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Original Article

Diagnostic value of serum 25-hydroxyvitamin D, hs-CRP, and adiponectin metabolic dysfunction-associated steatotic liver disease in patients with severe obesity: a retrospective study

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Abstract: Objective: To investigate the clinical value of serum 25-hydroxyvitamin D [25(OH)D], high-sensitivity C-reactive protein (hs-CRP), and adiponectin in the assessment of metabolic dysfunction-associated fatty liver disease (MAFLD) in patients with severe obesity. Methods: The clinical data of 338 patients with severe obesity (BMI ≥ 30 kg/m^2) who attended the Health Management Center of West China Hospital, Sichuan University, from January 2022 to January 2024 were retrospectively analyzed. The patients were divided into a MAFLD group and a simple obesity group according to the presence or absence of metabolic dysfunction-related fatty liver disease. Clinical data were collected for all subjects, including fasting serum levels of 25(OH)D, hs-CRP, and adiponectin. Multivariate logistic regression analysis was used to evaluate the independent predictive value of each indicator. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive performance of each marker alone and in combination. Results: Compared to the simple obesity group, patients with MAFLD had significantly lower levels of serum 25(OH)D and adiponectin, while the level of hs-CRP was significantly higher (all $P < 0.05$). Multivariate logistic regression analysis showed that low 25(OH)D level (OR = 2.354, 95% CI: 1.282-4.325), low adiponectin level [OR = 2.565, 95% CI: 1.408-4.673], and high hs-CRP level (OR = 3.402, 95% CI: 1.845-6.274) were independent risk factors for MAFLD in patients with severe obesity. ROC curve analysis showed that the combined model demonstrated the highest predictive value, with an area under the curve (AUC) of 0.960 (95% CI: 0.921-0.999). Conclusion: Serum 25(OH)D, hs-CRP, and adiponectin are closely associated to MAFLD in patients with severe obesity. A combined predictive model incorporating these three biomarkers demonstrates excellent predictive performance.

Keywords: Obesity, 25-hydroxyvitamin D, high-sensitivity C-reactive protein, adiponectin, metabolic dysfunction-associated steatotic liver disease, prediction

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a metabolic disorder driven primarily by overnutrition, insulin resistance, and genetic susceptibility [1, 2]. With the rising prevalence of obesity, the incidence of MASLD has increased [3]. The disease spectrum of MASLD includes metabolic dysfunction-related steatosis, steatohepatitis, liver fibrosis, cirrhosis, and even hepatocellular carcinoma, and it has become the predominant form of chronic liver disease globally [4, 5]. Currently, the diagnosis of MASLD relies mainly on the detection of hepatic steatosis, which may delay timely treatment. Therefore, early prevention and accurate diagnosis are of great significance for improving patient prognosis [6].

Evidence indicates multiple circulating biomarkers are related to the occurrence and progression of MASLD. Previous studies have reported that the inflammatory state plays a critical role in MASLD pathogenesis, and high-sensitivity C-reactive protein (hs-CRP) serves as an important serum marker of systemic inflammation [7,

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8]. In addition, 25-hydroxyvitamin D [25(OH)D] has been shown to exhibit anti-inflammatory effects and contribute to the regulation of insulin sensitivity [9, 10]. Given the aberrant expression of adiponectin in obese patients, it is of great significance to investigate the circulating levels of these biomarkers in patients with MASLD. Therefore, this study retrospectively analyzed the serum levels of 25(OH)D, hs-CRP, and adiponectin in patients with MASLD, aiming to provide targets for improving tertiary prevention strategies.

Patients and methods

General information

The clinical data of 338 patients with severe obesity (BMI ≥ 30 kg/m^2) who attended the Health Management Center of West China Hospital, Sichuan University, Guangzhou Ciming Outpatient Department Co., Ltd and Guangzhou Eighth People's Hospital, Guangzhou Medical University from January 2022 to January 2024 were retrospectively analyzed. Patients were divided into a MASLD group (n = 218) and a simple obesity group (n = 120) according to whether they had metabolic dysfunction-related fatty liver disease. This study was approved by the Ethics Committee of the Eighth Affiliated Hospital of Guangzhou Medical University (approval number: KYLL-20241108). The diagnosis of MASLD was established based on the 2023 MASLD consensus criteria.

Sample size calculation

The sample size was determined based on the events per variable (EPV) principle for logistic regression analysis. According to the widely accepted rule of thumb, a minimum of 10 outcome events are required for each predictor variable included in the multivariate model to ensure reliable and stable estimates.

In this study, the primary outcome was the presence of MASLD. Based on previous literature, the prevalence of MASLD among individuals with severe obesity is approximately 60-70% [2]. With a total sample size of 338 patients, it was estimated that about 203 to 237 patients would have MASLD. The multivariate logistic regression analysis included 3-5 candidate predictors, including 25(OH)D, hs-CRP, adiponectin, and potential confounders such as age and sex.

Assuming 5 predictors in the final model, the minimum required number of outcome events would be 50 (5 predictors × 10 events). The estimated number of MASLD cases (≥ 200) far exceeded this requirement, indicating that the sample size was sufficient for the planned analyses.

