

# Integrating Oxidative Stress Biomarkers with Non-Invasive Fibrosis Scores (FIB-4, APRI) for Enhanced Assessment of Liver Disease Severity in Chronic Hepatitis B and C

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## ABSTRACT

**Background:** Chronic hepatitis B and C are leading causes of progressive liver fibrosis, largely mediated by persistent inflammation and oxidative stress. Non-invasive indices such as Fibrosis-4 (FIB-4) and Aspartate Aminotransferase to Platelet Ratio Index (APRI) are commonly used for fibrosis assessment; however, their relationship with oxidative stress biomarkers remains underexplored.

**Methods:** This cross-sectional study was conducted on 100 patients with chronic hepatitis B and C at tertiary care hospitals in Peshawar, Pakistan, from February 2022 to February 2023. Liver fibrosis was assessed using FIB-4 and APRI scores. Serum oxidative stress biomarkers, including malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH), were measured using ELISA. Statistical analysis was performed using Pearson correlation and multivariate regression.

**Results:** The mean FIB-4 and APRI scores were  $1.92 \pm 0.85$  and  $1.08 \pm 0.52$ , respectively. MDA levels showed a strong positive correlation with FIB-4 ( $r = 0.66$ ,  $p < 0.001$ ) and APRI ( $r = 0.61$ ,  $p < 0.001$ ). In contrast, SOD and GSH demonstrated significant negative correlations with both fibrosis indices ( $p < 0.01$ ). Patients with advanced fibrosis exhibited significantly higher MDA levels and reduced antioxidant levels compared to those with mild fibrosis. Multivariate analysis identified MDA as an independent predictor of fibrosis severity, while SOD and GSH were negative predictors.

**Conclusion:** Oxidative stress is significantly associated with liver fibrosis severity in chronic hepatitis B and C patients. Integration of oxidative stress biomarkers with non-invasive fibrosis scores may enhance the accuracy of liver disease assessment and monitoring.

**Keywords:** Chronic hepatitis B, Chronic hepatitis C, Liver fibrosis, FIB-4, APRI, Oxidative stress, Malondialdehyde, Superoxide dismutase, Glutathione

## INTRODUCTION

Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) remain major global health challenges, contributing significantly to the burden of chronic liver disease, liver fibrosis, cirrhosis, and hepatocellular carcinoma<sup>1</sup>. Despite advances in antiviral therapies, a substantial proportion of patients continue to progress toward advanced liver disease due to persistent inflammation and ongoing hepatocellular injury<sup>2</sup>. Early detection and accurate assessment of liver fibrosis are therefore critical for disease monitoring, prognostication, and therapeutic decision-making<sup>3</sup>.

Traditionally, liver biopsy has been regarded as the gold standard for assessing hepatic fibrosis<sup>4</sup>. However, its invasive nature, potential complications, sampling variability, and limited patient acceptance have led to increasing reliance on non-invasive methods<sup>5</sup>. Among these, the Fibrosis-4 (FIB-4) index and the Aspartate Aminotransferase to Platelet Ratio Index (APRI) are widely used due to their simplicity, cost-effectiveness, and reasonable diagnostic accuracy<sup>6</sup>. These scoring systems utilize routine laboratory parameters to estimate fibrosis severity and have been validated in various populations with chronic viral hepatitis<sup>7</sup>.

Oxidative stress has emerged as a fundamental mechanism underlying the pathogenesis and progression of chronic liver diseases<sup>8</sup>. It results from an imbalance between the generation of reactive oxygen species (ROS) and the capacity of antioxidant defense systems<sup>9</sup>. In chronic HBV and HCV infections, viral replication, immune-mediated cytotoxicity, and mitochondrial dysfunction collectively enhance oxidative stress, leading to lipid peroxidation, protein modification, and DNA damage<sup>10</sup>. These processes contribute directly to hepatocyte injury and indirectly to the activation of hepatic stellate cells, which play a central role in fibrogenesis<sup>11</sup>.

Biomarkers of oxidative stress provide valuable insight into the biochemical environment of the liver<sup>12</sup>. Malondialdehyde (MDA), a byproduct of lipid peroxidation, is widely used as an indicator of oxidative damage<sup>13</sup>. In contrast, antioxidant enzymes such as superoxide dismutase (SOD) and reduced glutathione (GSH) reflect the body's capacity to neutralize reactive oxygen species<sup>14</sup>. Alterations in these biomarkers have been consistently reported in chronic liver diseases and are believed to correlate with disease severity and progression<sup>15</sup>.

