



Association of Oxidative Stress and Inflammatory Biomarkers with Liver Enzyme Alterations in Patients with Non-Alcoholic Fatty Liver Disease

1Sama Amer Abbas El-Tekreti, 2Yousif khalid hameed, 3Samar Abdulwahab Abdulla

1 College of Health and Medical Techniques Al-Dour, Northern Technical University Iraq 2 Tikrit University, college of medicine, Tikrit, Iraq 3 College of Science, Diyala University, Diyala, Iraq

Article Information

Received: 4-8-2026

Accepted: 28-8-2026

Published: 1-10-2026

Abstract

Background: Oxidative stress and chronic inflammation contribute to hepatocellular injury in NAFLD, potentially explaining altered liver enzymes and progressive disease severity. **Aims of the study:** This study aimed to evaluate oxidative-stress and inflammatory biomarkers in patients with non-alcoholic fatty liver disease and determine their relationships with liver-enzyme alterations. It also assessed whether MDA, SOD, GSH, hs-CRP, IL-6, and TNF- α were independently associated with ALT levels after adjustment for relevant metabolic factors. **Methodology:** This analytical case-control study enrolled 150 adults at Al-Nasiriyah General Hospital and affiliated clinics between October 2025 and May 2026: 100 ultrasonographically confirmed NAFLD patients and 50 controls. After 8–12-hour fasting, venous blood was collected, processed, and stored at -80°C . Metabolic and liver parameters were measured using automated enzymatic methods. MDA, SOD, GSH, and hs-CRP were assessed colorimetrically or immunoturbidimetrically, while IL-6 and TNF- α were quantified by duplicate sandwich ELISA. Appropriate exclusion criteria and internal quality controls were applied throughout. **Result:** NAFLD patients had higher BMI, fasting glucose, triglycerides, ALT, AST, GGT, MDA, hs-CRP, IL-6, and TNF- α , but lower HDL-C, SOD, and GSH than controls. Oxidative and inflammatory biomarkers correlated significantly with liver enzymes; MDA and IL-6 showed the strongest positive relationships with ALT. Multivariable analysis identified MDA, IL-6, BMI, and hs-CRP as independent positive correlates of ALT, whereas SOD was inversely associated. Triglycerides and TNF- α were nonsignificant after adjustment, supporting a combined oxidative, inflammatory, and metabolic contribution to hepatic injury. **Conclusions;** NAFLD was associated with adverse metabolic features, elevated liver enzymes, increased lipid peroxidation, weakened antioxidant defenses, and systemic inflammation. MDA, IL-6, hs-CRP, BMI, and SOD independently correlated with ALT, supporting their potential complementary value in assessing hepatocellular injury and activity.

Keyword: Non-alcoholic fatty liver disease; oxidative stress; inflammation; liver enzymes; malondialdehyde.

Introduction:

Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver disease in which the liver contains excess fat, but in people who do not have an obvious other cause of liver fat accumulation or excessive alcohol intake. NAFLD is a recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD), based on an international consensus that highlights the strong association between NAFLD and obesity, insulin resistance, dyslipidemia, hypertension, and T2DM (Rinella et al., 2023). However, NAFLD is still being used in well-established clinical databases and research protocols. The disease can be a wide spectrum of clinicopathological conditions ranging from isolated steatosis to non-alcoholic steatohepatitis, fibrosis, cirrhosis and HCC (Powell et al., 2021).

Today, NAFLD is a significant health threat to the world. A recent meta-analysis estimated that around 30% of the global adult population in the world suffers from NAFLD, and there is a higher prevalence in the Middle East and North Africa (MENA) region in particular (Younossi et al., 2023). Currently, it is rapidly gaining its importance as obesity, sedentary lifestyle, unhealthy eating habits and the prevalence of type 2 diabetes rise (Quek et al., 2023). Patients with simple steatosis have no significantly higher risk of liver-related complications, cardiovascular disease, malignancy or premature mortality, while those with steatohepatitis and fibrosis have much higher risk of these conditions (Huang et al., 2021). Therefore, it is clinically relevant to identify clinically accessible biomarkers that reflect hepatocellular injury and disease progression that can be attributed to the biological processes.

Several factors are involved in the pathogenesis of NAFLD, and a multiple hit model of insulin resistance, adipose tissue dysfunction, hepatic lipid accumulation, lipotoxicity, mitochondrial abnormalities, oxidative stress, inflammation, intestinal dysbiosis and genetic susceptibility best explains this (Loomba et al., 2021). The insulin resistance enhances adipose-tissue lipolysis and hepatic supply of free fatty acids, and stimulates de novo lipogenesis. Excessive lipid accumulation in the liver leads to the accumulation of toxic lipid intermediates that affect the function of mitochondria and endoplasmic reticulum (Geisler & Renquist, 2021). These disturbances lead to an increase in the production of ROS and to a decrease of the endogenous antioxidant system, thus making oxidative stress a key factor in hepatocellular damage (Karkucinska-Wieckowska et al., 2022).