Therefore, the final sample of 338 patients with severe obesity (with an expected MASLD prevalence of approximately 60-70%) provided sufficient statistical power to identify independent predictors and to evaluate the predictive performance of the combined model.

Inclusion criteria

Participants were eligible for inclusion if they met all of the following criteria: (1) Age between 18 and 75 years; (2) Body mass index (BMI) ≥ 30 kg/m^2; (3) Completion of all required physical examinations at our hospital; (4) First-time physical examination at our institution; (5) No history of significant alcohol consumption, defined as a self-reported alcohol intake of < 20 g/day for men and < 10 g/day for women, and no history of regular heavy drinking (≥ 5 drinks per occasion) within the past 5 years.

Exclusion criteria

Participants were excluded if they met any of the following criteria: (1) BMI < 30 kg/m^2; (2) Incomplete clinical data or missing key laboratory indicators; (3) History of treatment for liver disease, including any pharmacologic or interventional therapy; (4) Infection within the past two weeks, defined by any of the following: (a) Clinical symptoms such as fever (≥ 38.0°C), cough, sputum production, dysuria, or localized signs of inflammation; (b) Laboratory evidence including a white blood cell count > 10 × 10^9/L or < 4 × 10^9/L, neutrophil percentage > 75%, or C-reactive protein (CRP) > 10 mg/L; (c) Radiologic evidence of infection (e.g., pulmonary infiltrates); or (d) A confirmed diagnosis of infection requiring antibiotic or antiviral treatment; (5) History of rheumatic or autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus); (6) Major cardiovascular or cerebrovascular diseases (e.g., myocardial infarc-

tion, stroke, heart failure); (7) Parathyroid dysfunctions (e.g., hyperparathyroidism or hypoparathyroidism); (8) Use of medications that may affect vitamin D, glucose, or lipid metabolism within the past 3 months (e.g., vitamin D supplements, corticosteroids, lipid-lowering drugs, antidiabetic agents).

Data collection

No missing data were observed for any variables included in this study, including serum biomarkers and baseline clinical characteristics. Only patients with complete medical records were included in the final analysis. Demographic and clinical data were collected from the electronic medical record system, including baseline data, liver and renal function indicators, and inflammatory markers.

Fasting venous blood samples were collected from all participants at admission. Routine hematologic data, including white blood cell (WBC) counts, were measured using an automated hematology analyzer (Model: Sysmex XN-9000). Serum interleukin-6 (IL-6) levels were detected using chemiluminescence immunoassay (Roche Cobas e801). C-reactive protein (CRP) levels were measured using immunoturbidimetry (Beckman Coulter AU5800). Liver function parameters, kidney function, and blood lipid levels were evaluated using a Roche Cobas c702 biochemical analyzer. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the enzymatic rate method, while lipid profiles including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assessed using enzymatic colorimetric methods. Renal function was evaluated by measuring creatinine (SCR) by the enzymatic method and blood urea nitrogen (BUN) using the urease-glutamate dehydrogenase method. Serum levels of 25(OH)D and hs-CRP were measured using electrochemiluminescence immunoassay and immunoturbidimetric method, respectively, on a Roche Diagnostics analyzer with corresponding kits (lot numbers: 25(OH)D, 08492700; hs-CRP, 07851901). Serum adiponectin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, lot number: DY1065). All assays were performed strictly according to the manufacturers' instructions by trained technicians blinded to the clinical data.

Quality control procedures were rigorously implemented. Internal quality control samples were included in each analytical run. The intra-assay coefficients of variation (CVs) were < 5% for 25(OH)D, < 3% for hs-CRP, and < 6% for adiponectin, while the inter-assay CVs were < 8%, < 5%, and < 9%, respectively. The laboratory participated in the National External Quality Assessment Scheme, and all results during the study period met acceptable standards. The reference ranges were as follows: 25(OH)D deficiency < 20 ng/mL, insufficiency 20-30 ng/mL, and sufficiency ≥ 30 ng/mL; hs-CRP < 3 mg/L; adiponectin 4-26 μg/mL.

Liver steatosis was evaluated using a real-time ultrasound-guided liver steatosis intelligent analysis (LISA) system integrated with a Vision Transformer encoder (VIT-Encoder). Patients were examined in a supine position using a convex array probe (3-5 MHz), with scanning performed through the right intercostal or subcostal margin. The LISA system acquired ultrasound image streams in real time and provided standardized guidance for image acquisition, including probe angle and depth adjustment. Only standard right liver intercostal views meeting predefined criteria (clearly displaying the liver capsule, hepatic parenchyma, and right renal cortex as a reference) were accepted for analysis.

After the probe position was stable, the optimal image frame was automatically selected and input into a pre-trained deep learning model. The system automatically completed feature extraction and analysis within seconds and generated liver steatosis grading results (S0-S3), together with quantitative data such as hepatorenal echo ratio [11, 12]. Detailed procedures are illustrated in **Figure 1**.

Observation indicators

Primary outcomes: Baseline characteristics were compared between the MASLD group and the simple obesity group. A prediction model for MASLD was subsequently developed, and its diagnostic performance was evaluated.

