Med. Sci. Innov. 72(3-4):43-55, 2025

Association of Non-Invasive Liver Fibrosis Biomarkers with NAFLD in an Apparently Healthy Population: A Matched Case-Control Analysis

Hiroyuki Saito,[1] *MH Mahbub,*[1,2] *Junki Shimokawa,*[3] *Natsu Yamaguchi,*[1] *Ryosuke Hase,*[1] *Masanori Harada,*[4] *Sunao Wada,*[1] *Fumie Kurokawa*[5] *and Tsuyoshi Tanabe*[1]

[1] Department of Public Health and Preventive Medicine, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[2] Division of Systems Medicine and Informatics, Research Institute for Cell Design Medical Science, Yamaguchi University, 1-1--1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[3] Department of General Medicine, Mine City Hospital, 11313-1 Ohminechohigashibun, Mine, Yamaguchi 759-2212, Japan
[4] Support Center for Rural Medicine, Yamaguchi Prefectural Grand Medical Center, 10077 Osaki, Hofu, Yamaguchi 747-8511, Japan
[5] Department of General Medicine and Primary Care, Yamaguchi University Hospital, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
(Received February 20, 2025, accepted April 30, 2025)
Correspondence to MH Mahbub, M.D., M.P.H., Ph.D. E-mail: hossain@yamaguchi-u.ac.jp.

Abstract This study examined the association between non-invasive liver fibrosis biomarkers and non-alcoholic fatty liver disease (NAFLD) in an apparently healthy population. A matched case-control design was used to analyze 145 pairs of participants with and without NAFLD. Six liver fibrosis indexes were evaluated: Aspartate Aminotransferase to Alanine Aminotransferase Ratio (AAR), Aspartate Aminotransferase to Platelet Ratio Index (APRI), fibrosis index based on four factors (FIB-4), modified FIB-4 (mFIB-4), Forns Index, and Gamma-Glutamyl Transpeptidase to Platelet Ratio (GPR). Adjusted logistic regression analyses showed significant associations between mFIB-4 and Forns Index with NAFLD, highlighting their potential as tools for early detection. These markers demonstrated consistency across multiple analyses, supporting their potential use for screening asymptomatic individuals, especially in resource-limited settings. However, traditional markers like APRI and GPR showed limited utility in this cohort, emphasizing the need for contextual biomarker selection. Future studies should validate these findings across diverse populations and investigate their diagnostic capabilities in prospective cohort studies to improve early NAFLD detection and intervention.

Key words: liver fibrosis biomarkers, NAFLD, association, case-control study

https://doi.org/10.60462/30394

Introduction

Liver fibrosis poses a significant global health challenge, especially within the context of non-alcoholic fatty liver disease (NAFLD), which affects an estimated 25% of the worldwide population.[1] NAFLD can progress to severe complications, including liver fibrosis, cirrhosis, and hepatocellular carcinoma, all of which increase morbidity and mortality rates.[1,2] The presence and extent of liver fibrosis are critical prognostic indicators in liver disease, as they significantly impact the risk of liver-related complications and mortality.[3] Therefore, early detection and accurate staging of liver fibrosis are essential to improve long-term outcomes and prevent the progression to advanced liver disease and liver failure.[4]

Although liver biopsy remains the gold standard for diagnosing and staging liver fibrosis, it is invasive, costly, and carries risks of complications, limiting its feasibility for routine clinical use.[5] These limitations have led to increasing interest in non-invasive biomarkers as alternative tools for assessing liver fibrosis. Especially in resource-limited settings, biomarkers derived from routine laboratory tests may offer a safer, more accessible, and cost-effective approach compared to biopsy. Additionally, biomarkers that enable early detection of liver fibrosis could prove extremely beneficial for both diagnosis and intervention. Commonly studied such non-invasive biomarkers include Aspartate Aminotransferase to Alanine Aminotransferase Ratio (AAR), Aspartate Aminotransferase to Platelet Ratio Index (APRI), fibrosis index based on four factors (FIB-4), Forns Index, and Gamma-Glutamyl Transpeptidase to Platelet Ratio (GPR).[6-9] These easy-to-use biomarkers have demonstrated good diagnostic performance across diverse clinical settings and are considered suitable for use in both resource-rich and resource-limited environments.[10]

However, their clinical utility remains a subject of debate and controversy, as their diagnostic accuracy and effectiveness have shown inconsistent results across different populations and stages of liver disease.[7,11] For example, while the World Health Organization recommends APRI and FIB-4 tests for assessing liver fibrosis in resource-limited settings, studies have shown that their accuracy in diagnosing fibrosis or cirrhosis can be inconsistent.[11-13] Additionally, biomarkers including Gamma-Glutamyl Transpeptidase (GGT), although widely used for evaluating liver fibrosis, are often criticized for their lack of specificity, as elevated GGT levels can result from conditions unrelated to liver fibrosis, including alcohol consumption and cardiovascular disease.[14] Conversely, NAFLD can sometimes be associated with normal alanine aminotransferase (ALT) values, further complicating the use of liver enzymes as reliable markers of fibrosis.[15]

These limitations underscore the need for a comprehensive evaluation of established non-invasive biomarkers across diverse populations, particularly among individuals who may not show overt liver disease symptoms. Such assessments are valuable for determining the effectiveness of these biomarkers in early detection, ultimately contributing to improved clinical management of at-risk populations. Therefore, our study aimed to compare the levels of established liver fibrosis indexes between individuals with and without NAFLD identified within an apparently healthy population undergoing routine health check-ups. The goal was to identify which biomarkers are most strongly associated with NAFLD. We hypothesize that an effective biomarker will demonstrate a robust association with NAFLD in this cohort, potentially serving as a valuable tool for the early identification of liver fibrosis in broader clinical practice.