Lipid peroxidation, protein oxidation, damage to mitochondrial DNA and hepatocyte apoptosis can all be triggered by oxidative stress. Malondialdehyde is often used as a marker of circulating lipid peroxidation products while superoxide dismutase, catalase, glutathione and glutathione peroxidase are key enzymes in the antioxidant defense system (Arroyave-Ospina et al., 2021). This could thus represent a 'transition' from simple steatosis to liver injury with inflammation, due to enhanced oxidant generation and decreased antioxidant capacity. This process can be exacerbated by mitochondrial dysfunction, which leads to reduced fatty acid oxidation and production of more ROS, leading to a vicious cycle of metabolic stress and cellular damage (Longo et al., 2021).

Oxidative injury also triggers nuclear factor- κ B activation, inflammasome activation, activation of kupffer cells and hepatic stellate cells, thus connecting oxidative stress to inflammation and fibrogenesis (Schuster et al., 2021). Tumor necrosis factor- α , interleucins 6 and 1 β and other inflammatory mediators are produced by activated immune and hepatic cells. A systematic review showed that circulating levels of pro-inflammatory cytokines, such as C-reactive protein, interleukin-1 β , interleukin-6, and tumor necrosis factor- α were associated with NAFLD, though their levels may differ based on obesity, diabetes, severity of disease, and analytical method (Duan et al., 2022). Clinical trials have also shown that inflammatory cytokines could separate the patients with fatty liver from the controls with similar metabolic characteristics (Duan et al., 2022).

Several tests are routinely used to assess liver damage including serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase and alkaline phosphatase. Alanine aminotransferase is moderately hepatotopic, whereas, aspartate aminotransferase can come from liver and extrahepatic

sources. Alk. phosph. could be a sign of cholestatic or more advanced liver disease and GGT level could be a sign of oxidative and metabolic stress (Rinella et al., 2023). Concentrations of aminotransferases, however, are not abnormal enough to rule out steatohepatitis or clinically significant fibrosis (Cusi et al., 2022). Current guidelines thus suggest looking at liver enzymes in conjunction with metabolic characteristics, imaging and non-invasive fibrosis testing. Other tests like fibrosis-4 index and transient elastography help to better stratify fibrosis risk but are not direct markers of oxidative and inflammatory activity (Cusi et al., 2022).

Combining assessment of oxidative stress markers with conventional liver enzymes along with inflammatory markers may give a more holistic picture of the metabolic injury, inflammation and hepatocellular damage (Mózes et al., 2022). However, across populations, the evidence of their relationships is not consistent, with some areas, especially those with a high metabolic-disease burden, lacking evidence (Amini-Salehi et al., 2024). The purpose of this study was to investigate the link between oxidative stress and inflammatory markers with changes in serum liver enzymes in patients with NAFLD, and their use as additional markers of liver damage.

Methodology:

The study was an analytical case patient control design that was carried out from 15 October 2025 to 30 May 2026 in Al-Nasiriyah General Hospital and its affiliated outpatient consultation clinics in Dhi Qar Province, Iraq. 150 adults were enrolled, 100 with non-alcoholic fatty liver disease (NAFLD) and 50 without apparent health without ultrasonographic evidence of hepatic steatosis. NAFLD was diagnosed by the clinical and biochemical assessment, which included increased hepatic echogenicity, vascular blurring or posterior beam attenuation seen on ultrasonography. People with viral hepatitis, other liver diseases (autoimmune or chronic), cirrhosis, malignancy, acute inflammatory or infectious diseases, pregnancy, recent major surgery, and who used drugs that can cause liver fat (hepatic steatosis) were excluded. A standardized form was used to record age, sex, and body mass index (BMI), smoking and history of hypertension. Venous blood was collected after 8 to 12 hours of fasting time from an antecubital vein, about 7 mL was transferred into a fluoride–oxalate tube for glucose measurement and 5 mL was transferred into a serum-separator tube. The serum samples were left to clot for 20-30 minutes, then centrifuged at 3000 g for 10 minutes and examined for hemolysis or lipemia, and aliquoted and stored at -80°C for later analysis. All these parameters such as fasting glucose, total cholesterol, triglycerides, HDL-C, LDL-C, ALT, AST, GGT, ALP, total bilirubin and albumin were measured by the automated chemistry analyzer by standard enzymatic and kinetic and colorimetric methods. The thiobarbituric-acid colorimetric method, the inhibition of chromogen reduction by the superoxide and the GSH reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) method were used to determine the MDA, SOD activity and GSH, respectively. hs-CRP was determined by latex-enhanced immunoturbidimetry, IL-6 and TNF- α were measured in duplicate by human sandwich ELISA kits, and the absorbance at 450 nm was determined and concentrations were calculated from the standard calibration curve. Internal quality control was used in each analytical run with duplicate samples if the CoV was greater than 10% then this is repeated.