Recent research suggests a potential link between oxidative stress and non-invasive fibrosis indices<sup>16</sup>. Elevated levels of oxidative stress markers have been associated with higher fibrosis stages, while reduced antioxidant levels are observed in advanced disease<sup>17</sup>. However, data specifically correlating oxidative stress biomarkers with FIB-4 and APRI scores in patients with chronic hepatitis B and C remain limited, particularly in resource-constrained settings such as Pakistan, where the burden of viral hepatitis is substantial<sup>18</sup>.

Understanding this relationship may provide a more comprehensive, integrated approach to evaluating liver disease by combining biochemical and clinical indicators<sup>19</sup>. Such an approach could enhance the accuracy of non-invasive fibrosis assessment, reduce dependence on invasive procedures, and offer new perspectives for monitoring disease progression and therapeutic response<sup>20</sup>.

Therefore, the present study aims to evaluate the correlation between liver fibrosis scores (FIB-4 and APRI) and oxidative stress biomarkers (MDA, SOD, and GSH) in patients with chronic hepatitis B and C<sup>14</sup>, with the objective of exploring their combined diagnostic and prognostic utility<sup>17</sup>.

## MATERIALS AND METHODS

This cross-sectional observational study was conducted at the Department of General Medicine, Lady Reading Hospital, and MTI Hayatabad Medical Complex, Khyber Girls Medical College, over a period of one year from February 2022 to February 2023. The

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study aimed to evaluate the correlation between non-invasive liver fibrosis scores and oxidative stress biomarkers in patients diagnosed with chronic hepatitis B and C. A total of 100 patients were enrolled using a non-probability consecutive sampling technique from both outpatient and inpatient departments of the participating centers, ensuring representation of real-world clinical cases commonly encountered in tertiary care settings.

Patients aged between 18 and 65 years with a confirmed diagnosis of chronic hepatitis B or hepatitis C of more than six months duration were included in the study. Diagnosis was established based on positive serological markers, including hepatitis B surface antigen (HBsAg) for hepatitis B and anti-HCV antibodies for hepatitis C. Only those patients who provided written informed consent were enrolled. Patients were excluded if they had a history of significant alcohol consumption, non-alcoholic fatty liver disease, autoimmune hepatitis, co-infection with human immunodeficiency virus or hepatitis D virus, hepatocellular carcinoma, or any other malignancy. Additionally, patients with severe systemic illnesses, renal failure, or those who had received antiviral therapy within the last six months were also excluded to minimize confounding factors.

Detailed demographic and clinical information, including age, gender, disease duration, and relevant medical history, was recorded using a structured data collection proforma. A comprehensive clinical examination was performed for each patient. Venous blood samples were collected under aseptic conditions for laboratory investigations. Routine biochemical tests included liver function tests such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), along with complete blood count to determine platelet counts, which are essential parameters for calculating fibrosis indices.

Non-invasive liver fibrosis was assessed using the Fibrosis-4 (FIB-4) index and the Aspartate Aminotransferase to Platelet Ratio Index (APRI), calculated using standard validated formulas incorporating age, AST, ALT, and platelet count values. Based on these scores, patients were stratified into categories representing mild and advanced fibrosis to allow comparative analysis.

To evaluate oxidative stress status, serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions. Malondialdehyde was used as a marker of lipid peroxidation, while SOD and GSH were assessed as indicators of enzymatic and non-enzymatic antioxidant defense systems, respectively. All samples were processed in a standardized laboratory environment to ensure accuracy and reproducibility of results.

Ethical approval was obtained from the Institutional Review Boards of both participating institutions prior to the commencement of the study, and all procedures were carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant after explaining the purpose and nature of the study, ensuring confidentiality and the right to withdraw at any stage without any consequences.

Statistical analysis was performed using SPSS version 26. Continuous variables were expressed as mean  $\pm$  standard deviation, while categorical variables were presented as frequencies and percentages. Pearson correlation analysis was applied to determine the relationship between liver fibrosis scores (FIB-4 and APRI) and oxidative stress biomarkers (MDA, SOD, and GSH). Independent sample t-tests were used to compare differences between groups, and multivariate linear regression analysis was conducted to identify independent predictors of fibrosis severity. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

A total of 100 patients with chronic hepatitis B and C were included in the study. The mean age of the study population was  $43.8 \pm 12.1$  years, with a male predominance of 60%. Biochemical evaluation

revealed elevated liver enzymes, with mean AST and ALT levels of  $78.5 \pm 28.4$  U/L and  $85.2 \pm 31.6$  U/L, respectively, indicating ongoing hepatic inflammation. The mean platelet count was  $165 \pm 52 \times 10^9/L$ . Non-invasive fibrosis assessment showed a mean FIB-4 score of  $1.92 \pm 0.85$  and an APRI score of  $1.08 \pm 0.52$ , suggesting that a considerable proportion of patients had moderate to advanced fibrosis. Evaluation of oxidative stress markers demonstrated elevated malondialdehyde (MDA) levels ( $6.1 \pm 1.7$  nmol/mL), along with reduced antioxidant levels, including superoxide dismutase (SOD) ( $2.0 \pm 0.6$  U/mL) and reduced glutathione (GSH) ( $3.3 \pm 1.0$   $\mu$ mol/L), reflecting a significant oxidative imbalance in the study population (Table 1).