Secondary outcomes: The calibration performance of the predictive model was evaluated, and its clinical utility was evaluated using a decision curve analysis (DCA).

Statistics analysis

SPSS23.0 statistical software was used for data analysis. Continuous variables meeting a

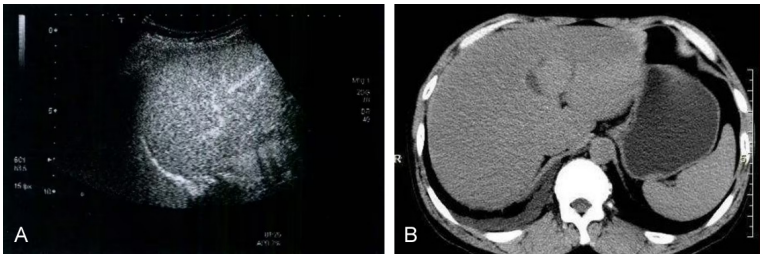

Figure 1. Representative liver Doppler and abdominal CT images of patient with MASLD. A: Color Doppler images; B: Liver CT images. Notes: MASLD, metabolic dysfunction-associated fatty liver disease.

normal distribution were expressed as Mean ± standard deviation (SD). Normality and homogeneity of variance were assessed prior to analysis. For comparisons between two groups, the independent-samples t-test was applied; otherwise, appropriate nonparametric tests were used. Categorical variables were expressed as counts and percentages [n (%)] and compared using the chi-square test.

Multivariate logistic regression analysis was used to identify independent predictors of MASLD, which were further incorporated to develop a predictive model. The predictive performance of the model was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was calculated. A two-tailed $P < 0.05$ was considered significant.

Variable selection for multivariate regression

Variables included in the multivariate logistic regression analysis were selected based on both statistical significance and clinical relevance. Specifically, variables with a *P* value < 0.10 in the univariate analysis were entered into the multivariate model. Additionally, clinically important variables, including age, sex, and BMI, were added to the model regardless of their univariate significance, since they are well-established risk factors for MASLD.

Collinearity diagnostics

Multicollinearity among the independent variables was assessed by calculating the variance inflation factor (VIF). A VIF > 10 was considered indicative of significant collinearity. In this study, all VIF values were < 3, suggesting no significant multicollinearity among the predictors included in the final model.

Results

Comparison of baseline characteristics between the two groups

A total of 338 patients with obesity (BMI ≥ 30 kg/m^2) were included in this study, including 120 patients in the simple obesity group and 218 in the MASLD group. The baseline characteristics are summarized in **Table 1**. There were no significant differences between the two groups in terms of age, sex distribution, height, weight, BMI, or waist circumference (all $P > 0.05$).

Regarding comorbidities, the prevalence of type 2 diabetes mellitus (23.8% vs. 12.5%, $P = 0.011$) and metabolic syndrome (65.6% vs. 35.0%, $P < 0.001$) was significantly higher in the MASLD group compared to the simple obesity group, while no significant difference was observed in hypertension prevalence ($P > 0.05$).

In terms of metabolic indicators, patients in the MASLD group exhibited significantly higher levels of glycated hemoglobin (HbA1c), fasting insulin (FINS), and insulin resistance index (HOMA-IR) compared to the simple obesity group (all $P < 0.05$). However, no significant differences were observed between the two groups in lipid profiles, including triglycerides, TC, LDL-C, HDL-C, nor in liver function markers (e.g., ALT and AST) (all $P > 0.05$).

Comparison of serum levels of 25(OH)D, hs-CRP, and adiponectin between the two groups

Significant differences were observed in serum markers related to metabolism and inflammation between the two groups (**Table 2**). Specifically, patients in the MASLD group had significantly lower serum levels of 25(OH)D (21.7 ± 5.8 vs. 28.4 ± 6.2 ng/mL, $P < 0.001$) and adiponectin (8.3 ± 2.4 vs. 12.5 ± 3.1 μg/mL, $P < 0.001$) compared to those in the simple obesity group. In contrast, the level of hs-CRP, a systemic inflammatory marker, was significantly higher in the MASLD group (4.8 ± 1.5 vs. 2.1 ± 0.9 mg/L, $P < 0.001$).

Moreover, the proportions of patients with 25(OH)D deficiency (< 20 ng/mL) and elevated