Statistical analysis:

Appropriate statistical software was used for the statistical analyses. Categorical data was presented as frequencies and percentages and continuous data was presented as mean $\pm$ standard deviation or median with IQR depending on the distribution of the data. Independent-samples t-test or Mann–Whitney U test were used to compare the differences between the groups for continuous variables, and chi-square or Fisher's exact test were used to compare the differences between the groups for categorical variables. Relationships between oxidative stress and inflammatory markers/liver enzymes were evaluated by Pearson's or Spearman's correlation analysis, depending on the circumstances. To find factors that are independent predictors of serum ALT level after accounting for other relevant metabolic and inflammatory

factors, multivariable linear regression analysis was conducted and the results are presented as standardized regression coefficients and 95% confidence intervals. The p value on each side was <0.05, if either or both were statistically significant.

Ethical approval:

The study was approved by the responsible human research ethics committee, and was carried out following the Declaration of Helsinki. Prior to involvement, all participants were informed of the purpose of the study and its procedures and gave their written informed consent. All the participants' personal and clinical information was kept confidential and anonymous during the study.

Results

Baseline comparison of demographic factors, cardiometabolic risk indicators, glycemic status, and lipid profiles between the study groups

As seen in Table 1, there were no significant differences between the patients with NAFLD and controls in terms of age, sex distribution, current smoking or the prevalence of hypertension (all $P > 0.05$). In contrast, patients with NAFLD had a significantly higher mean BMI than controls (29.4 ± 3.9 vs. 27.8 ± 3.7 kg/m²; $P = 0.017$), as well as higher fasting glucose (108.6 ± 17.8 vs. 101.4 ± 15.2 mg/dL; $P = 0.016$) and triglyceride concentrations (168.5 ± 52.4 vs. 142.7 ± 43.6 mg/dL; $P = 0.003$). HDL-C was significantly lower in patients (42.6 ± 9.4 vs. 47.1 ± 10.2 mg/dL; $P = 0.008$). The increase in total cholesterol was borderline significant ($P = 0.057$), while there was no significant difference in LDL-C ($P = 0.230$) between the groups. In general, NAFLD was linked to a more unfavorable metabolic profile - higher adiposity, higher fasting glucose, higher triglycerides, and lower HDL-C.

Table 1. Demographic, Clinical, and Metabolic Characteristics of Patients with NAFLD and Healthy Controls

Variable	NAFLD patients (n=100)	Controls (n=50)	P value
Age (years), mean $\pm$ SD	46.8 ± 10.2	45.1 ± 9.8	0.331
Male sex, n (%)	54 (54.0)	25 (50.0)	0.644
Female sex, n (%)	46 (46.0)	25 (50.0)	N/A
BMI (kg/m ²), mean $\pm$ SD	29.4 ± 3.9	27.8 ± 3.7	0.017
Current smoking, n (%)	18 (18.0)	7 (14.0)	0.536
Hypertension, n (%)	31 (31.0)	11 (22.0)	0.248
Fasting glucose (mg/dL)	108.6 ± 17.8	101.4 ± 15.2	0.016
Triglycerides (mg/dL)	168.5 ± 52.4	142.7 ± 43.6	0.003
Total cholesterol (mg/dL)	196.8 ± 38.7	184.3 ± 35.2	0.057
HDL-C (mg/dL)	42.6 ± 9.4	47.1 ± 10.2	0.008
LDL-C (mg/dL)	121.4 ± 32.6	114.7 ± 30.8	0.230

Comparison of hepatocellular enzymes, cholestatic markers, bilirubin, albumin, and the ALT-to-AST ratio between the study groups

As shown in Table 2, patients with NAFLD had significantly higher ALT (43.8 ± 18.6 vs. 27.4 ± 10.2 U/L), AST (35.7 ± 14.2 vs. 25.8 ± 9.1 U/L), and GGT concentrations (45.2 ± 22.4 vs. 29.6 ± 14.8 U/L) than controls (all $P < 0.001$). Patients also had a significantly elevated ALT/AST ratio (1.27 ± 0.39 vs. 1.08 ± 0.31 ;

P=0.003), suggestive of an ALT predominant hepatocellular enzyme pattern. For ALP, there was a near-significant difference between the groups (P=0.050), with levels in the NAFLD group being 93.6±25.7 U/L and 85.2±22.3 U/L in the controls. For total bilirubin and albumin, there was no significant difference between the groups (both P>0.05). The biochemical parameters indicate that among patients with NAFLD, there is evidence of hepatocellular injury with relatively intact hepatic synthetic and excretory function.

