (478 cases), IgA levels, IgA/G ratio, M2BPGi and FIB-4 were associated with LS levels (Fig. 3A). In the SLD group (169 cases), IgA levels and the IgA/G ratio were associated with LS levels (Fig. 3B); however, in the non-SLD group (309 cases), only M2BPGi was significantly associated with LS levels (Fig. 3C). In the SLD group, IgA levels, M2BPGi and FIB-4 were compared for their association with high LS (>8 kPa) using ROC analysis. IgA levels (AUC, 0.79362), M2BPGi (AUC, 0.84439) and FIB-4 (AUC, 0.78391) were equally useful for diagnosing high LS (Fig. 3D). The associations between IgA levels and CAP were evaluated, but no significant association was found (Table SIII). CAP values were positively correlated with BMI and negatively correlated with age and ALBI (Table SIII). IgA showed a correlation with FAST in both males (Fig. S2A) and females (Fig. S2B). However, there was no correlation between IgA and CAP in males (Fig. S2A and C), while a weak correlation with CAP was observed in females (Fig. S2B and D)

Next, the associations between IgA levels and body composition were evaluated (Tables III and SII). IgA levels were associated with SM, VAT, VSR and BMI (Table III). In particular, a weak correlation was observed between VAT and IgA, and between VSR and IgA in females (Fig. S3). No association was found between IgA and SM or IgA and BMI in women (Fig. S3). Since body composition is influenced by sex differences (19), the cut-off value for detecting high IgA levels was evaluated using ROC analysis. In males, the cut-off value (sensitivity) for high IgA level was 23.3 (0.525) for BMI, 121.3 (0.536) for SM and 1.2 (0.552) for VSR. No significant difference was observed in the area under the curve (AUC) among

