

RESEARCH ARTICLE

OPEN ACCESS

Diagnostic performance of serum hepcidin, soluble transferrin receptor, and heme oxygenase-1 in metabolic-dysfunction-associated steatotic liver disease and steatohepatitis

Abed RK¹[ID](#), Abass HA²[ID](#), AL-Shammaa AS³[ID](#)

¹Al-Zahraa Teaching Hospital, Wasit Health Directorate, Ministry of Health, Baghdad, Republic of Iraq

²Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq

³Department of Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Submitted: 11th April 2026

Accepted: 12th June 2026

Published: 31st October 2026

[ID](#): Orcid ID

Abstract

Objective: Iron dysregulation and oxidative stress are central to the pathogenesis of metabolic dysfunction-associated steatotic liver disease (MASLD) and its progression to steatohepatitis (MASH). This study evaluated the diagnostic utility of serum hepcidin, soluble transferrin receptor (sTfR), and heme oxygenase-1 (HO-1) in patients with MASLD and MASH.

Methods: A case-control study enrolled 120 adults aged 30–60 years, divided equally into simple steatosis (MASLD), MASH (assessed by FibroScan), and matched healthy controls. Serum hepcidin, sTfR, and HO-1 were quantified using sandwich ELISA. Diagnostic accuracy was assessed with receiver operating characteristic (ROC) curves, and independent associations were examined using Firth penalised logistic regression adjusted for age, body mass index (BMI), HbA1c, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).

Results: Hepcidin, sTfR, and HO-1 concentrations increased stepwise from controls to MASLD and MASH (all $p < 0.001$). For discriminating MASLD from controls, sTfR and HO-1 achieved areas under the curve (AUC) of 1.000 and 0.986, respectively. For differentiating MASH from controls, HO-1 (AUC = 1.000) and sTfR (AUC = 0.999) showed excellent performance. Multivariable analysis confirmed sTfR as an independent predictor of the MASLD spectrum after adjusting for metabolic risk factors.

Conclusions: Hepcidin, sTfR, and HO-1 are promising non-invasive biomarkers for MASLD and MASH, reflecting underlying iron dysregulation. However, the near-perfect diagnostic accuracy observed in this single-centre discovery cohort requires external validation before clinical application.

Keywords: Iron Overload, Biological Markers, Oxidative Stress, Insulin Resistance, Non-alcoholic Fatty Liver Disease

Plain English Summary

Metabolically-dysfunction-associated steatotic liver disease (MASLD) is a common condition where fat builds up in the liver. In some people, this fat causes inflammation and liver damage, a more serious stage called steatohepatitis (MASH). Currently, doctors often need liver biopsies or special scans to tell these stages apart. This study examined whether three blood markers, hepcidin, soluble transferrin receptor (sTfR), and heme oxygenase-1 (HO-1), could help distinguish between simple fatty liver and

Correspondence:

Abed Rehab K

Al-Zahraa Teaching Hospital, Wasit Health Directorate

Ministry of Health, Baghdad

Republic of Iraq

rehab.kadim.med25@nahrainuniv.edu.iq

the more dangerous inflammatory form. The researchers measured these markers in 120 adults: 40 with simple fatty liver, 40 with MASH, and 40 healthy volunteers. All three markers were higher in people with liver disease, and the levels rose as the disease became more severe. The markers were very good at telling healthy people from those with liver disease. However, because this was a relatively small study from a single hospital, these promising results need to be confirmed in larger, more diverse groups of patients before the markers can be used in routine clinical practice.

Introduction

Metabolically-dysfunction-associated steatotic liver disease (MASLD), previously termed non-alcoholic fatty liver disease (NAFLD), has emerged as one of the most prevalent chronic liver conditions worldwide, posing a substantial global health burden (1). The disease encompasses a histological spectrum ranging from simple hepatic steatosis to metabolic dysfunction-associated steatohepatitis (MASH), which can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (2). The rising global incidence of obesity, type 2 diabetes mellitus, insulin resistance, and metabolic syndrome has fuelled the increasing prevalence of MASLD and MASH across diverse populations (3).

The pathogenesis of MASLD is complex and multifactorial, involving metabolic dysregulation, chronic low-grade inflammation, oxidative stress, mitochondrial dysfunction, and disturbances in both lipid and iron metabolism (4). Among these mechanisms, iron dysregulation has garnered increasing attention because excess iron accumulation may potentiate the generation of reactive oxygen species (ROS), leading to lipid peroxidation, cellular damage, inflammation, and ultimately fibrosis progression (5). Understanding the role of iron-related pathways could open avenues for non-invasive diagnostic biomarkers. Hepcidin is the master regulator of systemic iron homeostasis, controlling intestinal iron absorption and tissue iron distribution (6). Altered hepcidin expression has been documented in inflammatory and metabolic liver disorders, including MASLD and MASH. Chronic hepatic inflammation and metabolic dysfunction may impair iron regulation, leading to abnormal hepcidin levels and hepatic iron overload (7).

