Obesity Facts

Research Article

Obes Facts
DOI: 10.1159/000530845

Received: October 31, 2022
Accepted: April 17, 2023
Published online: May 22, 2023

Clinical Features of Non-Alcoholic Fatty Liver Disease in the Non-Lean Population

Min-ran Li[a] Jin-zhong Li[a] Jie-ying Li[a] Cun-chuan Wang[b] Rui-kun Yuan[c] Li-hong Ye[d] Yun-yan Liu[e] Xu-jing Liang[a] Hai-cong Zhang[d] Zhi-quan Liu[d] Dong-yu Zeng[a] Xue-dong Zhang[f] De-hua Wang[e] Jun-qing Li[e] Tao-yuan Li[a] Liu Yang[a] Yang Cao[a] Yun Pan[a] Xun-ge Lin[a] Calvin Q. Pan[g, h] Er-hei Dai[e] Zhi-yong Dong[b]

[a]Division of Infectious Disease, The First Affiliated Hospital of Jinan University, Guangzhou, China; [b]Bariatric and Metabolic Surgery Center, The First Affiliated Hospital of Jinan University, Guangzhou, China; [c]Division of Infectious Disease, The Sixth Affiliated Hospital of Jinan University, Dongguan, China; [d]Division of Pathology, The Fifth Hospital of Shijiazhuang, Hebei Medical University, Shijiazhuang, China; [e]Division of Liver Disease, The Fifth Hospital of Shijiazhuang, Hebei Medical University, Shijiazhuang, China; [f]Autobio Diagnostics Co., Ltd, Zhengzhou City, China; [g]Department of Infectious Disease, The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China; [h]Division of Gastroenterology and Hepatology, NYU Langone Health, NYU Grossman School of Medicine, New York, NY, USA

Keywords
Non-alcoholic fatty liver disease · Overweight · Obese · Fibrosis

Abstract

Introduction: The prevalence of non-alcoholic fatty liver disease (NAFLD) in non-lean patients is significantly increased, and obesity significantly increases the risk of cirrhosis and HCC in NAFLD patients. However, whether there is a difference in clinical manifestations of NAFLD between overweight and obesity remains unclear. The objective of this study was to assess the clinical and histological features of NAFLD among a non-lean population. ***Methods:*** Current study enrolled consecutive non-lean (body mass index [BMI] >23 kg/m^2) patients with NAFLD and available liver biopsy results. Patients were stratified by BMI into two groups for the comparison of their clinical and histological variables, which included the overweight (BMI 23~<28 kg/m^2) and the obese (BMI ≥28 kg/m^2). Risk factors for moderate to severe fibrosis (stage >1) were also analyzed through the logistic regression model. ***Results:*** Among 184 non-lean patients with metabolic-associated fatty liver disease enrolled, 65 and 119 were overweight and obese, respectively. Patients in the obesity group had a significantly lower level of gamma-glutamyl transpeptidase, higher levels of platelet, glucose, prothrombin time, and more common of moderate to severe inflammatory activity when compared to those in the overweight group. However, a significant low frequency of moderate to severe fibrosis was found in the obesity group versus the overweight group (19.33% vs. 40.00%, $p = 0.002$). Binary logistics regression analysis of fibrosis found that aspartate transaminase (AST), BMI, alanine transaminase (ALT), and cholesterol (CHOL) were

Min-ran Li, Jin-zhong Li and Jie-ying Li contributed equally to this work.

karger@karger.com
www.karger.com/ofa

Correspondence to:
Zhi-yong Dong, dongzy2008@163.com
Er-hei Dai, daieh2008@126.com
Calvin Q. Pan, Panc01@NYU.edu

independent predictors for moderate to severe fibrosis in non-lean patients with NAFLD. Compared with the traditional fibrosis-4 (AUC = 0.77) and aminotransferase to platelet ratio index (AUC = 0.79) indexes, the combined index based on AST, BMI, ALT, and CHOL was more accurate in predicting moderate to severe fibrosis in non-lean patients with NAFLD (AUC = 0.87). ***Conclusions:*** Clinical and histological features differed between obesity and overweight patients with NAFLD. When compared to the traditional serum markers, the combination index including AST, BMI, ALT, and CHOL provided a better model to predict moderate to severe fibrosis in non-lean patients with NAFLD.