Table 1. Comparison of baseline characteristics between the two groups

Variable	Simple obesity group (n = 120)	MASLD group (n = 218)	Statistics value	*P* value
Demographic characteristics				
Age (years)	43.1 ± 9.2	45.3 ± 8.7	1.850	0.065
Male [n (%)]	60 (50.0)	125 (57.3)	1.720	0.190
Anthropometric indicators				
Height (cm)	165.8 ± 6.9	166.5 ± 7.3	0.825	0.411
Weight (kg)	91.5 ± 8.1	93.5 ± 8.7	1.950	0.052
BMI (kg/m^2)	33.3 ± 2.0	33.8 ± 2.3	1.820	0.070
Waist circumference (cm)	100.1 ± 7.9	103.7 ± 8.4	1.950	0.052
Key complications [n (%)]				
Type 2 diabetes mellitus	15 (12.5)	52 (23.8)	6.405	0.025
Hypertension	33 (27.5)	67 (30.7)	0.376	0.540
Metabolic syndrome	42 (35.0)	143 (65.6)	29.417	< 0.001
Glycated hemoglobin (HbA1c, %)	5.6 ± 0.5	6.2 ± 0.8	2.150	0.032
Fasting insulin (FINS, μU/mL)	12.4 ± 4.2	18.7 ± 5.9	2.340	0.020
Insulin resistance index (HOMA-IR)	3.2 ± 1.1	5.2 ± 1.8	2.420	0.016
Laboratory indicators				
Fasting blood glucose (mmol/L)	5.8 ± 1.3	6.3 ± 1.6	1.750	0.081
Triglyceride (mmol/L)	1.9 ± 0.9	2.5 ± 1.2	1.720	0.087
Total cholesterol (mmol/L)	5.0 ± 0.9	5.3 ± 1.0	1.550	0.122
Low density lipoprotein cholesterol (mmol/L)	3.1 ± 0.8	3.3 ± 0.9	1.480	0.140
High density lipoprotein cholesterol (mmol/L)	1.2 ± 0.3	1.1 ± 0.3	1.600	0.111
Alanine aminotransferase (U/L)	28.4 ± 10.1	44.1 ± 19.5	1.900	0.058
Aspartate aminotransferase (U/L)	26.5 ± 8.7	40.2 ± 16.3	1.880	0.061

Notes: BMI, Body mass index; MASLD, metabolic dysfunction-associated fatty liver disease.

Table 2. Comparison of serum 25(OH)D, adiponectin and hs-CRP levels between the two groups

Variable	Simple obesity group (n = 120)	MASLD group (n = 218)	Statistics value	*p* value
25(OH)D [ng/mL]	28.4 ± 6.2	21.7 ± 5.8	9.247	< 0.001
Adiponectin (μg/mL)	12.5 ± 3.1	8.3 ± 2.4	13.058	< 0.001
hs-CRP (mg/L)	2.1 ± 0.9	4.8 ± 1.5	18.329	< 0.001

Notes: 25(OH)D, 25-hydroxyvitamin D [25-(OH)D]; Hs-CRP, high-sensitivity C-reactive protein; MASLD, metabolic dysfunction-associated fatty liver disease.

hs-CRP (> 3 mg/L) in the MASLD group were 58.7% and 71.6%, respectively, significantly higher than 26.7% and 15.0%, respectively, in the simple obesity group (both P < 0.001). Moreover, the proportion of patients with decreased adiponectin (< 10 μg/mL) was 90.8%, significantly higher than 48.3% in the simple obesity group (P < 0.001) (**Table 3**).

Association between serum marker abnormalities and baseline characteristics

The distribution of abnormal serum markers across different baseline characteristics was further analyzed based on predefined clinical thresholds (**Table 4**). Sex-based analysis showed that the prevalence of 25(OH)D deficiency was significantly higher in males than in females (52.97% vs. 40.52%, P = 0.027), whereas no significant differences were observed between sexes in the prevalence of elevated hs-CRP or adiponectin levels (P > 0.05). No significant differences were observed in the positive rates of any markers between age groups using a cut-off of 45 years (P > 0.05). Stratification by obesity severity showed that patients with BMI ≥ 35 kg/m^2 had a significantly higher proportion with 25(OH)D deficiency (56.36% vs. 42.98%, P = 0.020) and decreased adiponectin (90.00% vs. 81.14%, P = 0.037) com-

Table 3. Comparison of the prevalence of abnormal serum 25(OH)D, hs-CRP, and adiponectin levels between the two groups

Variable	Positive judgement standard	Simple obesity group (n = 120)	MASLD group (n = 218)	χ^2 value	*P* value
25(OH)D deficiency [n (%)]	< 20 ng/mL	32 (26.7)	128 (58.7)	32.84	< 0.001
Elevated hs-CRP [n (%)]	> 3 mg/L	18 (15.0)	156 (71.6)	101.45	< 0.001
Decreased adiponectin [n (%)]	< 10 μg/mL	58 (48.3)	198 (90.8)	72.12	< 0.001

Notes: 25(OH)D, 25-hydroxyvitamin D [25-(OH)D]; Hs-CRP, high-sensitivity C-reactive protein; MASLD, metabolic dysfunction-associated fatty liver disease.