Table 2. Liver Enzyme and Biochemical Profiles of Patients with Non-Alcoholic Fatty Liver Disease and Healthy Controls

Parameter	NAFLD patients (n=100)	Controls (n=50)	P value
ALT (U/L)	43.8 ± 18.6	27.4 ± 10.2	<0.001
AST (U/L)	35.7 ± 14.2	25.8 ± 9.1	<0.001
GGT (U/L)	45.2 ± 22.4	29.6 ± 14.8	<0.001
ALP (U/L)	93.6 ± 25.7	85.2 ± 22.3	0.050
Total bilirubin (mg/dL)	0.74 ± 0.25	0.69 ± 0.22	0.233
Albumin (g/dL)	4.28 ± 0.42	4.35 ± 0.39	0.326
ALT/AST ratio	1.27 ± 0.39	1.08 ± 0.31	0.003

Comparison of lipid peroxidation, antioxidant defenses, and systemic inflammatory mediators between NAFLD patients and control participants

NAFLD patients showed significantly elevated markers of oxidative and inflammatory activity as compared to healthy controls (Table 3). Mean MDA concentration was significantly higher in patients (4.62±1.31 vs. 3.38±1.02 nmol/mL; P<0.001), whereas SOD activity (82.7±18.5 vs. 94.8±19.6 U/mL) and GSH concentration (5.8±1.4 vs. 6.7±1.5 µmol/L) were significantly lower (both P<0.001). Patients also had higher hs-CRP (3.42±1.86 vs. 2.18±1.34 mg/L; P<0.001), IL-6 (5.8±2.6 vs. 4.1±2.0 pg/mL; P<0.001), and TNF-α concentrations (18.7±6.8 vs. 15.4±5.9 pg/mL; P=0.004). These results suggest that NAFLD is associated with a state of increased lipid peroxidation, decreased antioxidant capacity and increased low-grade systemic inflammation.

Table 3. Oxidative Stress and Inflammatory Biomarker Profiles in Patients with NAFLD and Healthy Controls

Biomarker	NAFLD patients (n=100)	Controls (n=50)	P value
MDA (nmol/mL)	4.62 ± 1.31	3.38 ± 1.02	<0.001
SOD (U/mL)	82.7 ± 18.5	94.8 ± 19.6	<0.001
GSH (µmol/L)	5.8 ± 1.4	6.7 ± 1.5	<0.001
hs-CRP (mg/L)	3.42 ± 1.86	2.18 ± 1.34	<0.001
IL-6 (pg/mL)	5.8 ± 2.6	4.1 ± 2.0	<0.001
TNF-α (pg/mL)	18.7 ± 6.8	15.4 ± 5.9	0.004

Relationships between circulating redox and inflammatory biomarkers and biochemical indicators of hepatocellular injury in the NAFLD group

The correlation of MDA with ALT was moderately and positively correlated (r=0.46; P<0.001), as well as its correlation with AST (r=0.39; P<0.001) and GGT (r=0.41; P<0.001). SOD and GSH, on the other hand, were negatively correlated with all three enzymes, ranging from -0.25 to -0.34 (all P<0.05); therefore, reduced antioxidant capacity was correlated with increased biochemical liver injury. ALT and AST levels were positively correlated with inflammatory markers such as hs-CRP, IL-6 and TNF-α, while GGT had a positive correlation with only IL-6 and TNF-α. ALT had the highest inflammatory associations

with IL-6 ($r=0.42$; $P<0.001$). In general, liver-enzyme changes were significantly correlated with elevated levels of oxidative stress, reduced antioxidant capacity and higher systemic levels of inflammation in NAFLD patients.

Table 4. Correlations of Oxidative Stress and Inflammatory Biomarkers with Liver Enzymes Among Patients with NAFLD

Biomarker	ALT, r (P value)	AST, r (P value)	GGT, r (P value)
MDA	0.46 (<0.001)	0.39 (<0.001)	0.41 (<0.001)
SOD	-0.34 (<0.001)	-0.28 (0.005)	-0.31 (0.002)
GSH	-0.30 (0.002)	-0.25 (0.012)	-0.27 (0.007)
hs-CRP	0.38 (<0.001)	0.31 (0.002)	0.35 (<0.001)
IL-6	0.42 (<0.001)	0.35 (<0.001)	0.37 (<0.001)
TNF- α	0.33 (<0.001)	0.29 (0.003)	0.30 (0.002)

Adjusted contributions of metabolic, oxidative-stress, and inflammatory biomarkers to variation in alanine aminotransferase among affected patients