Introduction

With urbanization, modernization, and westernization of dietary structure, non-alcoholic fatty liver disease (NAFLD) has become the world's largest chronic liver disease [1]. NAFLD is the manifestation of metabolic syndrome (MetS) in the liver, and the latest international consensus recommended that it should be renamed as metabolic-associated fatty liver disease (MAFLD). According to the guidelines for the diagnosis and treatment of MAFLD issued by the Asia-Pacific Association for the Study of the Liver in 2020 [2], regardless of the presence or absence of MetS, overweight, and obese patients diagnosed with fatty liver by abdominal ultrasonography, liver biopsy pathology, or other imaging methods can also be clearly diagnosed with MAFLD. Body mass index (BMI) is closely related to the occurrence and development of NAFLD. With the increase of BMI, the prevalence of NAFLD increases significantly, and the increase in BMI is a risk factor for NAFLD [3, 4].

Increased BMI is not only a risk factor for NAFLD but also determines the severity of NAFLD. Lean NAFLD patients have a lower prevalence of insulin resistance and less hepatic fibrosis compared to non-lean (overweight/obese) NAFLD [5, 6]. At present, data on clinical characteristics, metabolic profiles, and histopathological severity in non-lean patients are yet to be fully explored. One recent study analyzed the clinical features between overweight and obese NAFLD and indicated that BMI was associated with hepatic steatosis and fibrosis [7]. The above results hinted that weight control can improve NAFLD patient outcome, while most of these patients were not undergoing with liver biopsy. Furthermore, validation of conventional non-invasive fibrosis scoring systems in NAFLD patients showed that the aspartate aminotransferase to platelet (PLT) ratio index (APRI) scores did not perform well in NAFLD, a new threshold of fibrosis-4 (FIB-4) index was needed, so novel non-invasive scoring systems for fibrosis are required for NAFLD [8]. With that in mind, we designed a cross-sectional study to compare the clinical presentation and pathological findings between overweight and obese patients with NAFLD. In addition, the study expects to develop, validate, and compare a non-invasive scoring system for moderate to severe fibrosis in non-lean NAFLD patients.

Methods

Study Population

Patients who were pathologically diagnosed with NAFLD by liver biopsy from April 1, 2017 to May 31, 2022 in the Fifth Hospital of Shijiazhuang and the First Affiliated Hospital of Jinan University were selected. Inclusion criteria: (1) liver biopsy histology showing fatty liver; (2) BMI >23 kg/m^2 with or without type 2 diabetes or metabolic dysfunction. Exclusion criteria: (1) patients with other viral liver diseases (hepatitis A, C, E) or HIV infection; (2) patients with alcoholic liver disease, autoimmune liver disease, drug-induced liver injury, hepatolenticular degeneration, total parenteral nutrition, and toxic liver disease; (3) patients with liver cancer and other malignant tumors; (4) patients who combined with other autoimmune diseases.

Data Assessment and Collection

All study objectives underwent medical history collection and clinical checkup. A medical history of alcohol consumption, details of personal medicine prescriptions, hypertension, diabetes, viral hepatitis, and autoimmune hepatitis were collected before a general examination.

Patients' body mass and height were assessed, and the BMI (kg/m^2) was calculated as body mass divided by height squared. Blood pressure was also assessed, and hypertension was defined as a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of 90 mm Hg, a self-reported history of hypertension, and/or the use of antihypertensive drugs. Diabetes was defined as having a fasting plasma glucose (Glu) ≥7.0 mmol/L, a self-reported history of diabetes, and/or undergoing treatment of oral antidiabetic agents. Fasting venous blood samples were obtained within 7 days before the biopsy and used for measurements of the following parameters by conventional laboratory techniques: the complete blood cell counts, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin, direct bilirubin, total protein, albumin, globulin, prealbumin, cholinesterase, blood Glu, total cholesterol (CHOL), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid (UA), urea, creatinine, and prothrombin time (PT).