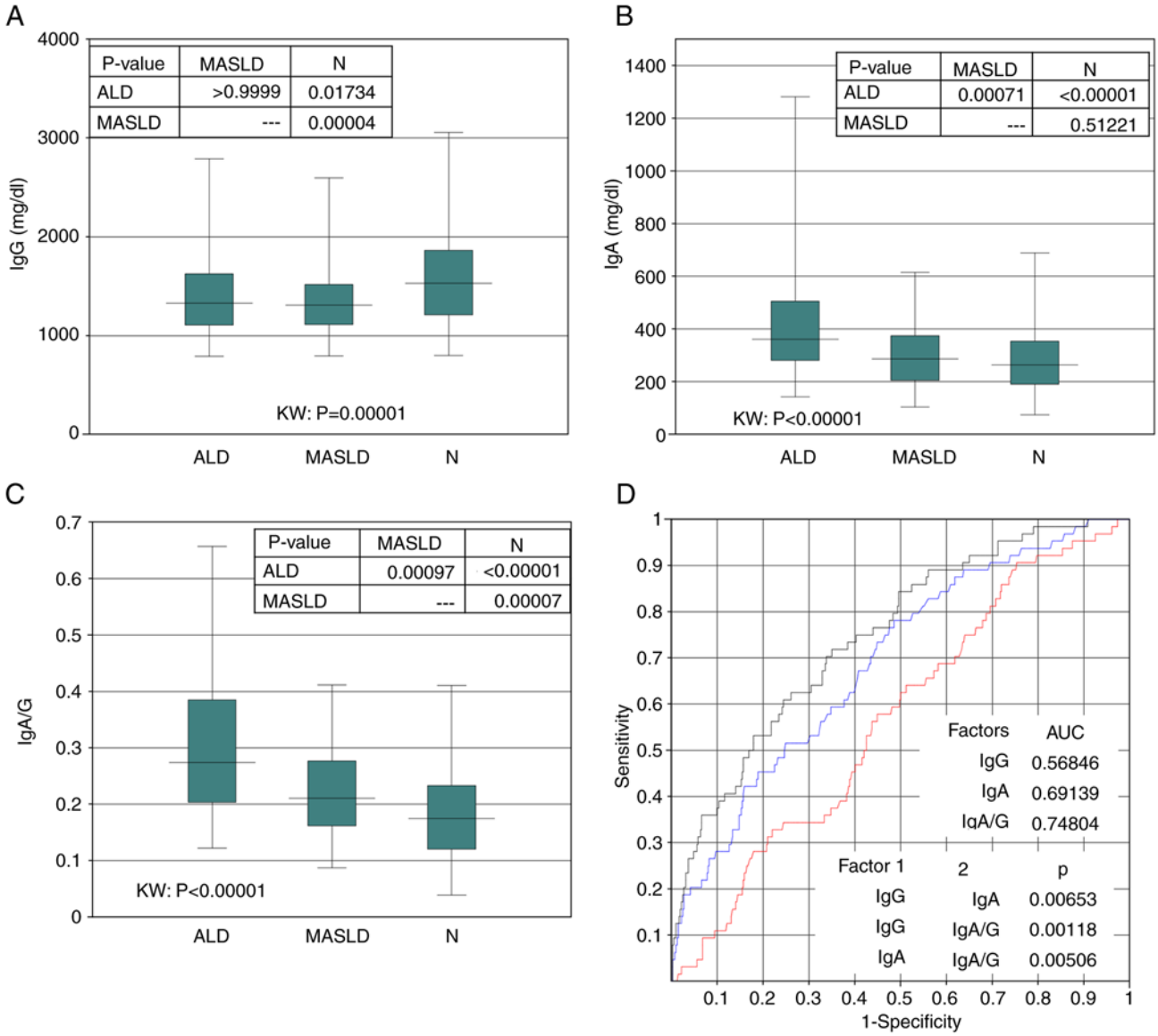


Figure 2. Comparison of IgA and IgG levels and IgA/G ratio among patients with ALD, MASLD and non-SLD. The P-values in the inset tables represent the results of the Dunn post hoc test. (A) Serum IgG titer (mg/dl), (B) serum IgA titer (mg/dl) and (C) the IgA/G ratio in the three groups. (D) Receiver operating characteristic analysis for ALD. The AUC is indicated. The black line represents the IgA/G ratio, the blue line represents IgA and the red line represents IgG. Ig, immunoglobulin; ALD, alcoholic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; N, non-steatotic liver disease; AUC, area under the curve; KW, Kruskal-Wallis.

the three groups (Table IV; Fig. S4A). In females, the cut-off value (sensitivity) for high IgA level was 23.25 (0.571) for BMI, 85.18 (0.504) for SM and 0.7 (0.741) for VSR. Similarly, no significant difference was observed in the AUC among the three groups (Table IV; Fig. S4B). In the multivariate logistic analysis, high VSR contributed to high IgA levels in females but not in males (Table V).

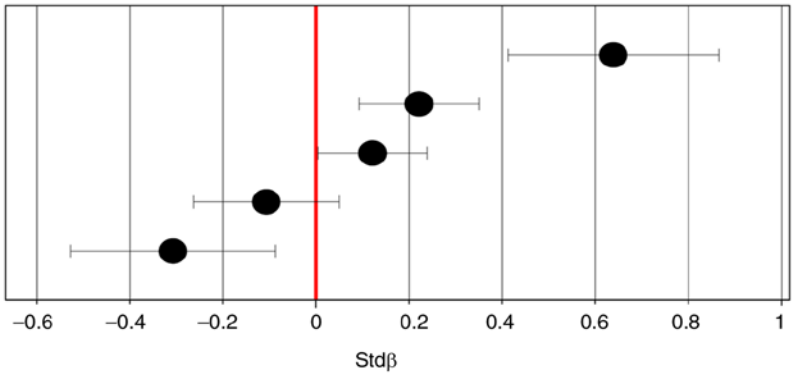
Discussion

The present study showed that in chronic liver disease (CLD), CPGBC, ALD, high IgG (>1,700 mg/dl), high M2BPGi (>1) and T2DM are associated with high IgA levels. The IgA/G ratio was the highest in patients with ALD, followed by those with MASLD and non-SLD. High LS was associated with high IgA levels, and IgA level was more strongly associated with LS than with M2BPGi and FIB-4. IgA level was associated with VSR and was particularly pronounced in females.