Further statistical analysis using Pearson correlation demonstrated a strong and statistically significant association between liver fibrosis scores and oxidative stress biomarkers. Malondialdehyde (MDA) showed a strong positive correlation with both FIB-4 ( $r = 0.66$ ,  $p < 0.001$ ) and APRI ( $r = 0.61$ ,  $p < 0.001$ ), indicating that lipid peroxidation increases with worsening fibrosis severity. In contrast, antioxidant markers exhibited inverse relationships with fibrosis indices. Superoxide dismutase (SOD) showed a negative correlation with FIB-4 ( $r = -0.51$ ) and APRI ( $r = -0.47$ ), while reduced glutathione (GSH) demonstrated similar negative correlations (FIB-4:  $r = -0.55$ , APRI:  $r = -0.50$ ), both statistically significant ( $p < 0.01$ ). These findings suggest that antioxidant defenses decline as liver fibrosis progresses, further supporting the role of oxidative stress in hepatic injury (Table 2).

To further evaluate the impact of fibrosis severity, patients were stratified into mild and advanced fibrosis groups based on standard FIB-4 and APRI cut-off values. Comparative analysis revealed that patients with advanced fibrosis had significantly higher levels of MDA ( $6.9 \pm 1.5$  nmol/mL) compared to those with mild fibrosis ( $5.0 \pm 1.2$  nmol/mL,  $p < 0.001$ ), indicating increased oxidative damage. Conversely, antioxidant markers were significantly reduced in the advanced fibrosis group, with SOD levels decreasing from  $2.4 \pm 0.6$  U/mL in mild fibrosis to  $1.7 \pm 0.5$  U/mL in advanced fibrosis ( $p < 0.001$ ), and GSH levels decreasing from  $4.0 \pm 1.1$   $\mu$ mol/L to  $2.8 \pm 0.9$   $\mu$ mol/L ( $p < 0.001$ ). These findings clearly demonstrate that oxidative stress intensifies while antioxidant defenses deteriorate with increasing fibrosis severity (Table 3).

In addition, multivariate linear regression analysis identified MDA as an independent positive predictor of liver fibrosis severity ( $\beta = 0.58$ ,  $p < 0.001$ ), whereas SOD ( $\beta = -0.42$ ,  $p = 0.003$ ) and GSH ( $\beta = -0.46$ ,  $p = 0.002$ ) were found to be independent negative predictors after adjusting for age, gender, and liver enzyme levels. These results further confirm that oxidative stress biomarkers are significantly associated with fibrosis progression and may serve as valuable adjuncts to non-invasive fibrosis scoring systems.

Table 1: Baseline Characteristics and Laboratory Parameters of Study Participants (n = 100)

Variable	Mean $\pm$ SD / Frequency (%)
Age (years)	$43.8 \pm 12.1$
Male	60 (60%)
Female	40 (40%)
AST (U/L)	$78.5 \pm 28.4$
ALT (U/L)	$85.2 \pm 31.6$
Platelet Count ( $\times 10^9/L$ )	$165 \pm 52$
FIB-4 Score	$1.92 \pm 0.85$
APRI Score	$1.08 \pm 0.52$
MDA (nmol/mL)	$6.1 \pm 1.7$
SOD (U/mL)	$2.0 \pm 0.6$
GSH ( $\mu$ mol/L)	$3.3 \pm 1.0$

Table 2: Correlation Between Liver Fibrosis Scores and Oxidative Stress Biomarkers

Parameter	FIB-4 (r-value)	APRI (r-value)	p-value
MDA	+0.66	+0.61	<0.001
SOD	-0.51	-0.47	<0.01
GSH	-0.55	-0.50	<0.01

Table 3: Comparison of Oxidative Stress Biomarkers Based on Fibrosis Severity

Variable	Mild Fibrosis	Advanced Fibrosis	p-value
MDA (nmol/mL)	5.0 ± 1.2	6.9 ± 1.5	<0.001
SOD (U/mL)	2.4 ± 0.6	1.7 ± 0.5	<0.001
GSH (µmol/L)	4.0 ± 1.1	2.8 ± 0.9	<0.001

## DISCUSSION

The present study demonstrates a strong and statistically significant association between non-invasive liver fibrosis scores (FIB-4 and APRI) and oxidative stress biomarkers in patients with chronic hepatitis B and C<sup>1</sup>. The findings highlight that oxidative stress plays a central role in the progression of liver fibrosis, with increasing lipid peroxidation and declining antioxidant defenses corresponding closely with worsening fibrosis severity<sup>2</sup>.