FibroScan was measured 1~30 days before liver biopsy. FibroScan was used to generate a liver stiffness measurement (LSM) and a controlled attenuation parameter (CAP) via transient

Table 1. Comparison of the general data between the overweight and obese MAFLD group

Group	All patients (n = 184)	Overweight group (n = 65)	Obese group (n = 119)	Statistics	*p value
Gender (male [%]/female [%])	80 (43.5)/104 (56.5)	36 (55.4)/29 (44.6)	44 (40)/75 (60)	χ^2 = 5.80	0.01
Age M (P25, P75)	36 (29, 49)	40 (28, 51)	34(30, 44)	Z = −1.83	0.07
Diabetes or abnormal blood sugar, %	28.8	21.5	32.8	χ^2 = 2.59	0.11
Hypertension, %	25.0	16.9	29.4	χ^2 = 3.50	0.06
Drinking history, %	9.8	4.6	12.6	χ^2 = 1.04	0.08

*p value for overweight group versus obese group.

elastography by trained operators in accordance with the manufacturer's instruction. A standard M probe was used in the first instance so that both LSM and CAP could be obtained. The XL probe was used in obese patients when the M probe failed. Ten successful acquisitions were performed in each patient. LSM and CAP values are expressed as the median of all valid measurements obtained.

Histological Data

Morbid obese patients underwent intra-operative liver biopsies during bariatric surgery, while other patients underwent liver puncture. The samples were fixed in formalin and embedded in paraffin, sections were cut and prepared by hematoxylin-eosin staining for morphological evaluation, Masson's trichrome staining, and reticulin staining for fibrosis assessment. All NAFLD sections were scored by two liver pathologists using FLIP-SAF, who were blind to the study protocol and the pathology report should include the presence and extent of hepatocyte steatosis, ballooning, intralobular inflammation, and liver fibrosis [9]. NAFLD was pathologically diagnosed if the steatosis area was >5%. Hepatic steatosis was assigned on a scale of 0–3 (S0: <5%; S1: 5–33%, S2: 34–66%, S3: >67%), ballooning of hepatocytes, and lobular inflammation was graded from 0 to 2, and fibrosis was assigned a score of 0, 1, 2, 3, or 4 (stage 0, no fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis; and stage 4, cirrhosis). The grade of activity (from A0 to A4) was calculated by addition of grades of hepatocytes ballooning and lobular inflammation. "Moderate to severity activity/fibrosis" was defined as activity/fibrosis score of more than 2.

Statistical Methods

SPSS 24.0 statistical software was applied. The measured data conforming to a normal distribution were expressed as mean ± SD, and t test was used for comparison between groups. The measured data that did not conform with the normal distribution was represented by the median M (P25, P75), and the rank sum test was used for comparison between groups. Enumeration data were expressed as the number of cases (percentage), and the comparison between groups was performed by χ^2 test. Binary logistic regression analysis was performed on relevant factors with statistical significance at the test level of 0.05 in univariate analysis. Logistic regression analysis used the likelihood ratio advance method to screen variables, and the test level of the introduced variables was α = 0.10. The independent factors affecting the occurrence of the disease were analyzed by the binary-adjusted OR value. The diagnostic efficacy was analyzed by receiver operating characteristic curve. $p < 0.05$ was considered to be statistically significant.

Results

Clinical Features of Overweight and Obese NAFLD

As a result of the obvious positive correlation between age and liver fibrosis, age adjustment was performed for the 2 groups of NAFLD patients. According to the above inclusion, exclusion criteria, and age adjustment, 184 non-lean NAFLD patients were finally included, including 65 overweight (BMI 23~<28 kg/m^2) and 119 obese (BMI ≥28 kg/m^2) NAFLD patients. Out of 184 non-lean NAFLD subjects, 80 were male and 104 were female. The median age was 36 years old. 28.8% non-lean NAFLD patients had diabetes or abnormal blood sugar, and 25.0% had hypertension. A small number of non-lean NAFLD patients (9.8%) had a drinking history. There were more women in the obese group than the overweight group. There was no significant difference between the two subgroups on the other mentioned items (Table 1).

The levels of WBC and PLT in the peripheral blood of obese NAFLD group were significantly higher than those of overweight NAFLD group; the difference was statistically significant, $p < 0.05$. The levels of ALT, AST, and GGT in the two groups were all higher than the upper limit of normal values, and the levels of ALT, AST, and GGT in the overweight NAFLD group were significantly higher than those in the obese NAFLD group ($p < 0.05$). The levels of total bilirubin, direct bilirubin, and albumin in the overweight NAFLD group were significantly higher than those in the obese NAFLD group, while the globulin and PT levels were lower, the differences were statistically

Table 2. Comparison of the laboratory parameters between the overweight and obese MAFLD groups