The soluble transferrin receptor (sTfR) is another key marker of iron metabolism that reflects cellular iron demand and erythropoietic activity (8). Unlike ferritin, sTfR is less affected by inflammatory conditions, making it a potentially reliable biomarker for assessing iron status in chronic liver diseases. Elevated sTfR levels may indicate altered iron utilisation and metabolic stress in patients with MASLD and MASH (9).

Oxidative stress is widely recognised as a critical driver of the transition from simple steatosis to steatohepatitis. Heme oxygenase-1 (HO-1) is a stress-inducible enzyme involved in heme degradation and cellular protection against oxidative injury (10). Increased HO-1 expression

has been linked to oxidative stress and inflammatory responses in liver diseases, suggesting its potential as a biomarker in MASLD and MASH (11).

Despite advances in understanding MASLD and MASH pathogenesis, there remains a pressing need for reliable non-invasive biomarkers that can improve disease diagnosis and staging. Liver biopsy, the current reference standard, is invasive, costly, and subject to sampling variability (12). Non-invasive scores such as FIB-4 and NFS have moderate accuracy but perform better for ruling out advanced fibrosis than for diagnosing steatohepatitis (13). Therefore, the present study aimed to evaluate the diagnostic performance of hepcidin, sTfR, and HO-1 in patients with MASLD and MASH and to explore their potential role as biomarkers reflecting iron dysregulation and oxidative stress.

Materials and Methods

Study design and setting

This case-control study was conducted at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, in collaboration with Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq, between November 2025 and March 2026.

Participants

A total of 120 adults aged 30–60 years were enrolled and divided equally into three groups (n = 40 each):

Group I (MASLD)

Patients with simple steatosis without fibrosis, confirmed by FibroScan (controlled attenuation parameter [CAP] ≥ 248 dB/m and liver stiffness measurement [LSM] < 8 kPa)

Group II (MASH)

Patients with steatohepatitis/fibrosis, confirmed by FibroScan (CAP ≥ 280 dB/m and LSM ≥ 8 kPa)

Group III (Control)

Healthy volunteers matched for age and sex, with normal liver biochemistry and no evidence of hepatic steatosis on ultrasound

Sample size consideration

A post hoc power calculation indicated that, with 40 participants per group, the study had 80%

power to detect a moderate effect size (Cohen's $d = 0.65$) at $\alpha = 0.05$ for the primary comparison of biomarker levels between groups. The sample size was practically determined by the recruitment capacity during the study period.

Inclusion and exclusion criteria

Inclusion criteria

Adults aged 30–60 years of either sex; non-alcoholic individuals (alcohol intake < 20 g/day for women and < 30 g/day for men); provision of written informed consent.

Exclusion criteria

Alcohol consumption exceeding the stated limits; viral hepatitis (positive HBsAg or anti-HCV); chronic renal failure; systemic inflammatory diseases; haemolytic anaemia; malignancy; autoimmune liver disease; drug-induced hepatitis; pregnancy or lactation; blood transfusion or iron supplementation within three months before sampling.

Clinical and anthropometric assessment

A detailed medical history was obtained from each participant. Anthropometric measurements, including body mass index (BMI, calculated as weight in kilograms divided by height in metres squared) and waist circumference (measured at the midpoint between the lowest rib and the iliac crest), were recorded.

Liver stiffness and steatosis were assessed using FibroScan (Echosens, Paris, France) with an M or XL probe according to the manufacturer's recommendations. All FibroScan examinations were performed after an overnight fast by a single experienced operator who was blinded to the clinical and laboratory data. A minimum of ten valid measurements was required, with an interquartile range/median ratio < 30%.

Laboratory measurements

Venous blood samples (5 mL) were collected after an overnight fast. Serum was separated by centrifugation at 4000 rpm for 10 minutes and stored at -80°C until analysis. The following parameters were measured:

Liver function tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin, and albumin using an automated biochemistry analyser (Cobas c311, Roche Diagnostics, Mannheim, Germany)

Metabolic markers

Fasting blood glucose, lipid profile (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol), serum iron, total iron-binding

capacity (TIBC), transferrin saturation (calculated as serum iron/TIBC $\times 100$), and insulin (ELISA kit, Diametra, Italy). HbA1c was measured from EDTA-anticoagulated whole blood using high-performance liquid chromatography (Bio-Rad D-10, Hercules, CA, USA). HOMA-IR was calculated as [fasting glucose (mg/dL) $\times$ fasting insulin ($\mu\text{IU/mL}$)] / 405.

Study biomarkers

Serum hepcidin, sTfR, and HO-1 were quantified using commercially available sandwich ELISA kits (Elabscience, Wuhan, China, for hepcidin and sTfR; Cusabio, Houston, TX, USA, for HO-1) according to the manufacturers' protocols. All samples were run in duplicate, and the mean values were used for analysis. Intra-assay coefficients of variation were < 8% for all assays.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) and SPSS version 26 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean $\pm$ standard deviation (SD). Normality was assessed using the Shapiro–Wilk test. Comparisons across three groups were performed using one-way ANOVA with Tukey's post-hoc test (for normally distributed variables) or the Kruskal–Wallis test with Dunn's post-hoc test (for non-normal variables). Categorical variables were compared using the chi-square test.