As shown in Table 5, MDA exhibited the strongest independent positive association with serum ALT (standardized $\beta=0.29$; 95% CI for B: 1.54–6.63; $P=0.002$), followed by IL-6 ($\beta=0.22$; 95% CI: 0.42–2.69; $P=0.008$). There was also an independent and positive association between BMI ($\beta=0.17$; $P=0.029$) and hs-CRP ($\beta=0.16$; $P=0.032$) with ALT. Lower antioxidant activity was however, associated with higher ALT levels, with a significant inverse association ($\beta=-0.18$; 95% CI: -0.37 to -0.03; $P=0.022$) in the case of SOD. However, after adjustment for these factors, there was no independent association between ALT and triglycerides or TNF- α ($P>0.05$). The findings indicate that altered enzymes in NAFLD are not just an effect of lipid peroxidation, but rather are also a result of impaired antioxidant defense and inflammation, and adiposity also plays its part.

Table 5. Multivariable Linear Regression Analysis of Factors Independently Associated with Serum ALT Levels in Patients with NAFLD

Predictor	Standardized β	95% CI for B	P value
BMI	0.17	0.08 to 1.46	0.029
Triglycerides	0.13	-0.01 to 0.10	0.091
MDA	0.29	1.54 to 6.63	0.002
SOD	-0.18	-0.37 to -0.03	0.022
hs-CRP	0.16	0.14 to 3.04	0.032
IL-6	0.22	0.42 to 2.69	0.008
TNF- α	0.09	-0.11 to 0.58	0.181

Discussion:

The present study showed that there was a specific metabolic, hepatic, oxidative, and inflammatory profile in the patients with NAFLD versus controls. The groups were similar with respect to age, sex, smoking and hypertension but NAFLD had higher BMI, fasting glucose and triglycerides and lower HDL-C. This pattern reinforces the notion that hepatic steatosis is not just a liver disease, but a closely coupled type of disease with adiposity, insulin resistance and atherogenic dyslipidemia. Chan et al. reported the same clustering of obesity, hyperglycemia, hypertriglyceridemia and low HDL-C in MASLD (Chan et al., 2023). The higher burden identified in Asian and Middle Eastern populations also partially reflects the high rate of growth of metabolic risk factors in these areas (Golabi et al., 2021). Likewise, Riazi et al. found that obesity, diabetes and dyslipidaemia were the key factors influencing the prevalence of fatty liver (Riazi et al., 2022). NAFLD is not necessarily associated with obesity, however—histologically proven lean NAFLD has been associated with significant hepatocellular damage (Patel et al., 2023). It is

a seemingly paradoxical finding that could be due to genetic susceptibility, to the composition of the body fat, to the nutritional quality of the diet, and/or to insulin resistance not captured by BMI.

ALT, AST, and GGT were significantly raised in NAFLD, while the ALP was only slightly elevated, and bilirubin and albumin levels were similar. This hepatocellular dominant pattern is typical of liver injury due to mild to moderate metabolic insult without a clinically significant decrease in synthetic function or liver cholestasis. In addition, the increased ALT/AST ratio is also likely in the early stages of NAFLD when AST tends to rise less than ALT. Increased ALT, AST and GGT levels were reported with the severity of NAFLD assessed by ultrasound and elastography by Popov et al. (Popov & Lim, 2024). Similarly, a large study revealed an increased risk for cirrhosis and HCC in NAFLD with elevated liver enzymes compared to normal liver enzymes (Lee et al., 2023). Andrade et al. also found AST related indices and GGT to be predictors for advanced fibrosis (Andrade et al., 2022). However, biochemical markers do not reliably differentiate at-risk disease and aminotransferases may be normal in patients with steatohepatitis or fibrosis; studies of the validation of the FAST score confirm this (Oeda et al., 2022). Results may vary between studies because of the variation in the laboratory ranges, sex-specific cut-offs, stage of fibrosis and diagnosis based on ultrasonography, elastography or biopsy.

No significant differences were seen in bilirubin or albumin levels suggest intact hepatic excretory and synthetic function in this study population. This is biologically reasonable as these parameters typically change later than aminotransferases. On the other hand, Pennisi et al. showed that a cholestatic biochemical pattern was associated with important liver outcomes in NAFLD (Pennisi et al., 2022). Their result is not necessarily in conflict with the present results as the cholestasis occurs when there is more advanced fibrosis or ductular injury and the current participants may have had less advanced disease.

