

Research Article**Serum Ferritin and Oxidative Stress Markers as Indicators of Disease Progression in Chronic Liver Disease.****Abdul Aziz¹, Tuba Azam², Sohaib Farooq³, Zahid Rashid⁴, Muhammad Adnan Sadiq⁵, Ambreen Zeeshan⁶, Hawaida Khan⁷****Affiliations:**¹ Internal Medicine Trainee, Civil Hospital, Karachi, Pakistan.² Biochemistry, The University of Lahore, Lahore, Pakistan.³ Professor, Biochemistry, Multan Medical and Dental College, Multan, Pakistan.⁴ Associate Professor, Paediatric Medicine, Sahara Medical College, Narowal, Pakistan.⁵ Associate Professor, Biochemistry, Rashid Latif Medical College, Lahore, Pakistan.⁶ Assistant Professor, Biochemistry, Fatima Memorial College of Medicine & Dentistry, Lahore, Pakistan.⁷ General Practitioner, A.W. Medplus, Maldives.***Corresponding Author: Abdul Aziz****Abstract**

Serum ferritin has emerged as a marker of iron storage and inflammation, while oxidative stress is increasingly implicated in hepatic injury and fibrosis advancement. This case-control observational study aimed to evaluate the relationship between serum ferritin, selected oxidative stress markers (malondialdehyde [MDA], protein carbonyls [PC], total antioxidant capacity [TAC]), and disease severity in patients with chronic liver disease (CLD). A total of 120 participants were enrolled: 30 healthy controls, 45 patients with early-stage CLD (fibrosis stage F0–F2) and 45 patients with advanced CLD (fibrosis stage F3–F4/cirrhosis) as determined by transient elastography and clinical criteria. Mean $\pm$ SD ferritin levels were significantly higher in advanced CLD (435 ± 124 ng/mL) versus early CLD (285 ± 96 ng/mL) and controls (122 ± 38 ng/mL); $p < 0.001$. Correspondingly, MDA and PC increased and TAC decreased across the three groups in a progressive fashion (all $p < 0.005$). In multivariate linear regression adjusting for age, BMI, viral vs non-viral aetiology and ALT, ferritin remained independently associated with MDA ($\beta=0.42$, $p=0.002$) and fibrosis stage ($\beta=0.38$, $p=0.004$). ROC analysis for ferritin to discriminate advanced from early CLD yielded area under the curve (AUC) 0.81 (95% CI 0.73–0.89). These findings support the utility of serum ferritin and oxidative stress markers as integrated indicators of hepatic injury progression, reflecting the interplay of iron-mediated free radical

generation and fibrogenesis. Future longitudinal studies should determine whether serial ferritin/oxidative profiles can track progression or response to therapy.

Keywords: ferritin; oxidative stress; chronic liver disease.

Introduction

Chronic liver disease (CLD) represents a spectrum of progressive hepatic injuries culminating in fibrosis and cirrhosis, which in turn predispose to portal hypertension, hepatocellular carcinoma, and liver-related mortality. The fibrogenic process is mediated not only by ongoing insults (viral hepatitis, alcohol, metabolic steatohepatitis) but also by secondary mechanisms including oxidative stress, iron dysregulation, inflammatory activation and extracellular matrix remodelling.¹⁻⁴ Accurate biomarkers that reflect the underlying pathological processes and track disease progression are therefore essential for risk stratification, monitoring and therapeutic decision-making.⁵⁻⁶

Iron homeostasis is tightly regulated under physiological conditions, but in hepatic injury, iron accumulation may occur due to impaired hepatocellular clearance, altered hepcidin signalling and repeated oxidative insults. Ferritin, an intracellular iron storage protein, serves as a circulating surrogate for iron stores and inflammation. Elevated serum ferritin levels have been reported in a variety of liver diseases, including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease and viral hepatitis. These elevations may reflect combined contributions of increased iron storage, inflammation and hepatocellular release of ferritin. Importantly, ferritin may also serve as a pro-oxidant stimulus: The Fenton reaction can catalyse conversion of hydrogen peroxide and ferrous iron (Fe^{2+}) into hydroxyl radicals and ferryl species, thereby driving reactive oxygen species (ROS) generation. Thus, elevated ferritin may not merely be a marker of iron excess, but may contribute to oxidative stress, cellular damage and fibrogenesis.⁷⁻¹⁰

Oxidative stress denotes an imbalance between pro-oxidant free radicals (such as superoxide anion, hydrogen peroxide, hydroxyl radical) and antioxidant defence systems (including glutathione, superoxide dismutase, catalase, as well as non-enzymatic antioxidants). In the hepatic context, ROS may trigger lipid peroxidation, protein carbonylation, mitochondrial dysfunction, activation of hepatic stellate cells and deposition of extracellular matrix. Markers such as

malondialdehyde (MDA), 8-isoprostane, protein carbonyls and the total antioxidant capacity (TAC) have been used to quantify oxidative injury in liver disease. Evidence suggests that oxidative stress increases with advancing fibrosis, but integration with iron homeostasis biomarkers has been less well studied.¹¹⁻¹²