Materials and Methods

Study Design and Population

This was a retrospective, single-center study. A flowchart detailing the study population is shown in Figure 1. We considered data from the first visit of adult patients who attended the Health Checkup Center at Ube Kohsan Central Hospital, Yamaguchi Prefecture, Japan, between April 2014 and March 2019. A total of 5292 participants underwent abdominal ultrasound examinations during this period. The health checkups included physical

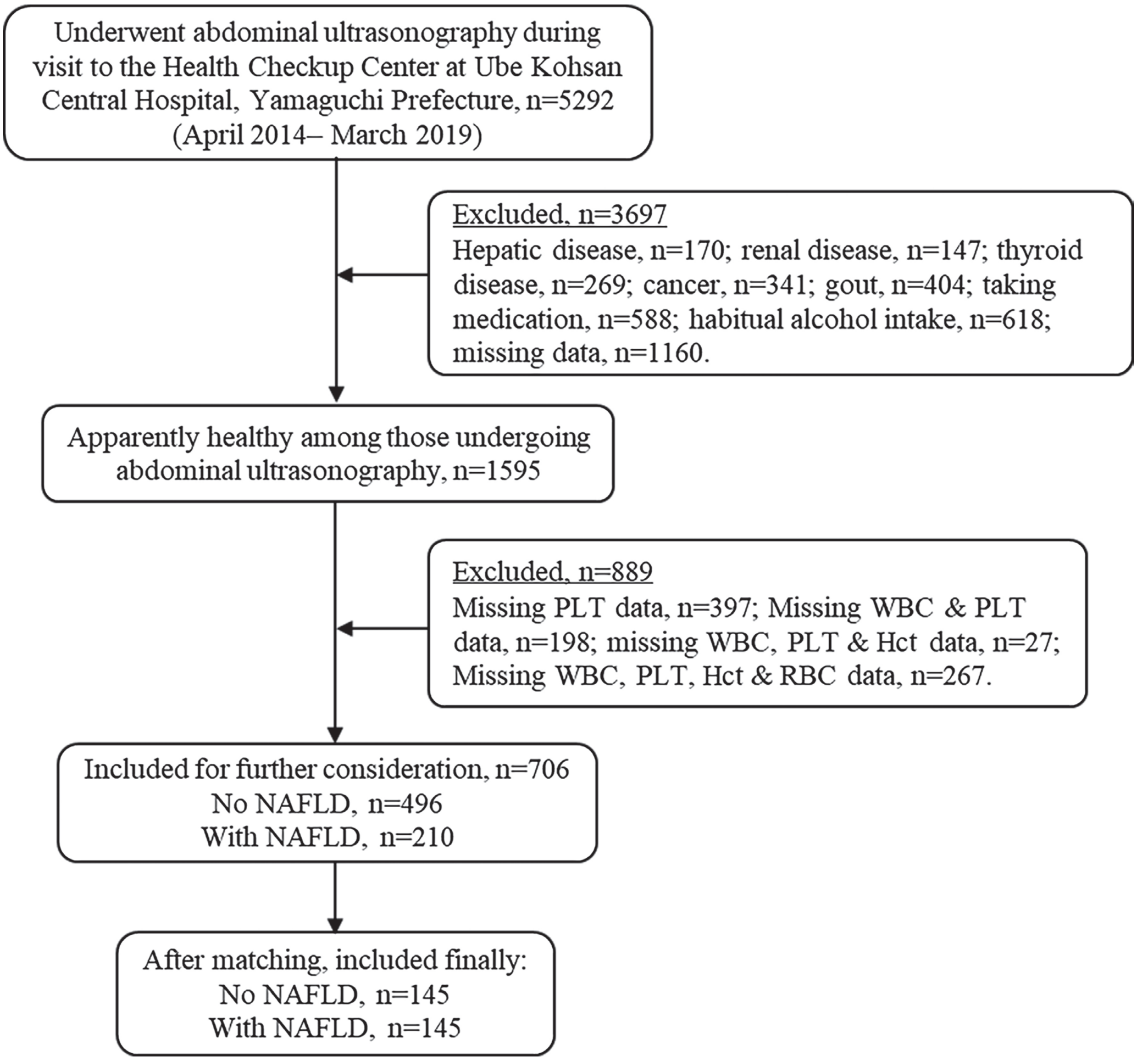

Fig. 1 Flowchart of current study population.

examinations, laboratory tests, and a self-administered questionnaire regarding medical and personal history.

To focus on a healthy population, a total of 1595 subjects without any major medical conditions or regular alcohol consumption, aside from fatty liver disease diagnosed via ultrasound, were selected. Subjects with a history of any diagnosed disease or incomplete data (n=3697), as well as those with missing values necessary for calculating liver fibrosis indexes (n=889), were excluded. This resulted in a final sample of 706 individuals, comprising 210 participants with NAFLD and 496 without NAFLD. Using a 1:1 matched case-control design, participants were matched based on age (±2 years) and Body mass index (BMI) (±1 kg/m^2), resulting in 145 matched pairs of subjects with and without NAFLD.

Data Collection

The medical data were anonymized for analysis. The health checkups included physical examinations and clinical laboratory tests, and participants provided information regarding personal and medical history through a questionnaire. For drinking history, participants were classified as either 'non-drinkers' (those reporting no alcohol consumption or only very rare and infrequent intake) or 'drinkers' (those reporting occasional alcohol consumption, defined as non-regular intake occurring on several days per month, without a consistent pattern or substantial quantity).

