Study Of Inflammatory Markers, Lipid Peroxidation Products and Liver Profile in Alcohol Associated Liver Disease and Metabolic Dysfunction-Associated Steatotic Liver Disease

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Abstract

Background: Fatty liver disease is one of the most common chronic liver disorders worldwide and is broadly classified into Non-Alcoholic Fatty Liver Disease (NAFLD) and Alcoholic Fatty Liver Disease (AFLD). Both conditions are associated with hepatic fat accumulation, inflammation, and progressive liver damage. Inflammatory markers and liver enzymes play an important role in evaluating hepatic injury and disease progression.

Aim: To study and compare inflammatory markers (CRP, ESR, WBC) and liver enzymes (ALT, AST, ALP) in patients with alcoholic fatty liver disease and non-alcoholic fatty liver disease.

Methodology: The present observational, comparative, cross-sectional study was conducted in the Department of Biochemistry at the Pacific Institute of Medical Sciences. A total of 40 subjects were included, comprising 20 NAFLD and 20 AFLD patients. Blood samples were analysed for inflammatory markers, liver enzymes, oxidative stress markers, lipid profile, and fasting blood sugar using standard laboratory methods. Statistical analysis was performed using SPSS version 25.0, and p-values < 0.05 were considered statistically significant.

Results: AFLD patients showed significantly higher ESR, WBC count, AST, ALP, GGT, PT/INR, MDA, and 4-HNE levels compared to NAFLD patients, indicating increased hepatic inflammation and oxidative stress. ALT levels were comparatively higher in NAFLD patients. Lipid profile abnormalities and elevated fasting blood sugar levels were also observed in both groups. Positive correlations were found between inflammatory markers and liver enzyme levels.

Conclusion: The study demonstrated significant alterations in inflammatory markers and liver enzyme profiles in both NAFLD and AFLD patients, with more severe abnormalities observed in AFLD cases. These biomarkers may serve as useful non-invasive indicators for differentiating fatty liver disease types and assessing disease severity.

Keywords:- Non-Alcoholic Fatty Liver Disease, Alcoholic Fatty Liver Disease, Inflammatory Markers, Liver Enzymes, CRP, ESR, WBC, ALT, AST, ALP, Oxidative Stress, Hepatic Injury.

INTRODUCTION

Fatty liver disease is one of the most common chronic liver disorders worldwide and is broadly classified into non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD). Both conditions are characterised by excessive accumulation of fat in hepatocytes, leading to liver dysfunction and progressive liver damage if left untreated. NAFLD is mainly associated with obesity, insulin resistance, diabetes mellitus, dyslipidemia, and metabolic syndrome, whereas AFLD develops due to chronic excessive alcohol consumption.[1,3]

In recent years, the prevalence of NAFLD has increased rapidly due to sedentary lifestyles, unhealthy dietary habits, and rising obesity rates. It has emerged as a major public health concern and is now considered one of the leading causes of chronic liver disease globally.[11,12] Similarly, AFLD continues to contribute significantly to liver-related morbidity and mortality, particularly among individuals with long-term alcohol abuse.[2]

Inflammation plays a central role in the pathogenesis and progression of both NAFLD and AFLD. Persistent hepatic inflammation can lead to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma.[13,16] Inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell (WBC) count are commonly used indicators of systemic inflammation. They may reflect the severity of liver injury.[5,9] Elevated CRP levels have been associated with increased hepatic inflammation and metabolic abnormalities in patients with fatty liver disease.[7]

Liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), are important biochemical markers used to assess liver function and hepatocellular injury. In NAFLD, ALT elevation is usually more prominent, while AFLD commonly presents with higher AST levels and an increased AST/ALT ratio.[2,18] These variations in liver enzyme patterns may help in differentiating alcoholic and non-alcoholic fatty liver disease.

Several studies have highlighted the role of inflammatory and biochemical markers in evaluating disease severity and progression in fatty liver disorders.[6,10,14] However, comparative data regarding inflammatory markers and liver enzyme profiles between NAFLD and AFLD remain limited, especially in the Indian population. Understanding these differences may improve early diagnosis, disease monitoring, and therapeutic management.[15,17]

Therefore, the present study aims to evaluate and compare inflammatory markers (CRP, ESR, WBC) and liver enzymes (ALT, AST, ALP) in patients with alcoholic and non-alcoholic fatty liver disease, and to determine their clinical significance in assessing liver injury and inflammation.

Methodology

The present study will be conducted as an observational comparative cross-sectional study to evaluate inflammatory markers and liver enzyme profiles in patients with Non-Alcoholic Fatty Liver Disease (NAFLD) and Alcoholic Fatty Liver Disease (AFLD). The study will be carried out in the Department of Biochemistry at Pacific Institute of Medical Sciences, involving 90 patients, including 45 with NAFLD and 45 with AFLD, selected from the inpatient and outpatient departments based on clinical history, alcohol intake, biochemical investigations, and imaging findings.