Table 4. Comparison of the prevalence of abnormal serum 25-(OH) D, hs-CRP and adiponectin levels according to patient characteristics

Variable	Number of cases	25(OH)D deficiency	Elevated hs-CRP	Decreased adiponectin
Sex				
Male	185	98 (52.97)	112 (60.54)	158 (85.41)
Female	153	62 (40.52)	84 (54.90)	126 (82.35)
χ^2 value		4.879	1.063	0.549
P value		0.027	0.302	0.459
Age				
< 45 years old	165	78 (47.27)	89 (53.94)	135 (81.82)
≥ 45 years old	173	82 (47.40)	107 (61.85)	149 (86.13)
χ^2 value		0.001	2.353	1.228
P value		0.982	0.125	0.268
BMI				
30-34.9 kg/m^2	228	98 (42.98)	124 (54.39)	185 (81.14)
≥ 35 kg/m^2	110	62 (56.36)	72 (65.45)	99 (90.00)
χ^2 value		5.384	3.663	4.372
P value		0.020	0.056	0.037
Metabolic syndrome				
Yes	204	118 (57.84)	152 (74.51)	188 (92.16)
No	134	42 (31.34)	44 (32.84)	96 (71.64)
χ^2 value		23.145	58.742	25.962
P value		< 0.001	< 0.001	< 0.001

Notes: 25(OH)D, 25-hydroxyvitamin D; Hs-CRP, high-sensitivity C-reactive protein; BMI, Body mass index.

pared to those with BMI 30-34.9 kg/m^2. Metabolic syndrome status was a key factor affecting all three markers. Patients with metabolic syndrome exhibited significantly higher proportion of 25(OH)D deficiency (57.84% vs. 31.34%, P < 0.001), elevated hs-CRP (74.51% vs. 32.84%, P < 0.001) and reduced adiponectin (92.16% vs. 71.64%, P < 0.001) than those without metabolic syndrome.

Multivariate analysis of factors associated with MASLD in obese patients

Multivariate logistic regression analysis showed that 25(OH)D deficiency (OR = 2.354, 95% CI: 1.282-4.325, P = 0.006), elevated hs-CRP (OR = 3.402, 95% CI: 1.845-6.274, P < 0.001), decreased adiponectin (OR = 2.565, 95% CI: 1.408-4.673, P = 0.002), diabetes mellitus (OR = 1.961, 95% CI: 1.101-3.492, P = 0.022), and severe obesity (BMI ≥ 35 kg/m^2) (OR = 2.198, 95% CI: 1.214-3.981, P = 0.009) were independent risk factors associated with MASLD in obese patients after adjustment for potential confounders (**Table 5**).

A combined predictive model was constructed using binary logistic regression. Based on the multivariate analysis results, three serum biomarkers with statistical significance-25(OH)D deficiency (x_1), elevated hs-CRP (x_2), and decreased adiponectin (x_3)-were included as inde-

Table 5. Multivariate analysis of factors associated with MASLD

Variable	β value	OR value	95% CI	*P* value
25(OH)D deficiency (x_1)	0.856	2.354	1.282-4.325	0.006
Elevated hs-CRP (x_2)	1.224	3.402	1.845-6.274	< 0.001
Reduced adiponectin (x_3)	0.942	2.565	1.408-4.673	0.002
Diabetes (x_4)	0.673	1.961	1.101-3.492	0.022
BMI ≥ 35 kg/m^2 (x_5)	0.788	2.198	1.214-3.981	0.009
Constant term	-4.352	0.013	0.001-0.125	< 0.001

Notes: 25(OH)D, 25-hydroxyvitamin D; Hs-CRP, high-sensitivity C-reactive protein; BMI, Body mass index; MASLD, metabolic dysfunction-associated fatty liver disease.

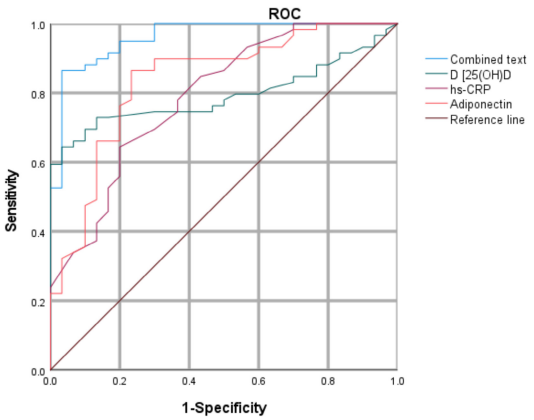

Figure 2. Predictive performance of serum 25(OH)D, hs-CRP, and adiponectin for MASLD analyzed using ROC Curve Analysis. Notes: ROC, Receiver Operating Characteristic curve; MASLD, metabolic dysfunction-associated fatty liver disease.

pendent variables, with the presence of MASLD as the dependent variable. The regression equation was as follows: Logit (P) = -4.352 + 0.856 × x_1 + 1.224 × x_2 + 0.942 × x_3, where x_1, x_2, and x_3 represent 25(OH)D deficiency (yes = 1, no = 0), elevated hs-CRP (yes = 1, no = 0), and decreased adiponectin (yes = 1, no = 0), respectively. The predicted probability was calculated as: P = 1/[1 + exp(-Logit(P))]. The predicted probability (P) generated from the logistic regression equation was then used as the composite indicator for ROC curve analysis.

Diagnostic performance of serum 25(OH)D, hs-CRP, and adiponectin

The ROC curves for 25(OH)D, hs-CRP, adiponectin, and their combined model are presented in **Figure 2**. The optimal cut-off values were determined by maximizing the Youden index.