Physical Examination

Measurements of height and weight were taken to the nearest 0.1 cm and 0.1 kg, respectively, and abdominal circumference was measured to the nearest 0.1 cm. BMI was calculated by dividing body weight (kg) by height squared (m^2). Systolic (SBP) and diastolic blood pressure (DBP) were measured using automated oscillometric devices in a quiet setting, with subjects seated and arms supported at heart level, following standardized guidelines.

Measurement of Blood Samples

Fasting blood samples were collected from the median cubital vein of seated participants. Hematological parameters, including hematocrit (Hct), hemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), and platelets (PLT), were measured using an automated hematology analyzer, the Sysmex XN-2000 (Sysmex, Kobe, Japan), following standard operating procedures. Biochemical analyses in this study included ALT, aspartate aminotransferase (AST), fasting plasma glucose (FPG), GGT, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG). These tests were performed using the HITACHI-7700 biochemical analyzer (Hitachi High-Technology Co., Tokyo, Japan). Serum uric acid (SUA) and serum creatinine (SCr) were measured using enzymatic methods on the same analyzer, following the manufacturer's protocols.

Ultrasonographic Examination and Diagnosis of NAFLD

Abdominal ultrasonography was performed by trained clinical laboratory technicians using the ProSound α5 and α7 devices (Aloka, Tokyo, Japan). Fatty liver disease was diagnosed based on the presence of at least one of the following ultrasonographic findings: bright liver, hepatorenal or hepatosplenic echo contrast, ultrasound signal attenuation, or vascular blurring. NAFLD was diagnosed based on established criteria: 1) imaging evidence of fatty liver; 2) absence of significant alcohol consumption; and 3) exclusion of other causes of steatosis, such as hepatitis, drug-induced liver disease, and alcohol-related liver disease. The diagnosis was confirmed by a consensus between two clinical laboratory technicians and one gastroenterologist.

Liver Fibrosis Indexes

This study evaluated six established liver fibrosis indexes to assess their association with NAFLD. The calculation formulas for each index are as follows:[6-9,16]

(1) AAR=(AST/ALT)
(2) APRI=((AST/30×100)/PLT)
(3) FIB-4=((Age × AST)/(PLT×$\sqrt{}$ALT))
(4) mFIB-4=((10 × Age×AST)/(PLT×ALT))
(5) Forns Index=(7.811-3.131×ln(PLT)+0.781×ln(GGT)+3.467×ln(Age)-0.014(TC))
(6) GPR=((GGT/50×100)/PLT)

Statistical Analyses

Because the data did not follow a normal distribution, the Wilcoxon signed-rank test was used to analyze matched continuous variables, and the McNemar's test was applied for matched categorical variables.

To investigate the association between liver fibrosis indexes and NAFLD, logistic regression analyses were performed. First, crude associations between each liver fibrosis index and NAFLD were evaluated. For the adjusted models, demographic and clinical variables that showed significant differences between the NAFLD and Non-NAFLD groups were included, except for the variables that were already part of the index calculation. This method was employed to prevent over-adjustment by factors inherent to the index formula.[17,18] Odds ratios (OR), 95% confidence intervals (CI), and p-values were calculated.

Statistical analysis was performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). All tests were two-tailed, and a p-value of less than 0.05 was considered statistically significant.

Ethical Approval and Informed Consent

The present study is a secondary analysis of the data from a research protocol approved by the Institutional Review Board of Yamaguchi University, Japan (approval number 2022-096) and adhered to the principles outlined in the Declaration of Helsinki. In accordance with Japanese law, individual

written informed consent is not required for research involving human biological specimens without intervention. Instead, an opt-out procedure was followed, with relevant details made available on the official website of Ube Kohsan Central Hospital, Yamaguchi Prefecture, Japan.

Results

Table 1 provides the demographic and clinical characteristics of the Non-NAFLD (control group) and NAFLD groups, each comprising 145 matched subjects. Most variables analyzed did not exhibit significant differences between the two groups, highlighting the overall similarities between individuals with and without NAFLD within an apparently healthy population undergoing routine health check-ups. The NAFLD group had a significantly higher proportion of individuals reporting alcohol consumption (32%) compared to the Non-NAFLD group (12%) ($p < 0.001$). Significant differences were also observed in BMI, abdominal circumference, platelet count, ALT, AST, FPG, and lipid profile components, HDL-C and TG. Specifically, NAFLD subjects showed a higher median BMI, abdominal circumference, elevated ALT and AST levels, and increased FPG ($p < 0.05$ to 0.001). Additionally, HDL-C level was significantly lower, while TG level was higher in the NAFLD group ($p < 0.001$).

Table 2 presents a comparison of six liver fibrosis indexes—AAR, APRI, FIB-4, mFIB-4, Forns Index, and GPR—between the NAFLD and Non-NAFLD groups. Among those indexes, significant differences were observed for AAR, FIB-4, and mFIB-4 ($p < 0.05$ to 0.001), while the Forns Index showed a near-significant difference ($p < 0.055$). NAFLD patients exhibited lower values for each of these established biomarkers compared to the Non-NAFLD group.

Table 3 displays the results of the logistic regression analysis, assessing the association between six liver fibrosis indexes (AAR, APRI, FIB-4, mFIB-4, Forns Index, and GPR) and NAFLD. In the unadjusted analysis (Model 1), significant inverse associations with NAFLD were observed for AAR, and mFIB-4 indicating that higher values were associated with lower odds of NAFLD [ORs of 0.71-0.97, 95% CIs of 0.57-0.95 (lower) and 0.88-0.99 (upper), $p < 0.05$ to 0.005]. Although Forns Index did not reach statistical significance in the unadjusted model, it showed a modest inverse association with NAFLD (OR 0.82, 95% CI 0.66-1.02, $p = 0.074$). After adjusting for covariates in Model 2, mFIB-4 maintained its significant association with NAFLD, with an adjusted OR of 0.97 (95% CI 0.94-0.99, $p < 0.01$). Notably, Forns Index demonstrated a stronger inverse association in the adjusted model, achieving statistical significance (OR 0.64, 95% CI 0.49-0.83, $p < 0.001$).