Previous reports have shown that ALD is associated with high serum IgA levels (5,6,29). High IgA levels are related to severe liver disease, including ALD, and high IgG levels are also associated with decompensated cirrhosis (5). IgA levels are elevated in ALD, and an increased IgA/IgG ratio is highly suggestive of ALD (29). IgA/G ratio >0.2 (sensitivity, 0.6715) was more valuable than IgG and IgA levels in distinguishing patients with ALD from those with non-ALD. We consider that IgA level, in combination with IgG level, can be used as a biomarker for ALD. By contrast, SLD, including ALD and MASLD, did not contribute to high IgA levels in the present study. Unlike pathogenic bacteria, commensal bacteria do not induce a systemic IgG response but only a mucosal IgA response, which is different from the response to non-invasive

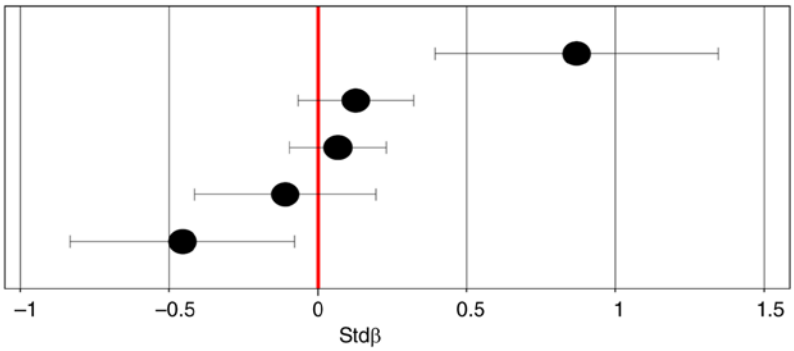
A All

Factors	Stdβ	P-value
IgA	0.6385	<0.00001
M2BPGi	0.2211	0.00085
FIB-4	0.1209	0.04285
IgG	−0.1069	0.18020
IgA/G	−0.3076	0.00624



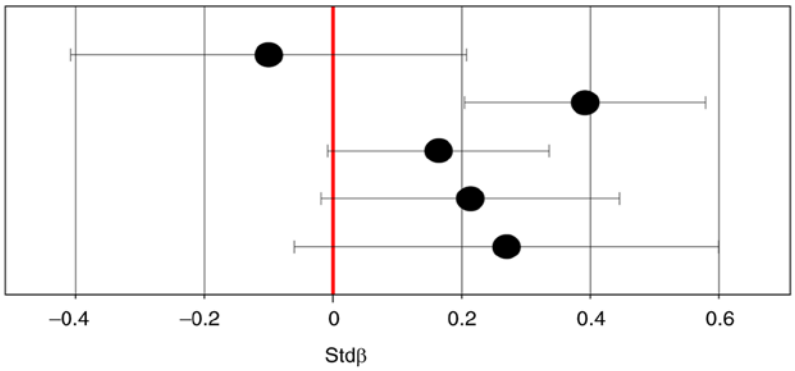
B SLD

Factors	Stdβ	P-value
IgA	0.8701	0.00041
M2BPGi	0.1277	0.19451
FIB-4	0.0672	0.41792
IgG	−0.1098	0.47747
IgA/G	−0.4555	0.01816

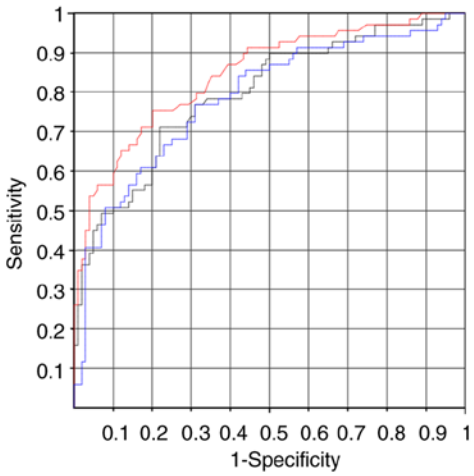


C Non SLD

Factors	Stdβ	P-value
IgA	−0.1004	0.52036
M2BPGi	0.3918	0.00006
FIB-4	0.1643	0.06126
IgG	0.2134	0.07097
IgA/G	0.2697	0.10842