One of the key findings of this study is the strong positive correlation between malondialdehyde (MDA) and fibrosis indices<sup>3</sup>. MDA, a well-established marker of lipid peroxidation, reflects oxidative damage to cellular membranes and hepatocytes<sup>4</sup>. The observed increase in MDA levels with higher FIB-4 and APRI scores suggests that oxidative injury intensifies as fibrosis advances<sup>5</sup>. This is consistent with previous studies, such as Najafi et al. (2024), who reported that higher fibrosis stages were associated with significantly elevated oxidative stress markers, indicating progressive hepatocellular damage in chronic viral hepatitis<sup>6</sup>. Similarly, Rungta et al. demonstrated that MDA levels were markedly increased in patients with advanced liver fibrosis, reinforcing its role as a surrogate marker of disease severity<sup>7</sup>.

In contrast, antioxidant biomarkers such as superoxide dismutase (SOD) and reduced glutathione (GSH) showed significant inverse relationships with fibrosis scores in the present study<sup>8</sup>. This decline in antioxidant levels reflects exhaustion of the body's defense mechanisms against reactive oxygen species (ROS)<sup>9</sup>. These findings are supported by studies conducted by Verma et al., who reported reduced SOD activity and glutathione levels in patients with chronic hepatitis, particularly in those with advanced fibrosis and cirrhosis<sup>10</sup>. The depletion of antioxidants not only exacerbates oxidative damage but also contributes to the activation of hepatic stellate cells, which are central mediators of fibrogenesis<sup>11</sup>.

The comparative analysis between mild and advanced fibrosis groups in this study further strengthens the role of oxidative stress in disease progression<sup>12</sup>. Patients with advanced fibrosis exhibited significantly higher MDA levels and markedly reduced SOD and GSH levels<sup>13</sup>. This pattern is in agreement with findings from Górski et al. (2024), who demonstrated that oxidative stress imbalance is directly linked with tissue remodeling and fibrosis progression in chronic liver diseases<sup>14</sup>. The progressive increase in oxidative damage alongside declining antioxidant capacity suggests a shift toward a pro-oxidant state, which accelerates fibrogenesis and liver dysfunction<sup>15</sup>.

Another important observation is the identification of MDA as an independent positive predictor of fibrosis severity, while SOD and GSH were independent negative predictors<sup>16</sup>. This indicates that oxidative stress biomarkers have prognostic significance beyond conventional biochemical parameters<sup>17</sup>. Similar conclusions were drawn by earlier studies, which emphasized that integrating oxidative stress markers with non-invasive scoring systems improves diagnostic accuracy and risk stratification in chronic hepatitis patients<sup>18</sup>.

From a clinical perspective, the findings of this study suggest that combining oxidative stress biomarkers with established non-invasive fibrosis indices such as FIB-4 and APRI may provide a more comprehensive assessment of liver disease<sup>19</sup>. This integrated approach could enhance early detection of fibrosis progression, reduce reliance on invasive liver biopsy, and assist clinicians in monitoring therapeutic response more effectively, particularly in resource-limited settings like Pakistan where access to advanced diagnostic tools may be restricted<sup>20</sup>.

Despite its strengths, this study has certain limitations<sup>13</sup>. The cross-sectional design limits the ability to establish causality between oxidative stress and fibrosis progression<sup>9</sup>. The sample size, although adequate, was relatively modest and derived from a limited number of centers, which may affect generalizability<sup>7</sup>. Additionally, liver biopsy, the gold standard for fibrosis assessment, was not performed, and fibrosis staging relied solely on non-invasive indices<sup>11</sup>. Future multicenter longitudinal studies with larger sample sizes and histopathological correlation are recommended to validate these findings and further explore the mechanistic pathways linking oxidative stress to liver fibrosis<sup>14</sup>.

## CONCLUSION

In conclusion, the present study demonstrates a significant association between oxidative stress and liver fibrosis severity in patients with chronic hepatitis B and C. Elevated levels of malondialdehyde and reduced levels of antioxidant markers such as superoxide dismutase and glutathione correlate strongly with higher FIB-4 and APRI scores. These findings suggest that oxidative stress plays a crucial role in the pathogenesis and progression of liver fibrosis. Incorporating oxidative stress biomarkers alongside non-invasive fibrosis indices may enhance the accuracy of liver disease assessment and provide a valuable, cost-effective approach for monitoring disease progression in clinical practice.

**Authors' Contributions:** M.I.K conceived and designed the study. A.U.R and M.B collected data and performed laboratory work. I.A.S conducted statistical analysis. M.A.R assisted in manuscript drafting. M.F contributed to interpretation and revision. All authors approved the final manuscript.

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