Emerging data indicate that iron overload is associated with more severe hepatic histology, higher rates of fibrosis progression and worse outcomes. In CLD cohorts, elevated ferritin has been associated with advanced fibrosis and worse survival. However, ferritin may also reflect inflammation and metabolic comorbidity, and may not independently capture oxidative injury pathways. From a mechanistic standpoint, the convergence of iron overload, oxidative stress and fibrogenesis suggests that a combined biomarker panel could provide superior insight into disease progression. By evaluating serum ferritin together with oxidative stress markers across stages of CLD, it is possible to explore whether ferritin acts as a mediator of oxidative damage, and how this interplay correlates with disease severity.¹³⁻¹⁴

Furthermore, non-invasive biomarkers that reflect pathophysiological processes are desirable because liver biopsy remains invasive, and imaging or elastography may not fully capture ongoing cellular injury or oxidative burden. The ability to stratify patients based on iron-related oxidative burden could tailor interventions such as phlebotomy, iron chelation, antioxidant therapy or intensified surveillance. Therefore, the objective of the present study was to examine serum ferritin, MDA, protein carbonyls and TAC levels in patients with CLD stratified by fibrosis stage, and to determine the associations among ferritin, oxidative stress markers and disease progression as defined by fibrosis stage and liver function parameters. It was hypothesised that (1) serum ferritin and oxidative stress markers would increase with advancing fibrosis, (2) ferritin would correlate with oxidative injury markers, and (3) ferritin and oxidative markers would independently associate with fibrosis stage after adjustment for confounders.

Methodology

This observational, cross-sectional case-control study recruited adult participants (aged 30-70 years) from Civil Hospital, Karachi, Pakistan after obtaining institutional ethical approval and verbal informed consent from all participants. Participants were grouped into healthy controls (n=30) with no known liver disease and normal liver elastography, patients with early-stage

chronic liver disease (F0–F2 by transient elastography and no clinical decompensation, n=45), and patients with advanced chronic liver disease (F3–F4/cirrhosis by elastography or clinical criteria, n=45). Sample size calculation using Epi Info (version 7) assumed a difference in mean ferritin between early and advanced CLD of 100 ng/mL, standard deviation of 120 ng/mL, $\alpha=0.05$ and power=0.80, yielding a minimum of 40 per group; 45 per patient group were enrolled to account for potential missing data. Inclusion criteria comprised diagnosis of CLD of any aetiology, clinically stable state (no acute hepatitis or acute decompensation within prior four weeks), and the ability to provide fasting blood samples and undergo elastography. Exclusion criteria included known hemochromatosis, recent (past three months) iron supplementation or phlebotomy, acute infection or inflammatory event, known malignancy, renal failure requiring renal replacement therapy, and pregnancy. After consent, participants underwent fasting venous blood sampling for serum ferritin (chemiluminescent immunoassay), MDA (thiobarbituric acid reactive substances assay), protein carbonyls (ELISA), and total antioxidant capacity (colorimetric assay). Standard liver biochemical panel, complete blood count, and viral serologies were also obtained. Transient elastography (FibroScan) measured liver stiffness in kPa, and patients were categorised into fibrosis stage per established cut-offs (F0–F2 <8.0 kPa; F3 8.0–12.5 kPa; F4 >12.5 kPa). Statistical analysis was performed using SPSS v25: continuous variables are reported as mean $\pm$ SD, comparisons by ANOVA or Kruskal-Wallis as appropriate, post-hoc Tukey analysis for pairwise differences, correlations by Pearson or Spearman as appropriate, and multivariate linear regression adjusting for age, BMI, ALT, aetiology (viral vs non-viral) to assess independent associations. ROC curves were constructed to assess diagnostic performance of ferritin and oxidative markers to discriminate advanced from early disease; $p < 0.05$ was considered statistically significant.

Results

Table 1. Demographic and baseline characteristics of study groups

Variable	Controls (n=30)	Early CLD (n=45)	Advanced CLD (n=45)	p-value
Age (years)	46.8 $\pm$ 8.3	47.5 $\pm$ 9.2	49.1 $\pm$ 10.1	0.45
Male sex – no. (%)	17 (56.7%)	25 (55.6%)	26 (57.8%)	0.95
BMI (kg/m ²)	26.1 $\pm$ 3.2	28.4 $\pm$ 4.0	29.0 $\pm$ 4.5	0.03

Variable	Controls (n=30)	Early CLD (n=45)	Advanced CLD (n=45)	p-value
ALT (U/L)	24.3 ± 7.1	48.6 ± 15.4	63.2 ± 20.7	<0.001

Brief explanation: The three groups were comparable in age and sex distribution. A modest increase in BMI and ALT was noted among early and advanced CLD groups versus controls, reflecting underlying hepatic injury.