D SLD



Factors	AUC	Cut off value	Sensitivity	PPV	NPV
IgA	0.79362	312	0.71	51.1	71.3
M2BPGi	0.84439	1.1	0.75362	49.8	51.6
FIB-4	0.78391	2.4	0.71	37.4	57.7

Factor 1	2	p
IgA	M2BPGi	0.20535
IgA	FIB-4	0.81115
M2BPGi	FIB-4	0.07485

Figure 3. Association between LS and clinical factors. (A-C) Multiple regression analysis for LS: (A) All patients, (B) SLD and (C) non-SLD. The stdβ and 95% CI values are indicated on the x-axis. The explanatory variables lies on the y-axis. (D) Receiver operating characteristic analysis for high LS (>8 kPa) in SLD. The AUC is indicated. The P-value is the difference in AUC between groups 1 and 2. The black line represents IgA, the blue line represents FIB-4 and the red line represents M2BPGi. The cut-off value is the point with equal sensitivity and 1-specificity. PPV, positive prediction value; NPV, negative prediction value; AUC, area under the curve; Ig, immunoglobulin; LS, liver stiffness; SLD, steatotic liver disease; CI, confidence interval; FIB-4, fibrosis-4; M2BPGi, macrophage galactose-specific lectin-2 binding protein glycosylation isomer.

Table IV. Cut-off value for BMI, SM and VSR for high serum immunoglobulin A level as per the receiver operating characteristic analysis.

	Male					Female				
Factors	Object	Control	AUC	Cut-off value	Sensitivity	Object	Control	AUC	Cut-off value	Sensitivity
BMI	40	97	0.52178	23.33	0.525	14	119	0.60864	23.25	0.571
SM	40	97	0.54240	121.3	0.536	14	119	0.57413	85.18	0.5
VSR	39	96	0.57051	1.2	0.552	12	116	0.78161	0.7	0.741

BMI, body mass index; SM, skeletal muscle; VSR, visceral-to-subcutaneous fat ratio; AUC, area under the curve.

Table V. Association between body composition and high immunoglobulin A level analyzed using multivariate logistic analysis.

	Multivariate logistic analysis			Adjusted multivariate logistic analysis[a]		
Factor	P-value	Odds ratio	95% CI	P-value	Odds ratio	95% CI
Females (n=128)						
VSRH	0.00263	9.451	2.187-40.847	0.00887	11.581	1.850-72.500
SMH	0.28494	2.246	0.510-9.895	0.21309	3.501	0.487-25.174
BMIH	0.59621	0.665	0.147-3.008	0.78905	0.770	5.245
Males (n=135)						
VSRH	0.27928	1.557	0.698-3.473	0.26382	1.632	0.691-3.852
SMH	0.15928	0.532	1.281	0.27658	0.602	1.501
BMIH	0.57518	1.300	0.520-3.251	0.66776	1.230	3.168

[a]Adjusted for Child-Pugh group A/BC, alcoholic liver disease and diabetes mellitus. CI, confidence interval; VSRH, high visceral-to-subcutaneous fat ratio; BMIH, high body mass index; SMH, high skeletal muscle.

strains of *Salmonella*, which are treated differently compared with pathogenic strains, even if the commensal bacteria are non-invasive (30). Since IgA levels in patients with ALD were higher than those in patients with MASLD in the present study, we hypothesized that alcohol consumption and metabolic abnormalities may have different effects on the gut microbiota, which may be reflected in the differences in IgA and IgG levels.