Diagnostic performance was evaluated by receiver operating characteristic (ROC) curve analysis; the area under the ROC curve (AUC), 95% confidence interval (CI), optimal cut-off value (determined by the Youden index), sensitivity, and specificity were reported. Because of the small sample size and the potential for separation in logistic regression (particularly with perfectly discriminating biomarkers), Firth penalised logistic regression was used to assess independent associations between candidate biomarkers and the MASLD spectrum (combined MASLD + MASH groups vs. controls) after adjustment for age, BMI, HbA1c, and HOMA-IR. Adjusted odds ratios (ORs) and 95% CIs are reported. All tests were two-sided, and a p-value < 0.05 was considered statistically significant.

Because the study generated a large number of correlation coefficients, these analyses were considered exploratory, and no multiplicity adjustment was applied; thus, the correlation results should be interpreted cautiously.

Results

Demographic and anthropometric characteristics

Sex distribution did not differ significantly among the control, MASLD, and MASH groups (male: 62.5%, 72.5%, and 62.5%, respectively; $p = 0.553$). Mean age was higher in the MASLD (45.4 ± 10.0 years) and MASH (46.1 ± 10.1 years) groups than in controls (38.6 ± 8.9 years), but this difference was not statistically significant ($p =$

0.14). BMI showed no significant difference across groups (control: 27.5 ± 2.5 kg/m²; MASLD: 28.8 ± 2.1 kg/m²; MASH: 28.6 ± 2.8 kg/m²; $p = 0.085$). In contrast, waist circumference was markedly higher in the MASLD (101.4 ± 8.4 cm) and MASH (95.3 ± 11.7 cm) groups compared with controls (77.8 ± 7.7 cm; $p < 0.001$) (Table 1).

Table 1: Demographic and anthropometric characteristics of the study groups

Parameter	Control (n=40)	MASLD (n=40)	MASH (n=40)	p-value
Male, n (%)	25 (62.5%)	29 (72.5%)	25 (62.5%)	0.553
Female, n (%)	15 (37.5%)	11 (27.5%)	15 (37.5%)	
Age (years), mean $\pm$ SD	38.6 ± 8.9	45.4 ± 10.0	46.1 ± 10.1	0.14
BMI (kg/m ²), mean $\pm$ SD	27.5 ± 2.5	28.8 ± 2.1	28.6 ± 2.8	0.085
Waist circumference (cm), mean $\pm$ SD	77.8 ± 7.7	101.4 ± 8.4	95.3 ± 11.7	<0.001

Biochemical and metabolic parameters

As shown in Table 2, participants with MASLD and MASH exhibited significant disturbances in lipid and glucose metabolism compared with controls. Total cholesterol increased from 168.9 ± 18.9 mg/dL in controls to 191.0 ± 32.5 mg/dL in MASLD and 189.0 ± 29.9 mg/dL in MASH ($p < 0.001$). Triglycerides were substantially higher in MASLD (170.4 ± 46.1 mg/dL) and MASH (169.7 ± 55.5 mg/dL) than in controls (100.5 ± 30.9 mg/dL; $p < 0.001$). HDL-cholesterol was lower, and LDL-cholesterol was higher, in both disease groups. Fasting blood glucose, fasting insulin,

HOMA-IR, and HbA1c were all significantly elevated in MASLD and MASH compared with controls ($p < 0.001$ for each).

Liver enzymes (ALT, AST, GGT) and total bilirubin were higher in the disease groups, while albumin levels were lower in MASH (39.8 ± 5.6 g/L) compared with controls (42.3 ± 5.2 g/L; $p < 0.001$), indicating more advanced hepatocellular injury. Serum iron and transferrin saturation were higher in MASLD and MASH, whereas TIBC was lower, particularly in MASH (272.3 ± 47.0 μ g/dL vs. 330.1 ± 51.2 μ g/dL in controls; $p = 0.0013$).

Table 2: Biochemical parameters across study groups

Parameter	Control (n=40)	MASLD (n=40)	MASH (n=40)	p-value
Total cholesterol (mg/dL)	168.9 ± 18.9	191.0 ± 32.5	189.0 ± 29.9	<0.001
Triglycerides (mg/dL)	100.5 ± 30.9	170.4 ± 46.1	169.7 ± 55.5	<0.001
HDL-cholesterol (mg/dL)	56.9 ± 13.9	45.9 ± 11.0	42.1 ± 11.3	<0.001
LDL-cholesterol (mg/dL)	85.6 ± 11.7	113.3 ± 24.3	109.0 ± 26.0	<0.001
Fasting glucose (mg/dL)	90.5 ± 12.2	117.1 ± 36.2	103.8 ± 24.8	<0.001
Fasting insulin (μ U/mL)	6.84 ± 1.70	13.78 ± 5.06	13.97 ± 4.32	<0.001
HOMA-IR	1.55 ± 0.51	3.68 ± 1.12	3.40 ± 0.89	<0.001
HbA1c (%)	5.64 ± 0.44	6.41 ± 0.98	6.33 ± 0.97	<0.001
ALT (U/L)	19.2 ± 8.0	42.8 ± 16.7	47.4 ± 20.4	<0.001
AST (U/L)	17.0 ± 6.5	25.1 ± 7.8	37.8 ± 15.4	<0.001
Albumin (g/L)	42.3 ± 5.2	42.1 ± 4.1	39.8 ± 5.6	<0.001
Total bilirubin (mg/dL)	0.77 ± 0.26	0.91 ± 0.23	0.92 ± 0.73	<0.001
GGT (U/L)	25.8 ± 6.9	28.9 ± 10.1	35.6 ± 9.9	<0.001
Serum iron (μ g/dL)	89.9 ± 23.7	105.0 ± 37.9	105.7 ± 60.6	0.007
TIBC (μ g/dL)	330.1 ± 51.2	311.3 ± 53.1	272.3 ± 47.0	0.0013
Transferrin saturation (%)	28.8 ± 11.2	36.9 ± 18.9	37.6 ± 18.4	0.0024