Project	Overweight group (n = 65)	Obese group (n = 119)	Statistics	p value
WBC (M [P25, P75], $\times10^9$/L)	5.93±1.64	7.70±2.80	5.37	**<0.01**
RBC (M [P25, P75], $\times10^{12}$/L)	4.79±0.56	4.96±0.73	1.59	0.11
Hb, mean ± SD, g/L	144.39±18.65	139.84±20.43	−1.48	0.14
PLT, mean ± SD, $\times10^9$/L	226.03±74.97	274.19±84.60	3.81	**<0.01**
ALT, U/L	145.93 (59.45, 172.30)	85.22 (32.00, 113.05)	−4.10	**<0.01**
AST, U/L	84.65 (39.45, 94.15)	49.49 (21.50, 57.10)	−5.15	**<0.01**
TB, μmol/L	16.98 (10.00, 19.00)	13.43 (9.35, 15.10)	−2.31	**0.02**
DB, μmol/L	6.02 (3.70, 6.73)	4.20 (2.40, 4.90)	−4.04	**<0.01**
ALB, g/L	47.33±4.56	43.23±4.01	−6.15	**<0.01**
PA, g/L	261.21±68.93	241.03±62.04	−1.81	0.07
GLB, g/L	27.09±5.75	29.21±4.85	2.54	**<0.01**
GGT, U/L	128.23 (43.98, 140.03)	65.52 (27.00, 80.50)	−4.16	**<0.01**
ALP, U/L	91.28±44.20	89.73±42.06	−0.22	0.83
UA, μmol/L	365.08±97.79	422.71±120.91	2.95	**<0.01**
Glu, mmol/L	6.06±1.32	7.54±4.93	3.04	**<0.01**
TG, mmol/L	2.80 (1.14, 2.50)	2.29 (1.30, 2.32)	−0.16	0.87
CHOL, mean ± SD, mmol/L	4.94±0.94	5.25±1.12	1.87	0.06
HDL-C, mmol/L	1.08±0.23	1.11±0.60	0.35	0.73
LDL-C, mean ± SD, mmol/L	3.09±0.7	3.13±0.91	0.30	0.77
CHE	9,625.69±2,053.87	10,224.81±2,087.72	1.71	0.09
Urea	4.45±1.00	4.40±1.11	−0.25	0.80
Cr	62.87±13.21	60.95±15.30	−0.66	0.51
PT	11.15±0.78	12.56±1.26	8.97	**<0.01**
INR	0.97±0.11	0.98±0.09	0.91	0.36
AFP	5.50 (2.09, 4.37)	3.00 (2.13, 3.67)	−0.77	0.44

WBC, white blood cell count; RBC, red blood cell; Hb, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; PA, prealbumin; GLB, globulin; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; UA, Uric Acid; Glu, blood glucose; TG, triglyceride; CHOL, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CHE, cholinesterase; Cr, creatinine; PT, prothrombin time; INR, international normalized ratio; AFP, alpha fetoprotein.

Table 3. Binary logistic regression analysis for the prediction of obese MALFD in non-lean patients

Influencing factors	*B*	*OR*	p value	95% *CI*
PLT	0.005	1.005	0.105	0.999–1.011
GGT	−0.007	0.993	0.045	0.986–1.000
Glu	0.330	1.392	0.046	1.005–1.926
PT	1.451	4.265	**<0.01**	2.462–7.388

PLT, platelet; GGT, gamma-glutamyl transpeptidase; Glu, blood glucose; PT, prothrombin time.

significant ($p < 0.05$), but they were all within the normal range. The levels of UA and Glu in the two groups were higher than normal levels, and the levels of UA and Glu in the obese NAFLD group were higher than those in the overweight NAFLD group ($p < 0.05$). However, there was no statistical significance for blood lipid, renal function, and other indicators ($p > 0.05$), as shown in Table 2.

Binary logistic regression analysis found that PLT, GGT, Glu, and PT were independent predict factors for obese NAFLD in non-lean patients. Among them, the OR values of PLT, Glu, and PT were 1.005 (0.999, 1.011), 1.392 (1.005, 1.926), and 4.265 (2.462, 7.388), respectively, all of which were the risk factors for the occurrence of obese NAFLD (see Table 3). After removing the influence of the above four factors, there was no significant association between gender, ALT, AST, and other factors and the occurrence of obese NAFLD.