Furthermore, elevated IgA levels reflect the severity of liver disease, regardless of the cause of the liver disease (5). In the present study, as CPGBC contributed to high IgA levels, there was no contradiction to this result. Notably, M2BPGi, a marker of liver fibrosis, also contributed to high IgA levels. When examining the associations between LS, typical second-line NIT and IgA level, an association between high IgA level and high LS as a high-risk factor for advanced fibrosis (11-13) was observed in SLD. In SLD, IgA level was equivalent to M2BPGi and FIB-4 as a marker for discriminating advanced fibrosis. In a previous study using a mouse NASH model and patients with NASH, the levels of serum IgA secreted by the plasma cells of secondary lymphoid organs was shown to be elevated in patients with NAFLD and was an independent predictor of advanced fibrosis (6). In the present study, high IgA levels were associated with liver fibrosis in patients with SLD, including ALD. There are a variety of common mechanisms that cause the elevated IgA underlying both diseases, including alcoholic liver disease and NAFLD (31). A previous review (31) explored the similar downstream signaling events involved in the onset and progression of the two entities, which are not completely different, predominantly focusing on the gut microbiome. We hypothesize that among the downstream events, lipopolysaccharide and bacterial migration are associated with increased blood IgA. Therefore, we hypohesize that IgA level (>312 mg/dl) is a useful marker of advanced liver fibrosis in SLD.

T2DM also contributed to high IgA levels in the present study; however, BMI was not associated with IgA levels. Therefore, the association between IgA levels and body composition was evaluated. Poor glycemic control is reportedly associated with high IgA levels (17). Elevated VSR (≥1 in males and ≥0.5 in females) is an independent risk factor for T2DM development (18). Notably, VSR (>1.33 in males and >0.93 in females) independently predicted the outcomes (mortality) of hepatocellular carcinoma (21). In the present study, high VSR (≥0.7 in females) contributed to high IgA, but not in males. Sex differences in VSR were detected in previous studies (18,19), and other reports have described that high VSR, but not sex differences, predicts advanced fibrosis in NAFLD (32,33). In

a previous study, VSR evaluated using CT was independently associated with VAT inflammation, and VSR was significantly associated with histological VAT inflammation in cirrhotic males but not in females (34). In females, the association between high IgA and high VSR was close; however, such an association in males should be further evaluated in the future. In the present study, SM was not associated with high IgA. However, a limitation in the field of clinical investigation of sarcopenic patients is the lack of a generally accepted definition coupled with the difficulty of adopting common diagnostic criteria (35). The association between sarcopenia and IgA in liver disease is a future challenge.

The present study had several limitations. Differentiation between ALD and MASLD was performed using medical records, and met-ALD was included in MASLD. Therefore, the association between alcohol consumption and serum IgA levels should be examined in the future. Additionally, treatment for diabetes was not considered. Thus, although T2DM contributed to high IgA levels, the glycemic control levels could not be evaluated. Finally, this was a single-hospital, small, retrospective study, and body composition factors associated with IgA were unknown in males. These issues should to be further examined in the future.

In conclusion, the present study demonstrated the usefulness of serum IgA measurements in CLD. IgA levels, in combination with IgG levels, are useful for the differential diagnosis of ALD. In SLD, IgA level is comparable to known NITs (FIB-4 and M2BPGi) in its ability to discriminate patients with advanced LS. T2DM is associated with high IgA levels regardless of sex, and visceral obesity (high VSR) is associated with high IgA levels in females. In the current era of increasing SLD, the evaluation of serum IgA level as a new NIT is important for the assessment of liver disease.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Author's contributions