Data are mean $\pm$ SD

Candidate biomarker levels across groups

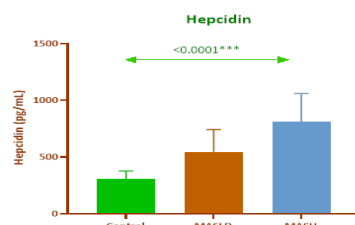
A clear stepwise and highly significant increase in all three candidate biomarkers was observed across the control, MASLD, and MASH groups (Table 3 and Figures 1–3). Serum hepcidin rose from 306.2 ± 70.7 pg/mL in controls to 545.1 ± 196.9 pg/mL in MASLD and 812.3 ± 246.9 pg/mL

in MASH ($p < 0.001$). HO-1 increased from 2.63 ± 1.02 ng/mL in controls to 5.91 ± 1.28 ng/mL in MASLD and 7.74 ± 2.04 ng/mL in MASH ($p < 0.001$). sTfR increased from 48.3 ± 16.4 ng/mL in controls to 129.5 ± 25.1 ng/mL in MASLD and 150.4 ± 35.7 ng/mL in MASH ($p < 0.001$).

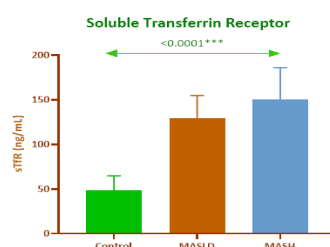
Table 3: Serum biomarker concentrations

Biomarker	Control (n=40)	MASLD (n=40)	MASH (n=40)	p-value
Hepcidin (pg/mL)	306.2 ± 70.7	545.1 ± 196.9	812.3 ± 246.9	<0.001
HO-1 (ng/mL)	2.63 ± 1.02	5.91 ± 1.28	7.74 ± 2.04	<0.001
sTfR (ng/mL)	48.3 ± 16.4	129.5 ± 25.1	150.4 ± 35.7	<0.001

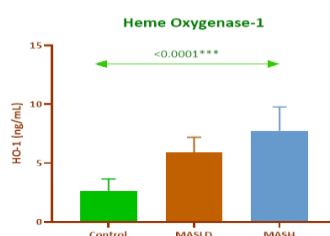
Data are mean ± SD

**Figure 1: Box and whisker plot of serum hepcidin concentrations in controls, MASLD, and MASH groups**

Horizontal lines indicate medians; boxes show interquartile ranges; whiskers extend to 5th and 95th percentiles

**Figure 2: Box-and-whisker plot of serum sTfR concentrations**

Horizontal lines indicate medians; boxes show interquartile ranges; whiskers extend to 5th and 95th percentiles

**Figure 3: Box-and-whisker plot of serum HO-1 concentrations**

Horizontal lines indicate medians; boxes show interquartile ranges; whiskers extend to 5th and 95th percentiles

Diagnostic accuracy for discriminating MASLD from controls

ROC analysis demonstrated excellent discriminatory performance of the three biomarkers for distinguishing MASLD from healthy controls (Table 4, Figures 4–6). sTfR achieved perfect discrimination in this dataset, with an AUC of 1.000 (95% CI: 1.00–1.00); the optimal cut-off value was ≥ 94.3 ng/mL, yielding

100.0% sensitivity and 100.0% specificity ($p < 0.001$). HO-1 also showed outstanding performance, with an AUC of 0.986 (95% CI: 0.96–1.00), a cut-off of ≥ 4.185 ng/mL, 97.5% sensitivity, and 92.5% specificity ($p < 0.001$). Hepcidin exhibited very good discriminatory ability, with an AUC of 0.944 (95% CI: 0.89–0.98), a cut-off of ≥ 397.1 pg/mL, 90.0% sensitivity, and 87.5% specificity ($p < 0.001$).