Non-Invasive Indicators of Steatosis and Fibrosis in Overweight and Obese NAFLD

The APRI and FIB-4 indexes of the overweight NAFLD patients were higher than those of the obese NAFLD group, and the difference was statistically significant ($p < 0.05$). There was no significant difference in the CAP and LSM between the two groups ($p > 0.05$) (Table 4).

Table 4. Comparison of non-invasive indicators of steatosis and liver fibrosis between the overweight and obese MAFLD groups

Group	APRI (M [P25, P75])	FIB-4 (M [P25, P75])	CAP, mean ± SD, dB/m	LSM, mean ± SD, kPA
Overweight group (n = 65)	1.18 (0.27, 1.46)	1.69 (0.66, 1.84)	283.50±31.63	13.36 (8.05, 17.00)
Obese group (n = 119)	0.67 (0.23, 0.72)	1.01 (0.39, 0.95)	306.71±43.59	12.38 (4.85, 15.98)
Statistics	−5.64	−4.67	−1.55	1.57
p value	<**0.01**	<**0.01**	0.12	0.12

FIB-4, fibrosis-4 score; APRI, aspartate aminotransferase to platelet ratio index; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Histopathological Characteristics of the Liver in Overweight and Obese NAFLD Groups

Compared with overweight NAFLD, the proportion of moderate to severe inflammatory activity (≥A2) in obese NAFLD patients was significantly higher, but the proportion of significant fibrosis (≥F2) in obese NAFLD patients was significantly lower ($p < 0.05$). However, there was no statistical difference in the proportion of significant steatosis (≥S2) between the two groups, as shown in Table 5.

Logistic Regression Analysis of Moderate to Severe Fibrosis in Non-Lean NAFLD

Taking the moderate to severe fibrosis (≥F2) in non-lean NAFLD as the dependent variable and the factors with statistical differences in univariate analysis as the independent variables, a binary logistic regression analysis was performed. The results showed that AST, BMI, ALT, and CHOL were independent influencing factors of fibrosis in non-lean NAFLD. Among them, the OR value of AST was 1.057 (1.024, 1.090), which was a risk factor for fibrosis in non-lean NAFLD (see Table 6).

Diagnostic Efficacy of Various Non-Invasive Methods for Moderate to Severe Fibrosis in Non-Lean NAFLD

Generate regression equations of AST, BMI, ALT, and CHOL according to the logistic regression model: $9.025 - 0.144 \times \text{BMI} - 0.014 \times \text{ALT} + 0.055 \times \text{AST} - 1.476 \times \text{CHOL}$. The regression equation was used as four combined indicators to diagnose moderate to severe fibrosis in non-lean NAFLD patients. The results showed that compared with the traditional FIB-4 (AUC = 0.77) and APRI (AUC = 0.79) indices, the new combined index was more effective in diagnosing moderate to severe fibrosis in non-lean NAFLD patients (AUC = 0.87, see Fig. 1).

Discussion

NAFLD is a newly proposed diagnosis of fatty liver disease that is more applicable to clinical practice than before [10]. According to BMI, non-lean NAFLD patients were divided to overweight and obese subgroups. Logistic regression models revealed that obese NAFLD was associated with PLT, GGT, Glu, and PT. Compared with overweight NAFLD patients, obese NAFLD patients had higher levels of peripheral blood PLT, PT, and Glu, and a higher proportion of histologically moderate to severe inflammatory activity levels. However, the GGT level in the obese NAFLD group was lower than that in the overweight NAFLD group.

With the increase in BMI and CAP, the PLT showed a decreasing trend [11, 12], but some studies have concluded the opposite [13]. PLTs participate in the inflammatory response of the liver and can promote leukocyte recruitment and the activation of effector cells through the hepatic sinuses. Moreover, the function and morphology of the PLTs will also change in patients with diabetes and MetS [14]. Average PLT volume is directly related to the histological severity of liver fibrosis [15]. GGT is a liver enzyme, which can help diagnose liver injury. Xing et al. [16] showed that there was a progressive increase in the prevalence of NAFLD with increasing tertiles of GGT/high-density lipoprotein cholesterol, which was not consistent with the study. Increased Glu is considered as an important component of MetS. The association between the prevalence of NAFLD and the impaired Glu metabolism has been reported in both children and adult. The prevalence of NASH in obesity children was closely related to high BMI, gender, insulin resistance, and hyperuricemia [17]. Fasting blood Glu was positively associated with BMI in adult patients with NAFLD [18]. Furthermore, type 2 diabetes increases the risk of serious NASH and advanced fibrosis in NAFLD patients [19]. Liver hepatocytes are involved in the synthesis of most blood coagulation factors. Activity is increased in patients with NAFLD, FVIII, FIX, FXI, and FXII. The relationships between NAFLD and these coagulation factors are independent of age, gender, and BMI, which suggest that NAFLD can contribute to the risk of thrombosis [20].