TIc wrote the manuscript, analyzed the data and designed the study. TIc, MY, SY, MK, YN, HY, OM, TIk, TO, KNag, KS, KNi and KNak collected the data. TIc and MY, confirm the authenticity of all the raw data. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki, which was approved by the Human Research Ethics Committee of the Nagasaki Harbor Medical Center (Nagasaki, Japan; approval no. H30-031). Informed consent was obtained from each patient included in the study, and they were guaranteed the right to leave the study if desired.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Pabst O, Cerovic V and Hornef M: Secretory IgA in the coordination of establishment and maintenance of the microbiota. Trends Immunol 37: 287-296, 2016.
2. Lycke NY and Bemark M: The regulation of gut mucosal IgA B-cell responses: Recent developments. Mucosal Immunol 10: 1361-1374, 2017.
3. Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, Kitami T, Taida T, Sasaki T, Negishi H, *et al*: Acetate differentially regulates IgA reactivity to commensal bacteria. Nature 595: 560-564, 2021.
4. Inamine T and Schnabl B: Immunoglobulin A and liver diseases. J Gastroenterol 53: 691-700, 2018.
5. Doi H, Hayashi E, Arai J, Tojo M, Morikawa K, Eguchi J, Ito T, Kanto T, Kaplan DE and Yoshida H: Enhanced B-cell differentiation driven by advanced cirrhosis resulting in hyperglobulinemia. J Gastroenterol Hepatol: 2018 (Online ahead of print).
6. Kotsiliti E, Leone V, Schuehle S, Govaere O, Li H, Wolf MJ, Horvatic H, Bierwirth S, Hundertmark J, Inverso D, *et al*: Intestinal B-cells license metabolic T-cell activation in NASH microbiota/antigen-independently and contribute to fibrosis by IgA-FcR signalling. J Hepatol 79: 296-313, 2023.
7. Tomita K, Teratani T, Yokoyama H, Suzuki T, Irie R, Ebinuma H, Saito H, Hokari R, Miura S and Hibi T: Serum immunoglobulin A concentration is an independent predictor of liver fibrosis in nonalcoholic steatohepatitis before the cirrhotic stage. Dig Dis Sci 56: 3648-3654, 2011.
8. Maleki I, Aminafshari MR, Taghvaei T, Hosseini V, Rafiei A, Torabizadeh Z, Barzin M and Orang E: Serum immunoglobulin A concentration is a reliable biomarker for liver fibrosis in non-alcoholic fatty liver. World J Gastroenterol 20: 12566-12573, 2014.
9. Danpanichkul P, Ng CH, Muthiah MD, Duangsonk K, Yong JN, Tan DJH, Lim WH, Wong ZY, Syn N, Tsusumi T, *et al*: The silent burden of non-alcoholic fatty liver disease in the elderly : A global burden of disease analysis. Aliment Pharmacol Ther 58: 1062-1074, 2023.
10. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E and Kamath PS: Global burden of liver disease: 2023 update. J Hepatol 79: 516-537, 2023.
11. Younossi ZM, Henry L, Isaacs S and Cusi K: Identification of high risk NAFLD patients in endocrinology clinics. Endocr Pract 29: 912-918, 2023.
12. Wattacheril JJ, Abdelmalek MF, Lim JK and Sanyal AJ: AGA clinical practice update on the role of noninvasive biomarkers in the evaluation and management of nonalcoholic fatty liver disease: Expert review. Gastroenterology 165: 1080-1088, 2023.
13. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Guha IN, Cobbold JF, Deeks JJ, Paradis V, *et al*: Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 156: 1717-1730, 2019.
14. Kiyoaki I, Sumida Y, Nakade Y, Okumura A, Nishimura S, Ibusuki M, Kitano R, Sakamoto K, Kimoto S, Inoue T, *et al*: Mac-2 binding protein glycosylation isomer, the FIB-4 index, and a combination of the two as predictors of non-alcoholic steatohepatitis. PLoS One 17: e0277380, 2022.
15. Guo J, Han X, Huang W, You Y and Jicheng Z: Interaction between IgA and gut microbiota and its role in controlling metabolic syndrome. Obes Rev 22: e13155, 2021.