Patients had a higher lipid peroxidation level (MDA) and a lower antioxidant defense level (SOD and GSH). Increased hepatic fatty acids lead to increased electron leakage and production of ROS in mitochondria, and lipid peroxidation leads to the production of MDA and other RAs, which leads to the damage of membranes, proteins and DNA. Similarly, Smirne et al., reported that the levels of oxidative products were elevated and antioxidant defenses (both enzymatic and non-enzymatic) were impaired in NAFLD (Smirne et al., 2022). Human metabolic studies also have linked impaired mitochondrial adaptation to increased liver insulin resistance and liver injury (Koliaki et al., 2024). These findings on vitamin E's improving effects on ALT, AST, steatosis, and lobular inflammation reinforce the importance of oxidative injury, but have been less consistent regarding fibrosis (Chee et al., 2024). Other intervention studies, however, have not shown consistent normalization of all the oxidative markers. These may occur due to differences in antioxidant levels in the diet, diabetes, smoking, medication effects, assay instability, and differences in the in vivo activities of the redox system in plasma versus liver.

Inflammatory activation was also seen with increased hs-CRP, IL-6 and TNF- α . Baek et al. reported that there was an increase in hs-CRP in each metabolically defined steatotic liver disease category, which is consistent with its role as a marker of low grade systemic inflammation (Baek et al., 2025). For the Fatty Liver in Obesity trial, dietary interventions led to improvement in the liver status, which was associated with improvement in inflammatory markers (Marin-Alejandro et al., 2024). From a mechanistic point of view, IL-6 and TNF- α are secreted by adipose-tissue macrophages and stressed hepatocytes, which activate kupffer cells, and increase insulin resistance and cell injury in the liver. Specific cytokine profiles have been found in steatosis and steatohepatitis by inflammatory protein-profiling studies (Govaere et al., 2025). But some studies have not detected TNF- α as an independent factor associated with NAFLD (Buzzetti et al., 2022). This lack of uniformity could be due to its short circulating half-life, its local intrahepatic biosynthesis, the sensitivity of the ELISA, obesity related confounding or the heterogeneity in disease stage.

The results of the correlation analysis further reinforce the biological consistency of the results. The correlation of MDA with ALT, AST and GGT was positive, whereas the correlation of SOD and GSH was negative, indicating that the increase of hepatocellular injury was accompanied by the increase of

lipid peroxidation and reduction of antioxidant protection. A similar relationship has been reported in studies that used a combination of redox-status markers and liver enzymes and circulating microRNAs (Milovanovic et al., 2025). In recent studies in diabetic MASLD, there was also an increase in oxidative-stress indices and systemic inflammatory abnormalities and fibrosis risk (Bălănescu et al., 2026). Correlations were moderate, however, suggesting that variation of liver enzymes is also shaped by adiposity, insulin resistance, genetic factors, physical activity and exposure to medications.

The positive correlations between levels of hs-CRP, IL-6, and TNF- α also reflect a coordinated inflammatory process in the body and liver damage. This is confirmed by therapeutic studies demonstrating concurrent decrease of inflammatory biomarkers and aminotransferases following metabolic improvement (Chee et al., 2024). However, the cross-sectional design does not allow conclusions to be drawn about whether the elevation of enzymes was due to oxidative stress/inflammation or was secondary to hepatocellular injury.

In the multivariable model, MDA, reduced SOD, IL-6, hs-CRP and BMI were independently associated with ALT, while triglycerides and TNF- α were not. The standardized effect was the greatest for MDA, suggesting that the variation in ALT could be due to lipid peroxidation in addition to the conventional metabolic factors. As expected IL-6 remained a stand-alone association independent of other cytokines as it had more continued systemic activity than TNF- α . Experimental and translational research indicates that enhancing mitochondrial function leads to a decrease in oxidative stress, inflammatory signaling and liver damage (Neve et al., 2024; Graupera et al., 2022). This may be because of the role of triglycerides as a mediator of loss or because of the correlation between triglycerides and BMI. Likewise, there can be a biological overlap between TNF- α and IL-6 and hscrp.

Conclusion:

This study shows that NAFLD has a negative metabolic profile, elevated liver enzymes, increased lipid peroxidation, decreased antioxidant status and systemic inflammation. Patients had greater ALT, AST, GGT, MDA, hs-CRP, IL-6 and TNF- α levels and lower levels of SOD and GSH when compared with the controls. There are consistent correlations between oxidative and inflammatory biomarkers and liver enzymes. Multivariable analysis confirmed that MDA, SOD, IL-6, hs-CRP and BMI were independently associated with ALT, indicating the relationship between adiposity, oxidative imbalance, and inflammation with liver injury. The results of the study are in favor of using some redox and inflammatory markers together with the conventional liver function tests for biochemical evaluation. Long-term studies with elastography or histology, however, are required to confirm the prognosis and clinically relevant thresholds of disease progression and treatment monitoring in NAFLD.