Table 5. Comparison of the histopathology between the overweight and obese MAFLD groups

Group	Steatosis		Activity		Fibrosis	
	S0 ~ S1	S2 ~ S3	A0 ~ A1	A2 ~ A4	F0 ~ F1	F2 ~ F4
Overweight group (n = 65), n (%)	33 (50.77)	32 (49.23)	25 (38.46)	40 (61.54)	39 (60.00)	26 (40.00)
Obese group (n = 119), n (%)	62 (52.10)	57 (47.90)	22 (18.49)	97 (81.51)	96 (80.67)	23 (19.33)
χ^2 value	0.03		8.82		9.19	
p value	0.86		<**0.01**		<**0.01**	

Table 6. Binary logistic regression analysis of fibrosis stage ≥F2 in non-lean MAFLD

Influencing factors	*B*	OR	*p* value	95% CI
AST	0.055	1.057	<**0.01**	1.024–1.090
BMI	−0.144	0.866	<**0.01**	0.783–0.958
ALT	−0.014	0.986	0.015	0.975–0.997
CHOL	−1.476	0.228	<**0.01**	0.097–0.541

AST, aspartate aminotransferase; BMI, body mass index; ALT, alanine aminotransferase; CHOL, cholesterol.

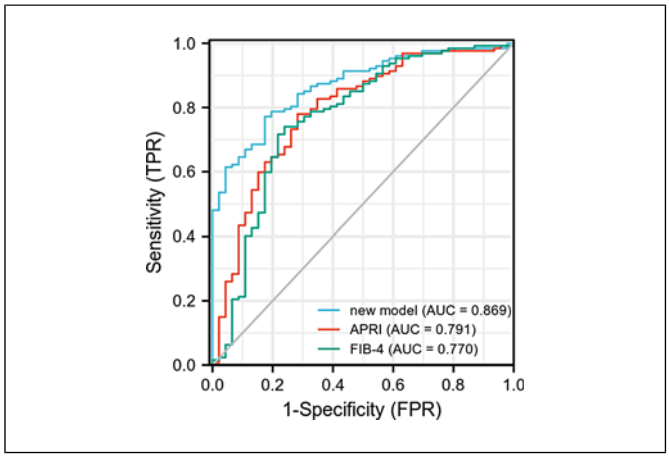

Fig. 1. ROC curves of novel model, APRI and FIB-4 for predicting fibrosis ≥F2 in non-lean patients. The new model is formed from AST, BMI, ALT, and CHOL: 9.025 − 0.144 × BMI − 0.014 × ALT + 0.055 × AST − 1.476 × CHOL. ROC, receiver operating characteristic.

Although previous meta-analyses have confirmed that obese NAFLD patients have a significantly higher metabolic-related serological markers than lean (BMI <25 kg/m^2) patients [21], a late-stage fibrosis relationship between non-obese and obese NAFLD raises a debate. A meta-analysis suggested that obesity may predict poor long-term prognosis in NAFLD patients; however, obesity may not be an independent factor for developing NASH or advanced fibrosis in NAFLD patients [22]. In the study, APRI and FIB-4 index levels were lower, and the proportion of histologically significant fibrosis was also lower in obese NAFLD compared to overweight NAFLD. We also found that the combined index composed of AST, BMI, ALT, and CHOL which was more effective in diagnosing significant fibrosis in non-lean NAFLD patients than the traditional FIB-4 and APRI. There are currently few non-invasive models for the assessment of NAFLD in non-lean population [8]. Compared with traditional fibrosis models, our model retains common ALT and AST and increases BMI and CHOL corresponding to body weight [23]. Published studies suggested that blood CHOL levels were relatively reduced in mice due to inflammation promoting abnormal accumulation of CHOL from the blood to the liver [24, 25]. Cheng-Maw Ho et al. also found that free CHOL (FC)and oxidized low-density lipoprotein co-localize on the portal vein wall, and their accumulation in periportal and sinus fibrosis was associated with local stellate cell activation and chicken-wire fibrosis. The accumulation of FC is associated with the activation of hematopoietic stem cells, and FC sensitizes cells to TGF-β through TLR4 upregulation and downregulation of the TGF-β pseudo-receptor, resulting in TGF-β-induced liver fibrosis [26, 27].