Discussion

The ability to identify early NAFLD in a population without overt symptoms is especially important, as the progression to more severe liver conditions, such as cirrhosis or hepatocellular carcinoma, often goes undetected until significant damage has occurred.[1,2] In line with this, the current study assessed the association between selected non-invasive liver fibrosis biomarkers and NAFLD in an apparently healthy population. While we did not evaluate diagnostic accuracy metrics such as sensitivity, specificity, or areas under the receiver operating characteristic curves, parameters essential for determining the clinical utility of each index, our primary objective was to identify biomarkers most strongly associated with NAFLD in this low-risk group. This approach was intended to contribute to the identification of biomarkers that could enhance the early diagnosis of NAFLD and potentially enable timely intervention.

In this study, key metabolic markers, including abdominal circumference, liver enzymes (ALT, AST, GGT), FPG, and TG, were significantly elevated in the NAFLD group. This pattern aligns with known metabolic dysfunctions associated with NAFLD, particularly central obesity, which is implicated in hepatic fat accumulation and insulin resistance.[19,20] Our observations reflect the research findings reported by Younossi et al. (2016),[1] who identified similar metabolic characteristics among NAFLD patients, sug-

Table 1 Demographic and clinical characteristics of the study subjects.

Variables	Non-NAFLD (n=145)		NAFLD (n=145)		P-value§
	Median or n	IQR or %	Median or n	IQR or %	
Age (Years)	53.0	15.0	53.0	14.5	0.419
Sex					
Male	95	66%	111	77%	0.057
Female	50	34%	34	23%	
Smoking status					
Non-smoker	126	87%	116	80%	0.144
Smoker	19	13%	29	20%	
Alcohol					
Non-drinker	127	88%	99	68%	<0.001
Drinker	18	12%	46	32%	
BMI (kg/m^2)	23.6	3.3	24.0	3.1	<0.001
Abd Circ	84.0	11.0	86.0	10.0	<0.001
RBC ($10^4/\mu L$)	453.0	74.5	461.0	72.5	0.115
WBC ($/\mu L$)	5180.0	1825.0	5390.0	2120.0	0.113
Platelets ($10^4/\mu L$)	22.8	6.0	23.7	7.5	0.004
Hb (g/dL)	14.2	2.0	14.6	2.4	0.394
Hct (%)	42.3	5.3	43.9	6.3	0.138
SBP (mmHg)	125.0	16.5	126.0	23.5	0.260
DBP (mmHg)	77.0	14.0	80.0	15.5	0.097
ALT (U/L)	20.0	13.5	24.0	14.0	0.005
AST (U/L)	20.0	7.5	21.0	8.0	0.049
FPG (mg/dL)	101.0	11.5	104.0	17.0	0.001
GGT (U/L)	30.0	41.0	34.0	36.5	0.096
HDL-C (mg/dL)	64.0	20.5	57.0	17.0	<0.001
LDL-C (mg/dL)	129.0	38.5	135.0	33.0	0.074
TC (mg/dL)	208.0	44.0	213.0	44.0	0.216
TG (mg/dL)	98.0	59.0	127.0	81.5	<0.001
SUA (mg/dL)	5.6	2.2	5.8	1.8	0.299
SCr (mg/dL)	0.9	0.3	0.8	0.2	0.690

Abd Circ, abdominal circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; Hb, hemoglobin; Hct, hematocrit; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RBC, red blood cell; SCr, serum creatinine; SUA, serum uric acid; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WBC, white blood cell.

Values have been expressed as median and IQR for the continuous variables, and as number (n) and percent (%) for the categorical variables.

§Two-tailed p-values were obtained by the Wilcoxon signed-Rank test for matched continuous variables and McNemar's test for matched categorical variables.

Table 2 Comparison of Liver Fibrosis Indexes Between NAFLD and Non-NAFLD Groups.

	Non-NAFLD (n=145)		NAFLD (n=145)		
Indexes	Median	IQR	Median	IQR	P-value[§]
AAR	1.0	0.5	0.9	0.3	0.003
APRI	3.0	1.2	3.0	1.4	0.922
FIB-4	10.5	5.6	9.7	4.7	0.027
mFIB-4	23.9	17.0	19.9	12.1	<0.001
Forns Index	11.6	1.3	11.2	1.8	0.055
GPR	2.8	3.4	2.9	3.1	0.333

AAR, Aspartate Aminotransferase to Alanine Aminotransferase Ratio; APRI, Aspartate Aminotransferase to Platelet Ratio Index; FIB-4, fibrosis index based on four factors; mFIB-4, modified FIB-4; GPR, Gamma-Glutamyl Transpeptidase to Platelet Ratio.
Values are shown as median and IQR.
[§]Two-tailed p-values were obtained by the Wilcoxon signed-Rank test.

Table 3 Logistic regression analysis for association between liver fibrosis biomarkers and NAFLD without and with adjustments for relevant potential confounding factors.