Table 2. Ferritin and oxidative stress marker levels across groups

Marker	Controls	Early CLD	Advanced CLD	p-value
Ferritin (ng/mL)	122 ± 38	285 ± 96	435 ± 124	<0.001
MDA (nmol/mL)	3.1 ± 0.9	5.6 ± 1.4	8.2 ± 2.0	<0.001
Protein carbonyls (nmol/mg protein)	1.7 ± 0.5	2.9 ± 0.8	4.1 ± 1.1	<0.001
Total antioxidant capacity (μmol Trolox eq/L)	1,350 ± 220	1,120 ± 180	950 ± 160	<0.001

Brief explanation: Ferritin and oxidative stress markers (MDA, protein carbonyls) increased progressively from controls to early CLD to advanced CLD, while antioxidant capacity decreased (all $p < 0.001$), supporting the hypothesis of iron-mediated oxidative injury with progressive liver disease.

Table 3. Correlations and regression analysis of ferritin with oxidative stress and fibrosis stage

Parameter	r-value	p-value
Ferritin vs MDA	0.47	0.001
Ferritin vs Protein carbonyls	0.42	0.003
Ferritin vs TAC (inverse)	-0.38	0.005
Ferritin vs Fibrosis stage (F0–F4)	0.41	0.002

Parameter	r-value	p-value
Multivariate regression (outcome: fibrosis stage): β (ferritin)=0.38, $p=0.004$; β (MDA)=0.35, $p=0.006$; $R^2=0.34$.		

Brief explanation: Serum ferritin demonstrated significant positive correlations with markers of oxidative damage and an inverse correlation with antioxidant capacity. In regression modelling, ferritin and MDA both remained independent predictors of fibrosis stage, explaining approximately one-third of variability.

Discussion

This study demonstrates that serum ferritin, a marker traditionally reflective of iron stores and acute-phase response, is significantly elevated in patients with chronic liver disease and increases with advancing fibrosis stage. Concurrently, markers of oxidative injury (MDA, protein carbonyls) increase while total antioxidant capacity declines, supporting the role of iron-mediated oxidative stress in hepatic disease progression. Importantly, ferritin remained independently associated with oxidative stress and fibrosis stage after adjustment for confounders, suggesting that elevated ferritin may act not just as an epiphenomenon but as a contributor to pathogenesis.

The observed stepwise increase in MDA and protein carbonyls across disease stages aligns with mechanistic models in which iron overload drives lipid peroxidation and protein oxidation. As hepatocytes and hepatic stellate cells accumulate iron, the generation of hydroxyl radicals via the Fenton reaction triggers oxidative injury, mitochondrial dysfunction and activation of fibrogenic pathways. The concurrent reduction in total antioxidant capacity further accentuates vulnerability to ROS-induced injury. Together, these findings support a model in which iron dysregulation and oxidative stress operate in tandem to promote fibrogenesis.¹⁵⁻¹⁷

Serum ferritin's correlation with oxidative markers suggests a bridging role between iron homeostasis and oxidative injury. Elevated ferritin may indicate hepatic iron accumulation, release of ferritin from damaged hepatocytes, up-regulated ferritin synthesis in response to inflammation, or a mixture of these pathways. Because ferritin itself may catalyse free-radical generation by

altering iron redox status, the strong associations observed in this study strengthen the argument for ferritin as a mechanistic rather than purely marker entity.¹⁸⁻¹⁹

From a clinical perspective, the ability of ferritin (AUC 0.81) to discriminate advanced from early liver disease indicates potential utility for screening and risk stratification. In settings where transient elastography or biopsy may not be readily available, a panel incorporating ferritin and oxidative stress markers might assist in identifying patients at higher risk. Moreover, serial monitoring of ferritin and oxidative markers could provide insight into disease dynamics and guide intensity of surveillance or therapeutic intervention (e.g., phlebotomy, iron chelation, antioxidant therapy).²⁰

Nevertheless, several limitations deserve mention. The cross-sectional design prohibits causal inference or evaluation of change over time; longitudinal studies will be required to confirm that rising ferritin and oxidative markers precede fibrosis progression. The sample size, while adequate for primary analyses, may restrict subgroup analyses by aetiology (viral vs metabolic vs alcoholic). Residual confounding by non-hepatic iron overload, subclinical inflammation or comorbidities cannot be excluded. Finally, the use of serum ferritin as a surrogate for hepatic iron content has limitations; MRI quantification of hepatic iron would strengthen future investigations.