Our study has several limitations. First, we did not assess the influence of body fat distribution (e.g., waist circumference, abdominal circumference, waist-to-hip ratio) and lifestyle factors. Second, there was a selection bias in the case. Most overweight NAFLD patients underwent liver biopsy due to elevated transaminase levels, while nearly half of obese NAFLD patients were young women with normal transaminase levels who underwent biopsy during bariatric surgery. Therefore, compared with overweight NAFLD, obese NAFLD patients were more representative of the general obese population. Third, the main body of the study is non-lean NAFLD, and there is a lack of normal-weight controls. More data or studies are needed to clarify the differences among non-obese, overweight, and obese NAFLD.

In conclusion, overweight and obese NAFLD have different clinical features in terms of laboratory indicators and pathology, especially obesity NAFLD manifests as low fibrosis level. AST, BMI, ALT, and CHOL provide a better model to predict moderate to severe fibrosis, and it is more effective in the diagnosis of fibrosis stage ≥F2 in non-lean patients with NAFLD patients.

Statement of Ethics

This study was approved by the Medical Ethics Committee of The Fifth Hospital of Shijiazhuang (approval number: LL2016003) and the Medical Ethics Committee of The First Affiliated Hospital of Jinan University (approval number: KY-2022-048). The study was conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent has been obtained from all patients prior to liver biopsy in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The study was funded by the Youth Program of the National Nature Science Foundation of China (grant number 82000556).

Author Contributions

Min-ran Li, Jin-zhong Li, and Jie-ying Li searched the literature and conceived of the study. Zhi-yong Dong, Er-hei Dai, and Cun-chuan Wang designed the study. and Min-ran Li and Jin-zhong Li interpreted the results and drafted the report. Li-hong Ye, Hai-cong Hai, and Zhi-quan Liu made pathological diagnoses of needle-biopsied liver tissue. Xue-dong Zhang performed the laboratory tests. Jie-ying Li, Yun-yan Liu, Dong-yu Zeng, De-hua Wang, Jun-qing Li, Liu Yang, Yang Cao, Yun Pan, and Xun-ge Lin collected the data. Min-ran Li, Jin-zhong Li, Rui-kun Yuan, Xu-jing Liang, and Tao-yuan Li analyzed the data. Calvin Pan revised the manuscript and addressed the reviewers' comments.