Indexes	Model 1				Model 2			
	OR	95% CI		P-value	OR	95% CI		P-value
		Lower	Upper			Lower	Upper	
AAR	0.41	0.2	0.82	0.016	0.58	0.26	1.28	0.175
APRI	1.07	0.91	1.24	0.431	1.01	0.77	1.31	0.961
FIB-4	0.95	0.9	1.01	0.1	0.96	0.90	1.03	0.219
mFIB-4	0.97	0.95	0.99	0.002	0.97	0.94	0.99	0.008
Forns Index	0.82	0.66	1.02	0.074	0.64	0.49	0.83	0.001
GPR	1.03	0.97	1.1	0.274	0.95	0.88	1.03	0.23

AAR, Aspartate Aminotransferase to Alanine Aminotransferase Ratio; APRI, Aspartate Aminotransferase to Platelet Ratio Index; FIB-4, fibrosis index based on four factors; mFIB-4, modified FIB-4; GPR, Gamma-Glutamyl Transpeptidase to Platelet Ratio.
Model 1, without adjustments.
Model 2, with adjustments: AAR for BMI, Abd Cicum, PLT, FPG, HDL-C, and TG; APRI for BMI, Abd Cicum, ALT, FPG, HDL-C, and TG; FIB-4 for BMI, Abd Cicum, FPG, HDL-C, and TG; mFIB-4 for BMI, Abd Cicum, FPG, HDL-C, and TG; Forns Index for BMI, Abd Cicum, ALT, AST, FPG, HDL-C, and TG; and GPR for BMI, Abd Circ, ALT, AST, FPG, HDL-C, and TG.

gesting a consistent metabolic profile associated with NAFLD across diverse populations. Observed increases in ALT and AST levels in the NAFLD group support the role of these enzymes in liver injury.[21,22] Their elevation in our cohort reinforces the notion that they can be useful indicators in conjunction with other markers, particularly for early-stage liver disease.[23,24]

In our study, BMI and abdominal circumference significantly differed between the study groups. In some previous works, the researchers suggested that BMI alone may not fully capture the risk of NAFLD, as it does not account for the distribution of adiposity, which is a critical factor in NAFLD pathogenesis.[20,25,26] Other researchers have reported that visceral fat, as indicated by abdominal circumference, is a stronger predictor of NAFLD than BMI, underscoring the importance of evaluating both general and central adiposity in clinical assessments.[27] Interestingly, despite matching participants for BMI within ± 1 kg/m^2, a significant difference was observed between groups using the Wilcoxon signed-rank test. This likely reflects the test's sensitivity to consistent directional differences across matched pairs, as it considers both the sign and rank of the differences, rather than just their median values. In contrast, the unpaired Mann-Whitney U test showed no significant difference ($p = 0.463$; results not shown), underscoring the importance of appropriate interpretation of statistical tests based on study design. Such residual differences are common in approximate matching and do not necessarily indicate poor matching quality.

Our findings showed a consistent trend across statistical analyses for mFIB-4 and Forns Index; however, it is essential to clarify that our primary conclusions are based on the adjusted logistic regression model (Model 2 in Table 3), which more robustly accounts for potential confounding factors. While we observed notable differences in mFIB-4 and Forns Index between the NAFLD and Non-NAFLD groups (Table 2), and the unadjusted analysis (Model 1 in Table 3) indicated statistical significance for mFIB-4 and a near-significant association for the Forns Index, it was in the adjusted Model 2 where both indices demonstrated statistically significant and independent associations with NAFLD. These results underscore the potential value of mFIB-4 and the Forns Index as non-invasive biomarkers for detecting early NAFLD in asymptomatic individuals, providing useful insights for early intervention and management. Our findings are in line with growing research that underscores the relevance of non-invasive biomarkers in detecting liver fibrosis across various liver disease etiologies.[4-8,16] It should be noted here that we observed lower values for each of these significant biomarkers in the NAFLD group. Comparisons with other studies are challenging, as most research focused on diagnostic performance across various liver disease conditions or compared fibrosis indexes within different fibrosis grades rather than between NAFLD and Non-NAFLD groups. However, Sugiyama et al. (2022) reported significantly lower FIB-4 index values in NAFLD patients compared to non-drinkers without fatty liver across all age groups ($p < 0.0001$), supporting our findings.[28]

mFIB-4, a modified version of the original FIB-4 index, emerged as a robust indicator in our study, showing a significant association with NAFLD. By integrating ALT, AST, and platelet counts, mFIB-4 provides a comprehensive assessment of liver function that appears particularly sensitive to early changes. Previous studies have validated mFIB-4's utility, especially in hepatitis B and C populations, demonstrating its reliability in predicting fibrosis.[8,29] In the study by Wang et al. (2017), compared to AAR, APRI, and FIB-4, mFIB-4 exhibited better diagnostic performance for liver cirrhosis in chronic hepatitis B and chronic hepatitis C patients.[29] Studies have also shown that mFIB-4 effectively differentiates fibrosis stages, with lower values typically associated with milder fibrosis in chronic liver disease.[29,30] The lower mFIB-4 values observed in our NAFLD group probably suggest its potential for identifying early-stage NAFLD, supporting its role as a screening marker in asymptomatic populations. Furthermore, our findings underscore mFIB-4's value for early detection in primary care settings, where its simplicity and diagnostic accuracy make it particularly valu-

able, especially in resource-limited areas. Its continued significance after covariate adjustment in our study highlights its robustness as a reliable biomarker among those without overt liver disease. Given that mFIB-4 incorporates age and liver enzyme levels, it may be especially useful in aging populations, as early liver changes related to NAFLD might otherwise go unnoticed.[31]

In our study, the Forns Index showed a significant inverse association with NAFLD after adjusting for confounders, suggesting its potential predictive value in this cohort. Originally validated by Forns et al. (2002) for hepatitis C patients,[6] the index has shown mixed utility across different liver disease contexts. For instance, Ballestri et al. (2021) found that the Forns Index slightly outperformed APRI and FIB-4 in predicting advanced fibrosis in patients with NAFLD and viral chronic liver disease,[32] whereas Adler et al. (2008) reported it to be less accurate than FIB-4 for diagnosing cirrhosis.[33] This variability may arise from the index's dependence on variables such as GGT and TC, which are highly susceptible to external factors, including medications, alcohol consumption, and dietary habits. Our findings indicate that while the Forns Index shows promise, its utility for screening asymptomatic populations for NAFLD requires further investigation.