Table 4: ROC analysis for MASLD vs. controls

Biomarker	AUC	95% CI	Optimal cut-off	Sensitivity (%)	Specificity (%)	p-value
Hepcidin (pg/mL)	0.944	0.89–0.98	≥ 397.1	90	87.5	<0.001
HO-1 (ng/mL)	0.986	0.96–1.00	≥ 4.185	97.5	92.5	<0.001
sTfR (ng/mL)	1	1.00–1.00	≥ 94.3	100	100	<0.001

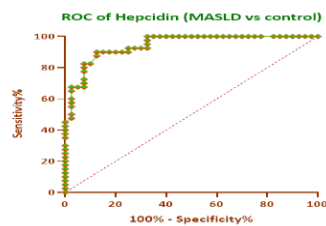


Figure 4: ROC curve for hepcidin discriminating MASLD from controls

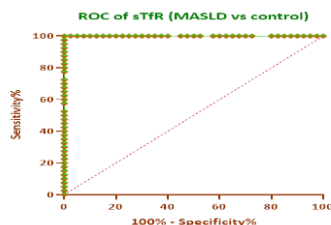


Figure 5: ROC curve for sTfR discriminating MASLD from controls

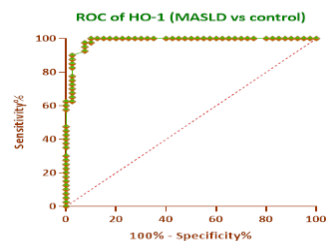


Figure 6: ROC curve for HO-1 discriminating MASLD from controls

Diagnostic accuracy for discriminating MASH from controls

All three biomarkers exhibited excellent diagnostic utility for differentiating MASH from healthy controls (Table 5, Figures 7–9). HO-1 provided perfect discrimination in this cohort, with an AUC of 1.000 (95% CI: 1.00–1.00); the optimal cut-off value was ≥ 5.45 ng/mL, yielding 100.0% sensitivity and 100.0% specificity ($p <$

0.001). sTfR displayed near-perfect performance, with an AUC of 0.999 (95% CI: 0.99–1.00), a cut-off of ≥ 95.5 ng/mL, 97.5% sensitivity, and 100.0% specificity ($p <$ 0.001). Hepcidin also showed excellent accuracy, with an AUC of 0.958 (95% CI: 0.89–0.98), a cut-off of ≥ 489.0 pg/mL, 90.0% sensitivity, and 100.0% specificity ($p <$ 0.001).

Table 5: ROC analysis for MASH vs. controls

Biomarker	AUC	95% CI	Optimal cut-off	Sensitivity (%)	Specificity (%)	p-value
Hepcidin (pg/mL)	0.958	0.89–0.98	≥ 489.0	90	100	<0.001
HO-1 (ng/mL)	1	1.00–1.00	≥ 5.45	100	100	<0.001
sTfR (ng/mL)	0.999	0.99–1.00	≥ 95.5	97.5	100	<0.001

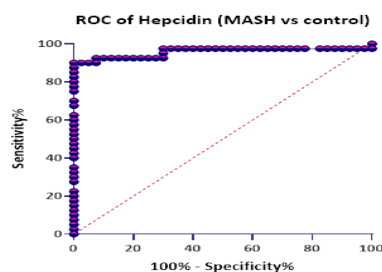


Figure 7: ROC curve for hepcidin discriminating MASH from controls

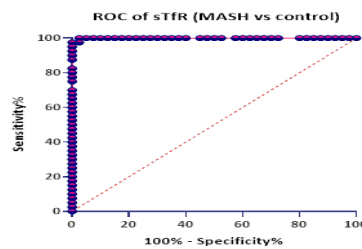


Figure 8: ROC curve for sTfR discriminating MASH from controls

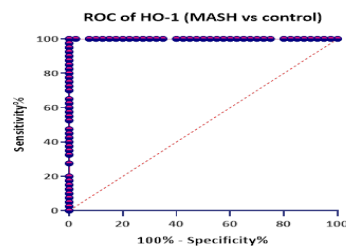


Figure 9: ROC curve for HO-1 discriminating MASH from controls.

Correlation analyses (exploratory)

Exploratory Pearson correlation analyses revealed clustering of liver injury markers in the MASLD and MASH groups. In the MASLD group, ALT correlated strongly with GGT ($r = 0.723$, $p < 0.001$), and AST correlated with GGT ($r = 0.748$, $p < 0.001$). Total cholesterol showed a very strong correlation with LDL-cholesterol ($r = 0.846$, $p < 0.001$) and with transferrin saturation ($r = 0.890$, $p < 0.001$). BMI was positively associated with LDL-cholesterol ($r = 0.539$, $p = 0.003$) and total cholesterol ($r = 0.390$, $p = 0.012$). In the MASH group, sTfR correlated positively with hepcidin ($r = 0.634$, $p < 0.001$) and with HO-1 ($r = 0.356$, $p = 0.02$), supporting the biological interconnection of iron and oxidative stress pathways. A full correlation matrix is available from the corresponding author upon request.

Multivariable analysis

Firth penalised logistic regression was used to evaluate whether the candidate biomarkers remained associated with the MASLD spectrum (combined MASLD + MASH vs. controls) after adjustment for age, BMI, HbA1c, and HOMA-IR. As shown in Table 6, sTfR demonstrated an independent positive association (adjusted OR = 1.041, 95% CI: 1.000–1.082, $p = 0.048$). Hepcidin showed a modest inverse adjusted association (adjusted OR = 0.992, 95% CI: 0.985–1.000, $p = 0.045$), which may reflect overlap among correlated iron-related pathways. HO-1 did not retain statistical significance after adjustment (adjusted OR = 1.788, 95% CI: 0.750–4.264, $p = 0.190$). None of the clinical covariates (age, BMI, HbA1c, HOMA-IR) reached statistical significance in the fully adjusted model.