As shown in **Table 6**, the combined model yielded the highest diagnostic performance, with an AUC of 0.960 (95% CI: 0.921-0.999). At the optimal cut-off value of 0.52, the model yielded a sensitivity of 90.0%, specificity of 86.7%, and overall accuracy of 88.9% for identifying MASLD in patients with severe obesity.

For individual markers, the optimal cut-off values were < 20 ng/mL for 25(OH)D (sensitivity: 72.5%, specificity: 73.3%), > 3 mg/L for hs-CRP (sensitivity: 71.7%, specificity: 76.7%), and < 10 μg/mL for adiponectin (sensitivity: 78.3%, specificity: 70.0%).

DeLong test (**Table 7**) demonstrated that the AUC of the combined model (0.960) was significantly higher than that of each individual biomarker (25(OH)D: 0.793, *P* = 0.001; hs-CRP: 0.789, *P* < 0.001; adiponectin: 0.836, *P* = 0.010). In contrast, no significant differences were observed among the individual biomarkers (all *P* > 0.05), suggesting comparable diagnostic performance and supporting the complementary value of their combination.

Internal validation and clinical utility of the predictive model

The calibration performance of the prediction model was assessed using a calibration curve. The results showed good agreement between the predicted probabilities and the observed outcomes, as shown in **Figure 3**. The clinical utility of the model was further evaluated using the DCA, showing that the predictive model provided a favorable net benefit across a wide range of threshold probabilities, suggesting good clinical applicability for identifying MASLD in patients with obesity (**Figure 4**).

Discussion

Epidemiologic evidence indicates that MASLD has surpassed viral hepatitis as the leading cause of chronic liver disease worldwide [13, 14]. MASLD is not only a common cause of cryptogenic cirrhosis but also represents a key hepatic manifestation of systemic metabolic disorders. Clinically, patients with early-stage MASLD may present symptoms such as fatigue and right upper abdominal discomfort, while advanced disease can progress to cirrhosis

Table 6. Diagnostic performance of individual biomarker and their combination for MASLD in obese patients

Biomarker	AUC (95% CI)	Cut-off value	Youden index	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)
25(OH)D	0.793 (0.701-0.885)	< 20 ng/mL	0.458	72.5	73.3	72.8	82.9
hs-CRP	0.789 (0.690-0.889)	> 3 mg/L	0.484	71.7	76.7	73.3	84.3
Adiponectin	0.836 (0.745-0.927)	< 10 μg/mL	0.483	78.3	70.0	75.6	82.5
Combined model	0.960 (0.921-0.999)	0.52	0.767	90.0	86.7	88.9	92.9

Notes: 25(OH)D, 25-hydroxyvitamin D; Hs-CRP, high-sensitivity C-reactive protein; BMI, Body mass index; AUC, Area under the curve; MASLD, metabolic dysfunction-associated fatty liver disease.

Table 7. Comparison of AUCs between the combined model and each individual biomarkers using DeLong test

	Difference in AUC	Z value	*P* value
Combined model vs. 25(OH)D	0.167	3.245	0.001
Combined model vs. hs-CRP	0.171	3.412	< 0.001
Combined model vs. Adiponectin	0.124	2.568	0.010
Adiponectin vs. 25-(OH)D	0.043	1.124	0.261
Adiponectin vs. hs-CRP	0.047	1.231	0.218
hs-CRP vs. 25-(OH)D	0.004	0.098	0.922

Notes: 25(OH)D, 25-hydroxyvitamin D; Hs-CRP, high-sensitivity C-reactive protein; BMI, Body mass index; AUC, Area under the curve.

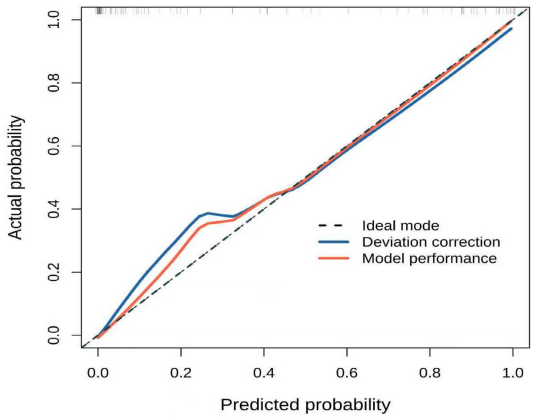

Figure 3. Calibration curve for the predictive model.

and even cancer [15, 16]. Therefore, early identification of MASLD in obese patients and timely intervention are of great clinical import.

In this study, patients with BMI ≥ 35 kg/m^2 exhibited a significantly higher prevalence of reduced adiponectin levels. This finding is consistent with the well-established inverse relationship between adiponectin and adiposity. Although adiponectin is exclusively secreted by adipocytes, its circulating levels paradoxically decrease as adipose tissue mass increases, particularly in visceral obesity. Several mechanisms may account for this phenomenon. First, adipocyte hypertrophy and hyperplasia in obesity can induce local hypoxia, endoplasmic reticulum stress, and chronic low-grade inflammation, thereby suppressing adiponectin gene expression and secretion. Second, pro-inflammatory cytokines elevated in obesity, such as TNF-α and IL-6, directly inhibit adiponectin production. Third, the accumulation of visceral adipose tissue, which is more metabolically active and prevalent in individuals with higher BMI, is associated with greater suppression of adiponectin secretion compared to subcutaneous adipose tissue.