In this study, AAR revealed significant differences between the NAFLD and Non-NAFLD groups and demonstrated a significant association with NAFLD in only the unadjusted logistic regression model. AAR is traditionally used to assess liver fibrosis and inflammation. Studies have shown that lower AAR values often correlate with milder fibrosis stages in chronic liver disease cohorts,[7,34] while AAR values exceeding 1.0 are typically associated with advanced fibrosis or cirrhosis.[16,34] The lower AAR values observed in the NAFLD group in our study suggest its potential utility in detecting early-stage NAFLD.

The lack of significance for indexes like APRI, FIB-4, and GPR in our study contrasts with findings in populations with chronic hepatitis and cirrhosis, where these indexes often correlate strongly with advanced hepatic fibrosis.[7,34] This suggests that APRI, FIB-4, and GPR may be more suited to detecting moderate-to-severe fibrosis rather than early-stage NAFLD, particularly in populations with low rates of advanced liver disease. The absence of significant associations in our study emphasizes the importance of considering disease etiology when selecting non-invasive biomarkers for liver fibrosis screening. Our findings indicate that mFIB-4, and potentially AAR and the Forns Index may offer more reliable results in low-risk populations.

A key consideration in this study relates to the diagnostic terminology we used, specifically NAFLD. In 2023, the term metabolic dysfunction-associated steatotic liver disease (MASLD) replaced NAFLD.[35] The revised diagnostic criteria require the presence of hepatic steatosis along with at least one cardiometabolic risk factor, to better reflect the metabolic underpinnings of fatty liver disease. However, in the present study, we have retained NAFLD as our diagnostic criterion for several scientifically valid reasons. First, our study data were collected from a period preceding the introduction of MASLD, and thus, the clinical assessments and data classification were performed under the established NAFLD framework. The retrospective nature of our study necessitates consistency with historical diagnostic criteria to ensure the validity of our findings. Second, as highlighted in recent literature, studies comparing MASLD and NAFLD have shown that while MASLD encompasses a broader metabolic spectrum, individuals meeting NAFLD criteria still represent a major subset of MASLD.[36] Importantly, those with NAFLD exhibit similar trends in non-invasive liver fibrosis scores, liver enzyme abnormalities, and metabolic comorbidities-factors crucial to our study objectives. Moreover, indexes validated for NAFLD (such as FIB-4 and Forns Index) have shown their applicability in MASLD, given the significant overlap in patient populations and metabolic risk factors.[37] It is noteworthy here that the term NAFLD is still commonly used in current scientific literature.[38] Thus, while the evolving nomenclature of MASLD aims to enhance disease characterization, our study findings remain highly relevant, as they provide valuable insights into fibrosis risk stratification among individuals with NAFLD, which in turn is applicable to

MASLD patients as well. Future studies may further investigate the applicability of our findings within the MASLD framework, but given the diagnostic framework available at the time of data collection, NAFLD remains the most appropriate term for this study.

This study also has a few additional limitations. Its retrospective design limits control over certain confounders, such as physical activity and diet, which are known to influence NAFLD risk.[39] However, the effects of these variables on the current results should be very limited as in this study, we included healthy subjects with similar socio-demographic characteristics. Another limitation is that while adjustments were made to account for demographic and clinical differences, these adjustments cannot fully eliminate the impact of potential confounders inherent to observational studies. The current study population, consisting exclusively of Japanese adults, may limit the generalizability of the findings. Also, we did not evaluate diagnostic accuracy metrics, such as sensitivity or specificity, which are essential for establishing the clinical utility of each index. Future prospective cohort studies incorporating these metrics would provide a clearer picture of the indexes' effectiveness in early NAFLD detection. Lastly, the diagnosis of NAFLD was based on ultrasonography. While practical, ultrasonography may not be as precise as other modalities like MRI or transient elastography in detecting early-stage liver changes.[40,41] Employing these advanced imaging techniques in future prospective research could enhance our understanding of these biomarkers' performance in early NAFLD.

In conclusion, our study suggests that mFIB-4 and the Forns Index are associated with NAFLD in an apparently healthy population, showing promise as non-invasive biomarkers for early NAFLD detection. These markers could support timely intervention and potentially improve clinical outcomes. Future prospective cohort studies should validate these markers in diverse populations, evaluate their diagnostic accuracy, and confirm their suitability for integration into routine screening for subclinical liver disease. For this purpose, a prospective cohort study should longitudinally follow an asymptomatic, ethnically diverse population with baseline assessments of mFIB-4 and Forns Index, tracking incident NAFLD development over time using imaging and metabolic profiling, to validate their predictive utility and assess temporal associations with disease onset.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016 Jul; 64(1): 73-84. doi: 10.1002/hep.28431. Epub 2016 Feb 22.
2. Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. Metabolism. 2020 Oct; 111S: 154170. doi: 10.1016/j.metabol.2020.154170. Epub 2020 Jan 30.
3. Ng CH, Lim WH, Hui Lim GE, Hao Tan DJ, Syn N, Muthiah MD, Huang DQ, Loomba R. Mortality outcomes by fibrosis stage in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2023 Apr; 21(4): 931-939. e5. doi: 10.1016/j.cgh.2022.04.014. Epub 2022 May 2.
4. Li C, Li R, Zhang W. Progress in non-invasive detection of liver fibrosis. Cancer Biol Med. 2018 May; 15(2): 124-36. doi: 10.20892/j.issn.2095-3941.2018.0018.
5. Chin JL, Pavlides M, Moolla A, Ryan JD. Non-invasive markers of liver fibrosis: adjuncts or alternatives to liver biopsy? Front Pharmacol. 2016 Jun 20; 7: 159. doi: 10.3389/fphar.2016.00159.
6. Forns X, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology. 2002 Oct; 36(4 Pt 1): 986-92. doi: 10.1053/jhep.2002.36128.
7. Huang R, Wang G, Tian C, Liu Y, Jia

B, Wang J, Yang Y, Li Y, Sun Z, Yan X, Xia J, Xiong Y, Song P, Zhang Z, Ding W, Wu C. Gamma-glutamyl-transpeptidase to platelet ratio is not superior to APRI, FIB-4 and RPR for diagnosing liver fibrosis in CHB patients in China. Sci Rep. 2017 Aug 17; 7(1): 8543. doi: 10.1038/s41598-017-09234-w.