References

- Amini-Salehi, E., Hassanipour, S., Joukar, F., et al. (2024). Global prevalence of nonalcoholic fatty liver disease: An updated systematic review and meta-analysis. *Revista de Gastroenterología de México* (English Edition), 89(3), 389–400. <https://doi.org/10.1016/j.rgmxen.2024.07.002>
- Andrade, T. G., de Oliveira, C. V., et al. (2022). Risk predictors of advanced hepatic fibrosis in patients with nonalcoholic fatty liver disease. *European Journal of Gastroenterology & Hepatology*, 34, 1134–1141.
- Arroyave-Ospina, J. C., Wu, Z., Geng, Y., & Moshage, H. (2021). Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: Implications for prevention and therapy. *Antioxidants*, 10(2), 174. <https://doi.org/10.3390/antiox10020174>
- Baek, S. U., Kang, S. H., Lee, H. W., et al. (2025). High-sensitivity C-reactive protein levels in metabolic dysfunction-associated steatotic liver disease. *Clinical and Molecular Hepatology*, 31, 253–267.

- Bălănescu, P., et al. (2026). Oxidative stress biomarkers and systemic inflammatory indices in diabetic patients with metabolic dysfunction-associated steatotic liver disease. *Antioxidants*, 15, 703.
- Buzzetti, E., Pinzani, M., & Tsochatzis, E. A. (2022). Treating inflammation to combat non-alcoholic fatty liver disease. *Trends in Molecular Medicine*, 28(10), 892–905.
- Chan, W. K., Chuah, K. H., Rajaram, R. B., Lim, L. L., Ratnasingam, J., & Vethakkan, S. R. (2023). Metabolic dysfunction-associated steatotic liver disease: A state-of-the-art review. *Journal of Obesity & Metabolic Syndrome*, 32(3), 197–213. <https://doi.org/10.7570/jomes23052>
- Chee, N. M. Z., Sinnanaidu, R. P., & Chan, W. K. (2024). Effects of antioxidant therapy on liver inflammation and aminotransferases in metabolic dysfunction-associated steatotic liver disease. *Journal of Gastroenterology and Hepatology*, 39(12), 2545–2554. <https://doi.org/10.1111/jgh.16723>
- Cusi, K., Isaacs, S., Barb, D., et al. (2022). American Association of Clinical Endocrinology clinical practice guideline for the diagnosis and management of nonalcoholic fatty liver disease. *Endocrine Practice*, 28(5), 528–562. <https://doi.org/10.1016/j.eprac.2022.03.010>
- Duan, Y., Luo, J., Pan, X., et al. (2022). Association between inflammatory markers and non-alcoholic fatty liver disease in obese children. *Frontiers in Public Health*, 10, 991393. <https://doi.org/10.3389/fpubh.2022.991393>
- Geisler, C. E., & Renquist, B. J. (2021). Hepatic lipid accumulation: Cause and consequence of dysregulated glucoregulatory hormones. *Journal of Endocrinology*, 249(1), R1–R21. <https://doi.org/10.1530/JOE-20-0513>
- Golabi, P., Paik, J. M., AlQahtani, S., Younossi, Y., Tuncer, G., & Younossi, Z. M. (2021). Burden of non-alcoholic fatty liver disease in Asia, the Middle East and North Africa: Data from Global Burden of Disease 2009–2019. *Journal of Hepatology*, 75(4), 795–809.
- Govaere, O., Cockell, S., Van Haele, M., et al. (2025). Inflammatory protein signatures as predictive disease-specific markers for non-alcoholic steatohepatitis. *Inflammation*, 48, 25–41.
- Graupera, I., Thiele, M., Serra-Burriel, M., et al. (2022). Low accuracy of FIB-4 and NAFLD fibrosis scores for screening for liver fibrosis in the population. *Clinical Gastroenterology and Hepatology*, 20(11), 2567–2576.e6. <https://doi.org/10.1016/j.cgh.2021.12.034>
- Huang, D. Q., El-Serag, H. B., & Loomba, R. (2021). Global epidemiology of NAFLD-related hepatocellular carcinoma: Trends, predictions, risk factors and prevention. *Nature Reviews Gastroenterology & Hepatology*, 18(4), 223–238. <https://doi.org/10.1038/s41575-020-00381-6>
- Karkucinska-Wieckowska, A., Simoes, I. C. M., Kalinowski, P., et al. (2022). Mitochondria, oxidative stress and nonalcoholic fatty liver disease: A complex relationship. *European Journal of Clinical Investigation*, 52(3), e13622. <https://doi.org/10.1111/eci.13622>
- Koliaki, C., Roden, M., et al. (2024). Loss of mitochondrial adaptation associates with deterioration of metabolic dysfunction-associated steatotic liver disease. *Cell Metabolism*, 36, 571–586.
- Lee, J., Kim, T., Yang, H., et al. (2023). Influence of nonalcoholic fatty liver disease with increased liver enzyme levels on risk of cirrhosis and hepatocellular carcinoma. *Clinical Gastroenterology and Hepatology*, 21(2), 433–441.e8.
- Longo, M., Paolini, E., Meroni, M., & Dongiovanni, P. (2021). Remodeling of mitochondrial plasticity: The key switch from NAFLD/NASH to HCC. *International Journal of Molecular Sciences*, 22(8), 4173. <https://doi.org/10.3390/ijms22084173>
- Loomba, R., Friedman, S. L., & Shulman, G. I. (2021). Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell*, 184(10), 2537–2564. <https://doi.org/10.1016/j.cell.2021.04.015>
- Marin-Alejandro, B. A., Cantero, I., Monreal, J. I., et al. (2024). Inflammatory biomarkers during a two-year dietary intervention in subjects with metabolic dysfunction-associated steatotic liver disease: Results from the FLiO trial. *Clinical Nutrition*, 43(8), 1845–1855. <https://doi.org/10.1016/j.clnu.2024.05.042>