Furthermore, the superior predictive performance of the combined model suggests potential synergistic interactions among 25(OH)D, hs-CRP, and adiponectin in the pathogenesis of MASLD, representing three interconnected pathological pathways: vitamin D metabolism, systemic inflammation, and adipose tissue function. Vitamin D deficiency contributes to MASLD development through multiple mechanisms, including the promotion of insulin resistance, enhancement of hepatic lipogenesis, and activation of inflammatory pathways. Elevated hs-CRP levels reflect the chronic low-grade inflammatory state characteristic of obesity and MASLD, in which adipose tissue dysfunction leads to increased release of pro-inflammatory cytokines that stimulate hepatic CRP production and promote hepatocyte injury. In contrast, adiponectin exerts protective effects through its insulin-sensitizing, anti-inflammatory, and anti-fibrotic properties. Mechanistically, adiponectin activates AMPK and PPAR-α

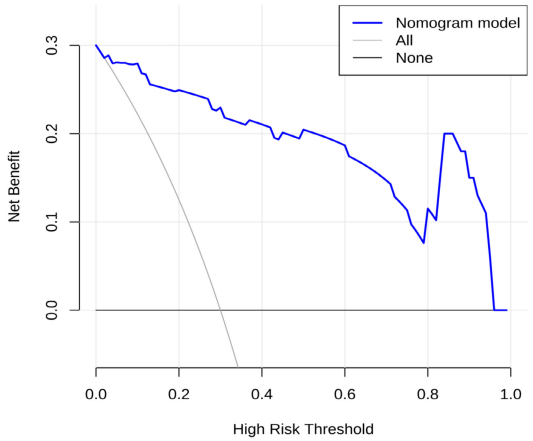

Figure 4. Decision curve analysis (DCA) for the predictive model.

pathways, promoting fatty acid oxidation, reducing hepatic steatosis, and inhibiting hepatic stellate cell activation. Importantly, these three biomarkers are interconnected: vitamin D deficiency may exacerbate systemic inflammation (reflected by elevated hs-CRP) and further suppress adiponectin production, creating a vicious cycle, while adequate vitamin D levels may enhance adiponectin expression and reduce inflammatory markers. Therefore, the combination of low 25(OH)D, low adiponectin, and high hs-CRP reflects the convergence of multiple pathogenic processes-metabolic dysregulation, chronic inflammation, and adipose tissue dysfunction - that collectively promote MASLD development and progression. This mechanistic interplays may explain why the combined model achieves superior predictive performance compared to any single biomarker alone.

Previous studies have confirmed that MASLD in obese patients is associated with baseline clinical characteristics [17]. In this study, serum levels of 25(OH)D, hs-CRP, adiponectin, and the presence of diabetes were identified as influencing factors for MASLD. Vitamin D is not only involved in calcium and phosphorus metabolism but also plays an important role in immune regulation, anti-inflammation, and insulin sensitivity. Decreased levels of 25(OH)D may aggravate insulin resistance and promote lipid deposition in hepatocytes. In addition, vitamin D deficiency may impair anti-fibrotic pathways, which facilitates hepatic inflammation and fibrogenesis [18, 19].

Elevated hs-CRP levels reflect a state of chronic inflammation, which represents a central pathological mechanism linking obesity and MASLD. Inflammation directly contribute to hepatic injury by promoting hepatocellular damage, steatosis, and fibrosis [20].

Diabetes was also identified as an independent factor associated with MASLD. Chronic hyperglycemia and insulin resistance in patients with diabetes aggravate hepatic lipid metabolism disorder through multiple mechanisms. Insulin resistance reduces the inhibitory effect of insulin on adipose tissue lipolysis, leading to increased release of free fatty acids into blood circulation, which in turn promotes hepatic lipid accumulation. In addition, hyperinsulinemia enhances de novo lipogenesis in the liver, while concomitant hyperglycemia provides abundant substrates, further exacerbating hepatic fat deposition. Consequently, the accumulation of advanced glycation end products (AGEs) in diabetic patients induce oxidative stress and liver inflammatory response, contributing to liver injury [21].

Adiponectin plays a protective role in liver metabolism through its insulin-sensitizing, anti-inflammatory, and anti-fibrotic effects. However, in obesity, adiponectin secretion is suppressed, resulting in reduced circulating levels and attenuation of its hepatoprotective functions. This reduction may increase susceptibility to hepatic injury and promote MASLD progression, consistent with previous reports [22, 23].

In the baseline comparison, patients in the MASLD group showed higher levels of ALT and AST compared to those in the simple obesity group, consistent with the expected pattern of hepatocellular injury. Although these differences did not reach statistical significance (ALT: $P = 0.058$; AST: $P = 0.061$), the observed trends are clinically meaningful and align with the pathophysiology of MASLD. The lack of statistical significance may be attributable to the inter-individual variability or the fact that liver enzyme elevations in MASLD are often mild and intermittent. These findings suggest that ALT and AST, while not independently significant in this cohort, may still serve as ancillary indicators of hepatic involvement. Further studies with larger sample sizes are needed to clarify their role in this population.