Table 6: Firth penalised multivariable logistic regression (MASLD spectrum vs. controls)

Parameter	Adjusted OR	95% CI	p-value
Hepcidin (pg/mL)	0.992	0.985–1.000	0.045
HO-1 (ng/mL)	1.788	0.750–4.264	0.19
sTfR (ng/mL)	1.041	1.000–1.082	0.048
Age (years)	0.982	0.867–1.113	0.781
BMI (kg/m ²)	1.121	0.776–1.619	0.543
HbA1c (%)	1.594	0.524–4.843	0.411
HOMA-IR	1.856	0.555–6.207	0.316

OR = odds ratio; CI = confidence interval

Discussion

This study evaluated the diagnostic performance of three serum biomarkers – hepcidin, sTfR, and HO-1 – in patients with MASLD and MASH. The principal findings are: (i) all three biomarkers increased significantly and stepwise from healthy controls to MASLD and then to MASH; (ii) they exhibited excellent to near-perfect discriminatory

accuracy in this single-centre discovery cohort; and (iii) sTfR remained independently associated with the MASLD spectrum after adjustment for conventional metabolic risk factors.

Metabolic disturbances and disease progression

The significantly higher fasting glucose, insulin, HOMA-IR, and HbA1c levels in the disease

groups confirm that insulin resistance is a core pathogenic feature of both MASLD and MASH. This aligns with the work of Jung and colleagues, who emphasised that MASLD is tightly linked to insulin resistance and that obesity-driven inflammation and lipotoxicity create a self-reinforcing cycle connecting fatty liver disease with type 2 diabetes (14). The biochemical pattern of elevated ALT, AST, GGT, and bilirubin, together with lower albumin in MASH, reflects progressive hepatocellular injury and worsening synthetic function as disease severity increases. Similar findings have been reported in large population-based studies (15, 16).

Iron metabolism abnormalities

Higher serum iron and transferrin saturation with lower TIBC in MASH suggest increased circulating iron availability combined with reduced binding reserve. While some studies have reported different patterns (17). These discrepancies underscore the complexity of iron metabolism in steatotic liver diseases, where markers may vary according to disease stage, inflammation, nutritional status, and whether serum or tissue parameters are measured (18, 19). Our findings are consistent with the concept that iron handling becomes increasingly abnormal as MASLD progresses towards MASH.

Biomarker patterns and biological plausibility

The progressive increase in hepcidin from controls to MASLD to MASH suggests an inflammatory and iron-regulatory response accompanying disease progression. Hepcidin, the master iron hormone, is induced by inflammation and iron overload (6). MASH, characterised by greater inflammatory burden than simple steatosis, would be expected to drive higher hepcidin expression (7). HO-1 elevation across the spectrum strongly supports escalating oxidative stress and hepatocellular injury (10, 11). Malaguarnera and colleagues previously reported higher HO-1 levels in NASH/MASH compared with simple steatosis (20). sTfR, reflecting cellular iron demand, also rose progressively, indicating altered iron utilisation at the cellular level in advancing disease (8, 9).

Diagnostic accuracy in context

The very high AUC values observed in this study (up to 1.000 for some comparisons) are striking. However, they must be interpreted with considerable caution. While several recently developed biomarker panels have shown strong performance, for example, the N3-MASH panel reported an AUROC of 0.954 for discriminating MASLD from controls (21), perfect discrimination in a single, relatively small case-control study is

unlikely to be replicated in real-world, heterogeneous populations (12, 13). The current results should therefore be viewed as hypothesis-generating and promising, requiring external validation in larger, multi-centre cohorts with histological reference standards.

Independent associations after adjustment

The multivariable analysis showed that sTfR remained a significant independent predictor of the MASLD spectrum after adjustment for age, BMI, HbA1c, and HOMA-IR. This suggests that sTfR captures disease-relevant information not fully explained by conventional metabolic risk factors, consistent with recent Mendelian randomisation studies showing that iron-related variables are associated with MASLD risk independently of obesity (22, 23). The modest inverse association for hepcidin after adjustment likely reflects complex interactions among correlated iron pathways and should not be overinterpreted.

Study limitations and future directions

Several limitations warrant consideration. First, this was a single-centre case-control study with a relatively modest sample size (40 per group). Second, the absence of liver biopsy as a reference standard is an important limitation; FibroScan provides validated surrogate measures of steatosis and fibrosis, but cannot definitively confirm steatohepatitis (24). Third, the near-perfect AUC values almost certainly represent overfitting; optimism-corrected estimates (e.g., via bootstrap resampling) or, ideally, validation in an independent external cohort would be necessary before any claim of clinical readiness. Fourth, the control group had a mean BMI in the overweight range and slightly elevated HOMA-IR, which may have underestimated true biomarker differences. Fifth, the exploratory correlation analyses were not adjusted for multiple comparisons, and some significant findings may be spurious. Sixth, the unit scaling for ORs was not standardised; future studies should report ORs per clinically meaningful increments or per standard deviation change.