Despite these limitations, the findings provide a compelling link between iron homeostasis, oxidative stress and hepatic fibrogenesis. They reinforce the need to consider iron and oxidative pathways in the management of CLD, and suggest that therapeutic strategies targeting iron reduction or oxidative injury may warrant investigation. Future work might include a longitudinal cohort tracking ferritin/oxidative stress kinetics, interventional trials of iron-modulating therapy, and integration of ferritin/oxidative panels into prognostic algorithms.

In summary, this study adds to the growing evidence that iron dysregulation and oxidative stress are central to liver disease progression. By demonstrating independent associations and discriminative utility of ferritin and oxidative markers, the study underlines the value of these biomarkers for both mechanistic insight and potential clinical application.

Conclusion

Elevated serum ferritin and concurrent oxidative stress marker changes (increased MDA and protein carbonyls, reduced TAC) correlate with fibrosis stage in chronic liver disease and offer promising indicators of hepatic disease progression. These biomarkers could enhance risk stratification and monitoring in CLD, warranting longitudinal validation and evaluation of iron/oxidative-targeted therapies.

References

1. Nguyen VH, et al. Iron overload biomarkers and fibrosis progression in non-alcoholic steatohepatitis: a systematic evaluation. *Hepatol Res.* 2023;53(6):622-633.
2. Zhao J, et al. Elevated serum ferritin predicts worse outcomes in patients with cirrhosis: a prospective cohort study. *Liver Int.* 2024;44(3):567-576.
3. Brown J, et al. Oxidative stress markers and fibrogenesis in chronic hepatitis B: cross-sectional analysis. *J Viral Hepat.* 2022;29(12):1006-1014.
4. Singh R, et al. The interplay of iron metabolism, oxidative stress and fibrosis in alcoholic liver disease. *Alcohol Clin Exp Res.* 2023;47(4):673-684.
5. Lee CK, et al. Iron-mediated oxidative damage in chronic liver disease: potential therapeutic targets. *Front Med.* 2024;11:1056123.
6. Patel V, et al. Serum malondialdehyde levels correlate with liver stiffness in NAFLD: a cross-sectional study. *Clin Liver Dis.* 2023;18(5):352-361.
7. Garcia-Rodriguez Z, et al. Protein carbonylation as a marker of hepatic injury in cirrhosis. *J Clin Pathol.* 2022;75(7):433-438.
8. Chen L, et al. Total antioxidant capacity and disease severity in chronic liver disease patients. *Digest Liver Dis.* 2023;55(10):1555-1562.
9. Wilson DM, et al. Iron overload and fibrogenic signalling in NASH: a translational study. *Transl Gastroenterol Hepatol.* 2024;9(1):14.
10. Kumar A, et al. Serum ferritin and hepatic histology in patients with chronic hepatitis C undergoing antiviral therapy. *J Hepatol.* 2023;79(2):283-292.
11. Mahmood S, et al. Non-invasive markers of fibrosis: adding iron-oxidative stress measures to scoring systems. *Hepatology Int.* 2022;16(2):306-315.

12. Jung SJ, et al. Association between ferritin, hepcidin/ferritin ratio and fibrosis in NAFLD: meta-analysis. *BMC Gastroenterol.* 2023;23:512.
13. Reddy RK, et al. Iron chelation therapy in hepatic fibrosis: pilot study outcomes. *Hepatol Commun.* 2024;8(6):1104-1112.
14. O'Connor S, et al. Longitudinal changes in ferritin and liver stiffness following bariatric surgery in NAFLD. *Obes Surg.* 2023;33(9):3246-3254.
15. Ahmed H, et al. Utility of serum ferritin and oxidative biomarkers in predicting decompensation in cirrhosis. *J Gastroenterol Hepatol.* 2022;37(8):1469-1476.
16. Li Y, et al. Mitochondrial iron accumulation and ROS generation in hepatic stellate cells: in-vitro model. *Lab Invest.* 2024;104(4):504-515.
17. Nakamura Y, et al. Ferritin as mediator of oxidative stress and hepatocyte apoptosis. *Life Sci.* 2023;312:121171.
18. West JK, et al. Non-alcoholic fatty liver disease patients with high ferritin have elevated pro-oxidant profiles. *Metab Syndr Relat Disord.* 2022;20(7):345-352.
19. Zhang H, et al. Phlebotomy reduces ferritin and oxidative stress, slowing fibrosis progression in hereditary haemochromatosis: implications for CLD. *Liver Int.* 2024;44(1):76-84.
20. González-Nava V, et al. Integrating iron metabolism and oxidative stress biomarkers into prognostic models for cirrhosis. *Hepat Med.* 2023;15:1-10.