An interesting finding of this study was the significantly higher prevalence of 25(OH)D deficiency in male patients compared with females. This sex-related difference may be attributed to several factors. First, hormonal differences, particularly estrogen, may enhance vitamin D receptor expression and influence vitamin D metabolism in females. Second, lifestyle-related factors, such as sun exposure and dietary habits, may differ between sexes, potentially leading to lower endogenous vitamin D synthesis or intake in males. Third, differences in body fat distribution may affect vitamin D sequestration in adipose tissue, influencing circulating 25(OH)D levels. These findings highlight the need for further research into the mechanisms underlying sex disparities in vitamin D status [17].

The present study demonstrated that serum levels of 25(OH)D, hs-CRP, and adiponectin differed significantly between obese patients with and without MASLD, and that their combined assessment exhibited excellent diagnostic performance (AUC = 0.960). This finding is consistent with previous studies indicating that predictive models based on baseline data possess substantial clinical value [24]. The superiority of this combined model may be attributed to its ability to capture multiple pathophysiological dimensions, including metabolic regulation, inflammatory status, and vitamin D metabolism, enabling a more comprehensive assessment of disease risk.

From a mechanistic perspective, several pathways may underlie these associations. First, the association between 25(OH)D deficiency and MASLD is supported by multiple pathophysiologic mechanisms. Beyond its classic role in regulating calcium and phosphorus metabolism, vitamin D has been increasingly recognized as a regulator of insulin sensitivity, adipocyte differentiation, and chronic inflammation [15]. Vitamin D deficiency may exacerbate insulin resistance by modulating insulin receptor expression and impairing downstream signaling pathways. Additionally, vitamin D deficiency may promote macrophage infiltration into adipose tissue, leading to increased secretion of pro-inflammatory cytokines and further aggravation of hepatic fat deposition and inflammation [25]. Second, hs-CRP, as a sensitive marker of systemic inflammation, directly reflects the low-grade inflammatory state commonly observed in metabolic diseases [16]. In individuals with obesity, expanded adipose tissue secretes abundant pro-inflammatory cytokines, which stimulate hepatic hs-CRP production. Beyond serving as a biomarker, hs-CRP may also contribute to disease progression by promoting endothelial dysfunction and activating inflammatory pathways, thereby exacerbating insulin resistance and hepatic injury [26, 27].

Third, adiponectin, a key protective hormone secreted by adipocytes, exerts protective effects, including improving insulin sensitivity and exerting anti-atherogenic and anti-inflammatory actions. Adiponectin activates the AMPK pathway, promotes fatty acid oxidation, inhibits hepatic gluconeogenesis, and suppresses pro-inflammatory macrophage polarization. In obese patients with MASLD, significantly decreased adiponectin levels weaken these protective effects, thereby facilitating hepatic lipid accumulation and sustaining inflammatory responses [28-30].

Importantly, there exist complex pathophysiologic interactions among these three biomarkers. Vitamin D deficiency may downregulate adiponectin expression and secretion by affecting adipocyte differentiation, while simultaneously promoting inflammatory factor release and elevating hs-CRP levels. Conversely, chronic inflammation can impair vitamin D receptor expression and activity through oxidative stress-related pathways, further exacerbating vitamin D deficiency and insulin resistance. Consequently, their combined assessment may provide a more comprehensive and accurate reflection of the overall metabolic disturbance in patients with obesity than any single biomarker, which aligns with previous reports [25, 26].

Several limitations of this study should be acknowledged. First, this was a retrospective, single-center study with a relatively limited sample size, which may have introduced selection bias and limit the generalizability of our findings. Second, important lifestyle factors (e.g., dietary patterns, physical activity, and sun exposure) that may have influenced both biomarker levels and MASLD risk were not assessed, and unmeasured confounding cannot be excluded. Third, subgroup analyses based on disease severity were not performed, and

the relationship between these biomarkers and different stages of MASLD remain to be elucidated. Fourth, the diagnosis of MASLD was based on ultrasonography rather than liver biopsy, the gold standard, which may have limited sensitivity for detecting mild steatosis and does not allow accurate assessment of inflammation or fibrosis. Finally, the cross-sectional design precludes causal inferences and limits our ability to assess the prognostic value of these biomarkers for disease progression. Future prospective, longitudinal, multi-center studies with larger sample sizes and external validation are warranted to confirm our findings.

Conclusion

In this cross-sectional study, serum levels of 25(OH)D, hs-CRP, and adiponectin were significantly associated with MASLD in patients with severe obesity. The combination of these three biomarkers demonstrated good discriminatory performance for MASLD identification.

From a clinical perspective, this model may aid in the early identification of high-risk individuals and facilitate risk stratification in patients with obesity, thereby supporting more targeted monitoring and management strategies. Biomarker-based assessments may also provide a cost-effective approach for MASLD screening, particularly in primary care settings where advanced imaging modalities are not readily available.

Disclosure of conflict of interest

None.

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