Data Availability Statement

All data generated during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Huang TD, Behary J, Zekry A. Non-alcoholic fatty liver disease: a review of epidemiology, risk factors, diagnosis and management. Intern Med J. 2020;50(9):1038–47.
2 Eslam M, Sarin SK, Wong VW, Fan JG, Kawaguchi T, Ahn SH, et al. The Asian Pacific Association for the Study of the Liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. Hepatol Int. 2020 Dec;14(6):889–919.
3 Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology. 2020 May;158(7):1999–2014.e1.
4 Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol. 2020 Jul;73(1):202–9.
5 Kumar R, Rastogi A, Sharma MK, Bhatia V, Garg H, Bihari C, et al. Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver disease in Indian patients with normal body mass index: do they differ from obese or overweight non-alcoholic fatty liver disease? Indian J Endocrinol Metab. 2013 Jul;17(4):665–71.
6 Chakrabarty M, Jha AN, Sharma DJ. Clinical characteristics and metabolic profiles of non-alcoholic fatty liver disease (NAFLD) in lean patients and their comparison with obese and overweight NAFLD. J Assoc Physicians India. 2022 Apr;70(4):11–2.
7 Li Q, Zeng A, Liu H, Wang S, Ding H, Li L. Clinical analysis of metabolically related fatty liver disease between overweight and obesity. Beijing Med. 2021;43(9):924–9.
8 Wu YL, Kumar R, Wang MF, Singh M, Huang JF, Zhu YY, et al. Validation of conventional non-invasive fibrosis scoring systems in patients with metabolic associated fatty liver disease. World J Gastroenterol. 2021 Sep 14;27(34):5753–63.
9 European Association for the Study of the Liver EASLEuropean Association for the Study of Diabetes EASDEuropean Association for the Study of Obesity EASO. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388–402.
10 Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, et al. Comparison of MAFLD and NAFLD diagnostic criteria in real world. Liver Int. 2020;40(9):2082–9.
11 Zheng M, Chen Y, Chen C, Gopal N, Jiao J. Clinical characteristics of platelets and its possible gender dimorphism in patients with non-alcoholic fatty liver disease (NAFLD). Postgrad Med. 2021 Apr;133(3):299–306.
12 Milovanovic Alempijevic T, Stojkovic Lalosevic M, Dumic I, Jocic N, Pavlovic Markovic A, Dragasevic S, et al. Diagnostic accuracy of platelet count and platelet indices in non-invasive assessment of fibrosis in nonalcoholic fatty liver disease patients. Can J Gastroenterol Hepatol. 2017;2017:6070135.
13 Shi JQ, Shen WX, Wang XZ, Huang K, Zou CC. Relationship between immune parameters and non-alcoholic fatty liver disease in obese children. Indian Pediatr. 2017 Oct 15;54(10):825–9.
14 Chauhan A, Adams DH, Watson SP, Lalor PF. Platelets: No longer bystanders in liver disease. Hepatology. 2016 Nov;64(5):1774–84.
15 Ozhan H, Aydin M, Yazici M, Yazgan O, Basar C, Gungor A, et al. Mean platelet volume in patients with non-alcoholic fatty liver disease. Platelets. 2010;21(1):29–32.
16 Xing Y, Chen J, Liu J, Ma H. Associations between GGT/HDL and MAFLD: a cross-sectional study. Diabetes Metab Syndr Obes. 2022 Feb 9;15:383–94.
17 Peng L, Wu S, Zhou N, Zhu S, Liu Q, Li X. Clinical characteristics and risk factors of nonalcoholic fatty liver disease in children with obesity. BMC Pediatr. 2021 Mar 12;21(1):122.
18 Ni L, Yu D, Wu T, Jin F. Gender-specific association between non-alcoholic fatty liver disease and type 2 diabetes mellitus among a middle-aged and elderly Chinese population: an observational study. Medicine. 2021 Feb 12;100(6):e24743.
19 Bian H, Zhu X, Xia M, Yan H, Chang X, Hu X, et al. Impact of type 2 diabetes on nonalcoholic steatohepatitis and advanced fibrosis in patients with nonalcoholic fatty liver disease. Endocr Pract. 2020 Apr;26(4):444–53.

20 Kotronen A, Joutsi-Korhonen L, Sevastianova K, Bergholm R, Hakkarainen A, Pietiläinen KH, et al. Increased coagulation factor VIII, IX, XI and XII activities in non-alcoholic fatty liver disease. Liver Int. 2011 Feb;31(2):176–83.
21 Furuhashi M. New insights into purine metabolism in metabolic diseases: role of xanthine oxidoreductase activity. Am J Physiol Endocrinol Metab. 2020;319(5):E827–34.
22 Lu FB, Hu ED, Xu LM, Chen L, Wu JL, Li H, et al. The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol. 2018;12(5):491–502.
23 Petta S, Wai-Sun Wong V, Bugianesi E, Fracanzani AL, Cammà C, Hiriart JB, et al. Impact of obesity and alanine aminotransferase levels on the diagnostic accuracy for advanced liver fibrosis of noninvasive tools in patients with nonalcoholic fatty liver disease. Am J Gastroenterol. 2019 Jun;114(6):916–28.
24 Mu YP, Ogawa T, Kawada N. Reversibility of fibrosis, inflammation, and endoplasmic reticulum stress in the liver of rats fed a methionine-choline-deficient diet. Lab Invest. 2010;90(2):245–56.
25 Liu Y. The effect of inflammatory stress on hepatic cholesterol accumulation and hepatic fibrosis in C57bl/6J mice (D). Chongqing Medical University; 2013.
26 Anavi S, Eisenberg-Bord M, Hahn-Obercyger M, Genin O, Pines M, Tirosh O. The role of iNOS in cholesterol-induced liver fibrosis. Lab Invest. 2015;95(8):914–24.
27 Tomita K, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, et al. Acyl-CoA:cholesterol acyltransferase 1 mediates liver fibrosis by regulating free cholesterol accumulation in hepatic stellate cells. J Hepatol. 2014;61(1):98–106.