8. Kim JH, Lee M, Park SW, Kang M, Kim M, Lee SH, Kim TS, Park JM, Choi DH. Validation of modified fibrosis-4 index for predicting hepatocellular carcinoma in patients with compensated alcoholic liver cirrhosis. Medicine (Baltimore). 2018 Nov; 97(48): e13438. doi: 10.1097/MD.0000000000013438.
9. Wang H, Wu J, Yang X, Liu J, Tao W, Hao Z, Wu B, Liu M, Zhang S, Wang D. Liver fibrosis indices associated with substantial hematoma expansion in Chinese patients with primary intracerebral hemorrhage. BMC Neurol. 2021 Dec 9; 21(1): 478. doi: 10.1186/s12883-021-02494-0.
10. Reinson T, Buchanan RM, Byrne CD. Noninvasive serum biomarkers for liver fibrosis in NAFLD: current and future. Clin Mol Hepatol. 2023 Feb; 29(Suppl): S157-S170. doi: 10.3350/cmh.2022.0348. Epub 2022 Nov 22.
11. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010 Sep; 59(9): 1265-9. doi: 10.1136/gut.2010.216077.
12. Elesawy BH, Abd El Hafez A, Dorgham LS, El-Askary A. Limited reliability of five non-invasive biomarkers in predicting hepatic fibrosis in chronic HCV mono-infected patients opposed to METAVIR scoring. Pathol Res Pract. 2014 Dec; 210(12): 922-8. doi: 10.1016/j.prp.2014.07.005. Epub 2014 Jul 22.
13. WHO Guidelines on Hepatitis B and C Testing: Geneva, World Health Organization. ANNEX 3: Guidelines for the screening, care, and treatment of persons with chronic hepatitis C infection-summary of recommendations. 2017.
14. Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci. 2001 Aug; 38(4): 263-355. doi: 10.1080/20014091084227.
15. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003 Jun; 37(6): 1286-92. doi: 10.1053/jhep.2003.50229.
16. Andrés-Otero MJ, De-Blas-Giral I, Puente-Lanzarote JJ, Serrano-Aulló T, Morandeira MJ, Lorente S, Lou-Bonafonte JM. Multiple approaches to assess fourteen non-invasive serum indexes for the diagnosis of liver fibrosis in chronic hepatitis C patients. Clin Biochem. 2016 May; 49(7-8): 560-5. doi: 10.1016/j.clinbiochem.2015.12.017. Epub 2016 Mar 9.
17. Greenland S, Robins JM. Confounding and misclassification. Am J Epidemiol. 1985 Sep; 122(3): 495-506. doi: 10.1093/oxfordjournals.aje.a114131.
18. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009 Jul; 20(4): 488-95. doi: 10.1097/EDE.0b013e3181a819a1.
19. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci. 2014 Apr 11; 15(4): 6184-223. doi: 10.3390/ijms15046184.
20. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol. 2014 Nov; 2(11): 901-10. doi: 10.1016/S2213-8587(14)70032-4. Epub 2014 Apr 7.
21. Beiriger J, Chauhan K, Khan A, Shahzad T, Parra NS, Zhang P, Chen S, Nguyen A, Yan B, Bruckbauer J, et al. Advancements in Understanding and Treating NAFLD: A comprehensive review of metabolic-associated fatty liver disease and emerging therapies. Livers. 2023 Nov 7; 3(4): 637-656. https://doi.org/10.3390/livers3040042.
22. Xuan Y, Wu D, Zhang Q, Yu Z, Yu J, Zhou D. Elevated ALT/AST ratio as a