Future research should prioritise: (i) external validation in larger, multi-ethnic cohorts with biopsy-proven MASLD/MASH; (ii) assessment of these biomarkers in combination with established non-invasive scores (FIB-4, NFS, ELF) to determine incremental value; (iii) evaluation of their ability to predict disease progression longitudinally; and (iv) investigation of whether these markers can guide patient selection for emerging pharmacotherapies for MASH.

Conclusion

Serum hepcidin, sTfR, and HO-1 are promising non-invasive biomarkers that reflect distinct but interconnected pathophysiological mechanisms – iron dysregulation and oxidative stress, in MASLD and MASH. Their stepwise elevation across the disease spectrum and retained significance in multivariable models suggest mechanistic relevance. However, the exceptionally high diagnostic accuracy observed in this discovery cohort is likely overestimated and requires independent external validation. At present, these biomarkers should not replace established diagnostic pathways but may, after further validation, complement existing non-invasive tests to improve disease stratification and reduce reliance on liver biopsy.

List of Abbreviations

ALT: Alanine aminotransferase
 AST: Aspartate aminotransferase
 AUC: Area under the curve
 BMI: Body mass index
 CAP: Controlled attenuation parameter
 CI: Confidence interval
 ELISA: Enzyme-linked immunosorbent assay
 GGT: Gamma glutamyl transferase
 HbA1c: Glycated haemoglobin
 HDL: High-density lipoprotein
 HO-1: Heme oxygenase 1
 HOMA-IR: Homeostatic Model Assessment for Insulin Resistance
 LDL: Low-density lipoprotein
 LSM: Liver stiffness measurement
 MASH: Metabolic dysfunction associated steatohepatitis
 MASLD: Metabolically associated steatotic liver disease
 NAFLD: Non-alcoholic fatty liver disease
 OR: Odds ratio
 ROC: Receiver operating characteristic
 sTfR: Soluble transferrin receptor
 TIBC: Total iron binding capacity

Declaration

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of Al-Nahrain University College of Medicine (approval number: IRB/COM/2025/112, dated 15 October 2025). Written informed consent was obtained from all individual participants included in the study.

Consent for Publication

All authors gave their explicit consent for the publication of this work under the Creative Commons Attribution Non-Commercial 4.0 International license.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request, subject to institutional data sharing policies and patient confidentiality restrictions.

Competing Interests

The authors declare that they have no competing interests. No pharmaceutical or commercial entity provided funding or had any role in the study design, data collection, analysis, interpretation, or manuscript preparation.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The study was supported by institutional resources from Al-Nahrain University and Al-Zahraa Teaching Hospital.

Author Contributions

ARK worked on the conceptualisation, investigation, data curation, writing the original draft and project administration.

AHA worked on the methodology, formal analysis, validation, writing the review and editing.

AAS worked on the conceptualisation, supervision, resources, final approval, writing the review and editing.

Acknowledgements

The authors are extremely grateful to the medical and laboratory staff of Al-Imamain Al-Kadhimain Teaching Medical City in Baghdad, Iraq, for their invaluable assistance with patient recruitment and sample collection. We also thank all the patients and healthy volunteers who participated in this study.

References

1. Eslam M, Sanyal AJ, George J, Sanyal A, Neuschwander-Tetri B, Tiribelli C, Kleiner DE, Brunt E, Bugianesi E, Yki-Järvinen H, Grønbaek H. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology*. 2020 May 1;158(7):1999-2014.
<https://doi.org/10.1053/j.gastro.2019.11.312>
2. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, Arrese M. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Annals of Hepatology*. 2024 Jan 1;29(1):101133.
<https://doi.org/10.1016/j.aohep.2023.101133>

3. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*. 2023 Apr 1;77(4):1335-1347. <https://doi.org/10.1097/HEP.0000000000000004>
4. Miller DM, McCauley KF, Dunham-Snary KJ. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): Mechanisms, Clinical Implications and Therapeutic Advances. *Endocrinology, Diabetes & Metabolism*. 2025 Nov;8(6):e70132. <https://doi.org/10.1002/edm2.70132>
5. Genslueckner S, Wernly B, Datz C, Aigner E. Iron, oxidative stress, and metabolic dysfunction—associated steatotic liver disease. *Antioxidants*. 2024 Feb 7;13(2):208. <https://doi.org/10.3390/antiox13020208>
6. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2012 Sep 1;1823(9):1434-43. <https://doi.org/10.1016/j.bbamcr.2012.01.014>
7. Chen WE, Wang X, Huang LI, Liu BO. Hepcidin in non-alcoholic fatty liver disease regulated by the TLR4/NF-κB signaling pathway. *Experimental and Therapeutic Medicine*. 2016 Jan;11(1):73-6. <https://doi.org/10.3892/etm.2015.2873>
8. Skikne BS. Serum transferrin receptor. *American Journal of Hematology*. 2008 Nov;83(11):872-5. <https://doi.org/10.1002/ajh.21279>
9. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. *Clinica Chimica Acta*. 2003 Mar 1;329(1-2):9-22. [https://doi.org/10.1016/S0009-8981\(03\)00005-6](https://doi.org/10.1016/S0009-8981(03)00005-6)
10. Rytter SW, Choi AM. Heme oxygenase-1/carbon monoxide: from metabolism to molecular therapy. *American Journal of Respiratory Cell and Molecular Biology*. 2009 Sep;41(3):251-60. <https://doi.org/10.1165/rcmb.2009-0170TR>
11. Malaguarnera L, Madeddu R, Palio E, Arena N, Malaguarnera M. Heme oxygenase-1 levels and oxidative stress-related parameters in non-alcoholic fatty liver disease patients. *Journal of Hepatology*. 2005 Apr 1;42(4):585-91. <https://doi.org/10.1016/j.jhep.2004.11.040>
12. Abdelhameed F, Kite C, Lagojda L, Dallaway A, Chatha KK, Chaggar SS, Dalamaga M, Kassi E, Kyrou I, Randeva HS. Non-invasive scores and serum biomarkers for fatty liver in the era of metabolic dysfunction-associated steatotic liver disease (MASLD): a comprehensive review from NAFLD to MAFLD and MASLD. *Current Obesity Reports*. 2024 Sep;13(3):510-31. <https://doi.org/10.1007/s13679-024-00574-z>
13. Marks LM, Jensen T, DeGrado TR. Noninvasive testing in the diagnosis of metabolic dysfunction-associated steatohepatitis. *Metabolism and Target Organ Damage*. 2024 Aug 22;4(3):N-A. <https://doi.org/10.20517/mtod.2024.44>
14. Jung I, Koo DJ, Lee WY. Insulin resistance, non-alcoholic fatty liver disease and type 2 diabetes mellitus: clinical and experimental perspective. *Diabetes & Metabolism Journal*. 2024 Feb 2;48(3):327-39. <https://doi.org/10.4093/dmj.2023.0350>
15. Chen H, Chen Z, Bai X, Li Z, Huang S, Lu D, Li J, Wang Y, Han L, Xia K, Huang S. Metabolic and hepatic biomarkers associated with MASLD in the Chinese population. *Scientific Reports*. 2025 Aug 27;15(1):31593. <https://doi.org/10.1038/s41598-025-17219-3>
16. Lekakis V, Papatheodoridis GV. Natural history of metabolic dysfunction-associated steatotic liver disease. *European Journal of Internal Medicine*. 2024 Apr 1;122:3-10. <https://doi.org/10.1016/j.ejim.2023.11.005>
17. Tan L, Zhou Q, Liu J, Liu Z, Shi R. Association of iron status with non-alcoholic fatty liver disease and liver fibrosis in US adults: a cross-sectional study from NHANES 2017–2018. *Food & Function*. 2023;14(12):5653-62. <https://doi.org/10.1039/D2FO04082D>
18. Zhou M, Lv J, Chen X, Shi Y, Chao G, Zhang S. From gut to liver: Exploring the crosstalk between gut-liver axis and oxidative stress in metabolic dysfunction-associated steatotic liver disease. *Annals of Hepatology*. 2025 Jan 1;30(1):101777. <https://doi.org/10.1016/j.aohep.2025.101777>
19. Bai W, Zhu Z. Multiorgan crosstalk in MASLD/MASH: from hepatic pathogenesis to systemic complications. *Frontiers in Endocrinology*. 2025 Dec 18;16:1720780. <https://doi.org/10.3389/fendo.2025.1720780>
20. Malaguarnera L, Madeddu R, Palio E, Arena N, Malaguarnera M. Heme oxygenase-1 levels and oxidative stress-related parameters in non-alcoholic fatty liver disease patients. *Journal of Hepatology*. 2005 Apr 1;42(4):585-91. <https://doi.org/10.1016/j.jhep.2004.11.040>
21. Zhang X, Zheng MH, Liu D, Lin Y, Song SJ, Chu ES, Liu D, Singh S, Berman M, Lau HC, Gou H. A blood-based biomarker panel for non-invasive diagnosis of metabolic dysfunction-associated steatohepatitis. *Cell Metabolism*. 2025 Jan 7;37(1):59-68. <https://doi.org/10.1016/j.cmet.2024.10.008>
22. Zeng Y, Wang X, Liao S, Li C, Chen J, He H. Iron Homeostasis as a Mediator Linking

- Central Obesity with MASLD and Primary Liver Cancer: A Two-Step Mendelian Randomization Study. *Biomedicines*. 2025 Jul 4;13(7):1641.
<https://doi.org/10.3390/biomedicines13071641>
23. Guo W, Weng T, Song Y. Association of serum iron status with MASLD and liver fibrosis. *Plos One*. 2025 Apr 1;20(4):e0319057.
<https://doi.org/10.1371/journal.pone.0319057>
24. Alkindy M, Alareedh M, Gataa Z. Hepatic steatosis and fibrosis in patients with thalassemia major: A cross-sectional study using transient elastography. *Babcock University Medical Journal*. 2025 Dec 31;8(2):518-23.
<https://doi.org/10.38029/babcockuniv.med.j..v8i2.1232>