- Milovanovic, Z., et al. (2025). Expression analysis of circulating miR-21, miR-34a and miR-122 and their relationship with redox status, lipids and liver enzymes in metabolic-associated steatotic liver disease. *Biomedicines*, 13, 602.
- Mózes, F. E., Lee, J. A., Selvaraj, E. A., et al. (2022). Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: An individual-patient-data meta-analysis. *Gut*, 71(5), 1006–1019. <https://doi.org/10.1136/gutjnl-2021-324243>
- Neve, E. P., et al. (2024). Modulation of mitochondrial amidoxime-reducing component 1 influences hepatic steatosis, oxidative stress, inflammation, and cell death. *Hepatology*, 80, 1164–1182.
- Oeda, S., Takahashi, H., Imajo, K., et al. (2022). Validation of the accuracy of the FAST score for detecting at-risk nonalcoholic steatohepatitis. *Frontiers in Medicine*, 9, 821924.
- Patel, A. H., Peddu, D., Amin, S., et al. (2023). Non-alcoholic fatty liver disease in lean individuals. *Journal of Clinical and Translational Hepatology*, 11(3), 630–638.
- Pennisi, G., Enea, M., Falco, V., et al. (2022). A cholestatic pattern predicts major liver-related outcomes in patients with non-alcoholic fatty liver disease. *Liver International*, 42(5), 1188–1199. <https://doi.org/10.1111/liv.15228>
- Popov, V. B., & Lim, J. K. (2024). Role of alanine transaminase and transient elastography in determining NAFLD severity. *Cureus*, 16, e51317.
- Powell, E. E., Wong, V. W., & Rinella, M. (2021). Non-alcoholic fatty liver disease. *The Lancet*, 397(10290), 2212–2224. [https://doi.org/10.1016/S0140-6736\(20\)32511-3](https://doi.org/10.1016/S0140-6736(20)32511-3)
- Quek, J., Chan, K. E., Wong, Z. Y., et al. (2023). Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in overweight and obese populations: A systematic review and meta-analysis. *The Lancet Gastroenterology & Hepatology*, 8(1), 20–30. [https://doi.org/10.1016/S2468-1253\(22\)00317-X](https://doi.org/10.1016/S2468-1253(22)00317-X)
- Riazi, K., Azhari, H., Charette, J. H., et al. (2022). The prevalence and incidence of NAFLD worldwide: A systematic review and meta-analysis. *The Lancet Gastroenterology & Hepatology*, 7(9), 851–861. [https://doi.org/10.1016/S2468-1253\(22\)00165-0](https://doi.org/10.1016/S2468-1253(22)00165-0)
- Rinella, M. E., Lazarus, J. V., Ratziu, V., et al. (2023). A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology*, 78(6), 1966–1986. <https://doi.org/10.1097/HEP.0000000000000520>
- Rinella, M. E., Neuschwander-Tetri, B. A., Siddiqui, M. S., et al. (2023). AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology*, 77(5), 1797–1835. <https://doi.org/10.1097/HEP.0000000000000323>
- Schuster, S., Cabrera, D., Arrese, M., & Feldstein, A. E. (2021). Triggering and resolution of inflammation in NASH. *Nature Reviews Gastroenterology & Hepatology*, 18(11), 757–774. <https://doi.org/10.1038/s41575-021-00477-1>
- Smirne, C., Croce, E., Di Benedetto, D., Cantaluppi, V., Comi, C., Sainaghi, P. P., et al. (2022). Oxidative stress in non-alcoholic fatty liver disease. *Livers*, 2(1), 30–76. <https://doi.org/10.3390/livers2010003>
- Younossi, Z. M., Golabi, P., Paik, J. M., Henry, A., Van Dongen, C., & Henry, L. (2023). The global epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: A systematic review. *Hepatology*, 77(4), 1335–1347. <https://doi.org/10.1097/HEP.0000000000000004>