16. Guo X, Meng G, Liu F, Zhang Q, Liu L, Wu H, Du H, Shi H, Xia Y, Liu X, *et al*: Serum levels of immunoglobulins in an adult population and their relationship with type 2 diabetes. Diabetes Res Clin Pract 115: 76-82, 2016.
17. Rafaqat S, Sattar A, Khalid A and Rafaqat S: Role of liver parameters in diabetes mellitus-a narrative review. Endocr Regul 57: 200-220, 2023.
18. Kim EH, Kim HK, Lee MJ, Bae SJ, Choe J, Jung CH, Kim CH, Park JY and Lee WJ: Sex differences of visceral fat area and visceral-to-subcutaneous fat ratio for the risk of incident type 2 diabetes mellitus. Diabetes Metab J 46: 486-498, 2022.
19. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D and Ross R: Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J Appl Physiol (1985) 85: 115-122, 1998.
20. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, *et al*: A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 79: 1542-1556, 2023.
21. Fujiwara N, Nakagawa H, Kudo Y, Tateishi R, Taguri M, Watadani T, Nakagomi R, Kondo M, Nakatsuka T, Minami T, *et al*: Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. J Hepatol 63: 131-140, 2015.
22. Shephard DA: The 1975 declaration of helsinki and consent. Can Med Assoc J 115: 1191-1192, 1976.
23. Tarantino G, Citro V, Esposit P, Giaquinto S, de Leone A, Milan G, Tripodi FS, Cirillo M and Lobello R: Blood ammonia levels in liver cirrhosis: A clue for the presence of portosystemic collateral veins. BMC Gastroenterol 9: 21, 2009.
24. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, O'Beirne J, Fox R, Skowronska A, Palmer D, *et al*: Assessment of liver function in patients with hepatocellular carcinoma: A new evidence-based approach-the ALBI grade. J Clin Oncol 33: 550-558, 2015.
25. Kamath P, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER and Kim WR: A model to predict survival in patients with end-stage liver disease. Hepatology 33: 464-470, 2001.
26. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H and Pol S: FIB-4: An inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and FibroTest. Hepatology 46: 32-36, 2007.
27. Newsome PN, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, Yilmaz Y, Czernichow S, Zheng MH, Wong VW, *et al*: FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: A prospective derivation and global validation study. Lancet Gastroenterol Hepatol 5: 362-373, 2020.
28. Mózes FE, Lee JA, Vali Y, Alzoubi O, Staufer K, Trauner M, Paternostro R, Stauber RE, Holleboom AG, van Dijk AM, *et al*: Performance of non-invasive tests and histology for the prediction of clinical outcomes in patients with non-alcoholic fatty liver disease: An individual participant data meta-analysis. Lancet Gastroenterol Hepatol 8: 704-713, 2023.
29. Torruellas C, French SW and Medici V: Diagnosis of alcoholic liver disease. World J Gastroenterol 20: 11684-11699, 2014.
30. Zagato E, Mazzini E and Rescigno M: The variegated aspects of immunoglobulin A. Immunol Lett 178: 45-49, 2016.
31. Tarantino G and Citro V: What are the common downstream molecular events between alcoholic and nonalcoholic fatty liver? Lipids Health Dis 23: 41, 2024.
32. Jung CH, Rhee EJ, Kwon H, Chang Y, Ryu S and Lee WY: Visceral-to-subcutaneous abdominal fat ratio is associated with nonalcoholic fatty liver disease and liver fibrosis. Endocrinol Metab (Seoul) 35: 165-176, 2020.
33. Hernández-Conde M, Llop E, Carrillo CF, Tormo B, Abad J, Rodriguez L, Perelló C, Gomez ML, Martínez-Porras JL, Puga NF, *et al*: Estimation of visceral fat is useful for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease. World J Gastroenterol 26: 6514-6705, 2020.
34. Ha NB, Cho SJ, Mohamad Y, Kent D, Jun G, Wong R, Swarnakar V, Lin S, Maher JJ and Lai JC: Visceral adipose tissue inflammation and radiographic visceral-to-subcutaneous adipose tissue ratio in patients with cirrhosis. Dig Dis Sci 67: 3436-3444, 2022.
35. Tarantino G, Sinatti G, Citro V, Santini SJ and Balsano C: Sarcopenia, a condition shared by various diseases: Can we alleviate or delay the progression? Intern Emerg Med 18: 1887-1895, 2023.