marker for NAFLD risk and severity: insights from a cross-sectional analysis in the United States. Front Endocrinol (Lausanne). 2024 Aug 26; 15: 1457598. doi: 10.3389/fendo.2024.1457598.
23. Miyake T, Kumagi T, Hirooka M, Koizumi M, Furukawa S, Ueda T, Tokumoto Y, Ikeda Y, Abe M, Kitai K, Hiasa Y, Matsuura B, Onji M. Metabolic markers and ALT cutoff level for diagnosing nonalcoholic fatty liver disease: a community-based cross-sectional study. J Gastroenterol. 2012 Jun; 47(6): 696-703. doi: 10.1007/s00535-012-0534-y. Epub 2012 Feb 14.
24. Watt J, Kurth MJ, Reid CN, Lamont JV, Fitzgerald P, Ruddock MW. Non-alcoholic fatty liver disease-A pilot study investigating early inflammatory and fibrotic biomarkers of NAFLD with alcoholic liver disease. Front Physiol. 2022 Dec 15; 13: 963513. doi: 10.3389/fphys.2022.963513.
25. Wang X, Rao H, Liu F, Wei L, Li H, Wu C. Recent advances in adipose tissue dysfunction and its role in the pathogenesis of non-alcoholic fatty liver disease. Cells. 2021 Nov 25; 10(12): 3300. doi: 10.3390/cells10123300.
26. Kuang M, Sheng G, Hu C, Lu S, Peng N, Zou Y. The value of combining the simple anthropometric obesity parameters, Body Mass Index (BMI) and a Body Shape Index (ABSI), to assess the risk of non-alcoholic fatty liver disease. Lipids Health Dis. 2022 Oct 20; 21(1): 104. doi: 10.1186/s12944-022-01717-8.
27. Dâmaso AR, do Prado WL, de Piano A, Tock L, Caranti DA, Lofrano MC, Carnier J, Cristofalo DJ, Lederman H, Tufik S, de Mello MT. Relationship between nonalcoholic fatty liver disease prevalence and visceral fat in obese adolescents. Dig Liver Dis. 2008 Feb; 40(2): 132-9. doi: 10.1016/j.dld.2007.09.009. Epub 2007 Dec 21.
28. Sugiyama A, Kurisu A, E B, Ouoba S, Ko K, Rakhimov A, Akita T, Harakawa T, Sako T, Koshiyama M, Kumada T, Tanaka J. Distribution of FIB-4 index in the general population: analysis of 75,666 residents who underwent health check-ups. BMC Gastroenterol. 2022 May 13; 22(1): 241. doi: 10.1186/s12876-022-02290-1.
29. Wang HW, Peng CY, Lai HC, Su WP, Lin CH, Chuang PH, Chen SH, Chen CH, Hsu WF, Huang GT. New noninvasive index for predicting liver fibrosis in Asian patients with chronic viral hepatitis. Sci Rep. 2017 Jun 12; 7(1): 3259. doi: 10.1038/s41598-017-03589-w. Erratum in: Sci Rep. 2018 Apr 11; 8(1): 6062. doi: 10.1038/s41598-018-24186-5.
30. Öznur M, Topçu B, Çelikkol A. Predictive value of noninvasive indices in chronic hepatitis B virus-related fibrosis. Eur J Gastroenterol Hepatol. 2021 Apr 1; 33(4): 577-582. doi: 10.1097/MEG.0000000000002045.
31. Ng CH, Lim WH, Chin YH, Yong JN, Zeng RW, Chan KE, Tan DJH, Fu CE, Tang ASP, Goh LH, Devi K, Chew NWS, Mak LL, Tamaki N, Huang DQ, Noureddin M, Siddiqui MS, Loomba R, Sanyal AJ, Muthiah M. Living in the non-alcoholic fatty liver disease silent epidemic: a qualitative systematic review of patients' perspectives. Aliment Pharmacol Ther. 2022 Aug; 56(4): 570-579. doi: 10.1111/apt.17121. Epub 2022 Jul 6.
32. Ballestri S, Mantovani A, Baldelli E, Lugari S, Maurantonio M, Nascimbeni F, Marrazzo A, Romagnoli D, Targher G, Lonardo A. Liver fibrosis biomarkers accurately exclude advanced fibrosis and are associated with higher cardiovascular risk scores in patients with NAFLD or viral chronic liver disease. Diagnostics (Basel). 2021 Jan 9; 11(1): 98. doi: 10.3390/diagnostics11010098.
33. Adler M, Gulbis B, Moreno C, Evrard S, Verset G, Golstein P, Frotscher B, Nagy N, Thiry P. The predictive value of FIB-4 versus FibroTest, APRI, FibroIndex and Forns index to noninvasively estimate fibrosis in hepatitis C and nonhepatitis C liver diseases. Hepatology. 2008 Feb; 47(2): 762-3. doi: 10.1002/hep.22085.
34. Park GJ, Lin BP, Ngu MC, Jones DB, Katelaris PH. Aspartate aminotransferase: alanine aminotransferase ratio in chronic hepatitis C infection: is it a useful predictor of cirrhosis? J Gastroenterol

Hepatol. 2000 Apr; 15(4): 386-90. doi: 10.1046/j.1440-1746.2000.02172.x.
35. 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. Ann Hepatol. 2024 Jan-Feb; 29(1): 101133. doi: 10.1016/j.aohep.2023.101133. Epub 2023 Jun 24.
36. Gofton C, Upendran Y, Zheng MH, George J. MAFLD: How is it different from NAFLD? Clin Mol Hepatol. 2023 Feb; 29(Suppl): S17-S31. doi: 10.3350/cmh.2022.0367. Epub 2022 Nov 29.
37. Kang SH, Cho Y, Jeong SW, Kim SU, Lee JW; Korean NAFLD Study Group. From nonalcoholic fatty liver disease to metabolic-associated fatty liver disease: Big wave or ripple? Clin Mol Hepatol. 2021 Apr; 27(2): 257-269. doi: 10.3350/cmh.2021.0067.
38. Zhang JW, Ullah K, Khan N, Pan HT. Comprehensive profiling of serum microRNAs in normal and non-alcoholic fatty liver disease (NAFLD) patients. Sci Rep. 2025 Jan 30; 15(1): 3766. doi: 10.1038/s41598-025-87791-1.
39. Oliveira CP, de Lima Sanches P, de Abreu-Silva EO, Marcadenti A. Nutrition and physical activity in nonalcoholic fatty liver disease. J Diabetes Res. 2016; 2016: 4597246. doi: 10.1155/2016/4597246. Epub 2015 Dec 7.
40. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol. 2014 Jun 21; 20(23): 7392-402. doi: 10.3748/wjg.v20.i23.7392.
41. Han MA, Saouaf R, Ayoub W, Todo T, Mena E, Noureddin M. Magnetic resonance imaging and transient elastography in the management of Nonalcoholic Fatty Liver Disease (NAFLD). Expert Rev Clin Pharmacol. 2017 Apr; 10(4): 379-390. doi: 10.1080/17512433.2017.1299573. Epub 2017 Mar 9.