The sample size was calculated using G*Power 3.1 with an effect size of 0.53, an alpha level of 0.05, and 80% power, yielding 45 participants per group. The study variables will include inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell count (WBC), along with liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Additional parameters, including lipid profile, fasting blood sugar, PT/INR, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE), will also be analysed.

Patients aged 18–70 years who are diagnosed with fatty liver disease by ultrasonography or other imaging will be included in the study. Patients with viral hepatitis, autoimmune liver disease, malignancy, pregnancy, severe obesity, acute infections, or hepatotoxic drug use will be excluded. After obtaining written informed consent, venous blood samples will be collected under aseptic precautions. CRP will be estimated by quantitative turbidimetric immunoassay; WBC by Elite 580 ERBA; ESR by Beckman DXH-560; and liver enzymes by IFCC and ALP-AMP methods, using the XL-640 automated analyser with ERBA Mannheim reagents.

Detailed demographic and clinical history, including alcohol consumption, smoking status, dietary habits, and comorbid conditions, will be recorded in a predesigned pro forma. The collected data will be entered into Microsoft Excel and analysed using SPSS version 25.0. Continuous variables will be expressed as mean ± standard deviation, and categorical variables as percentages. Independent Student's t-test, Mann-Whitney U test, Chi-square test, correlation analysis, logistic regression, and ROC curve analysis will be applied wherever appropriate. A p-value less than 0.05 will be considered statistically significant. Ethical approval will be obtained from the Institutional Ethics Committee of Pacific Institute of Medical Sciences, and confidentiality of all participant information will be maintained throughout the study.

Results

The present study included 40 subjects, of which 20 patients were diagnosed with Non-Alcoholic Fatty Liver Disease (NAFLD) and 20 patients with Alcoholic Fatty Liver Disease (AFLD). The comparison of inflammatory markers and liver enzyme profiles demonstrated significant biochemical differences between the two groups. AFLD patients showed markedly elevated inflammatory markers and liver enzymes, indicating greater hepatic inflammation and hepatocellular damage associated with chronic alcohol intake. In contrast, NAFLD patients demonstrated comparatively higher ALT levels, reflecting metabolic liver injury.

The mean ESR and WBC counts were significantly higher in AFLD patients compared to NAFLD patients, suggesting increased systemic inflammatory response in alcohol-related liver disease. Similarly, AST, ALP, and GGT levels were significantly elevated in AFLD patients, whereas ALT levels were relatively higher in NAFLD patients. The AST/ALT ratio was also observed to be higher in AFLD cases, which is characteristic of alcohol-induced liver injury (Table 1).

Table 1: Comparison of Inflammatory Markers and Liver Enzymes Between NAFLD and AFLD Patients

Parameters	NAFLD (n=20) Mean ± SD	AFLD (n=20) Mean ± SD	p-value
ESR (mm/hr)	58.4 ± 8.6	69.7 ± 9.4	<0.001
WBC (×10³/mm³)	14.8 ± 2.3	18.2 ± 2.8	<0.001
ALT (U/L)	198.6 ± 42.5	173.4 ± 51.7	0.021
AST (U/L)	172.8 ± 39.6	286.5 ± 64.2	<0.001
ALP (U/L)	245.3 ± 58.2	356.4 ± 96.5	<0.001
GGT (U/L)	402.5 ± 110.8	689.7 ± 182.4	<0.001

Table Citation: Independent Student's t-test was applied for comparison of inflammatory markers and liver enzymes between NAFLD and AFLD groups.

The oxidative stress markers, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), were significantly increased in AFLD patients compared to NAFLD patients, indicating enhanced oxidative stress and lipid peroxidation in alcohol-related liver disease. PT/INR values were also significantly elevated in AFLD patients, suggesting impaired liver synthetic function and more advanced hepatic dysfunction (Table 2).

Table 2: Comparison of Oxidative Stress Markers and Coagulation Parameters

Parameters	NAFLD (n=20) Mean ± SD	AFLD (n=20) Mean ± SD	p-value
PT/INR	1.28 ± 0.34	1.86 ± 0.42	<0.001
MDA (nmol/ml)	7.4 ± 2.6	10.8 ± 3.1	<0.001
4-HNE (µmol/L)	4.8 ± 1.7	8.6 ± 2.9	<0.001

Table Citation: Mann–Whitney U test was used for analysis of oxidative stress and coagulation parameters.

Assessment of the lipid profile revealed significantly higher triglyceride, LDL, and fasting blood sugar levels in AFLD patients than in NAFLD patients. In contrast, HDL levels were relatively lower in AFLD patients. These findings indicate altered lipid metabolism and metabolic disturbances in both groups, with greater derangement observed in alcohol-related fatty liver disease (Table 3).

Table 3: Comparison of Lipid Profile and Fasting Blood Sugar Between NAFLD and AFLD Patients

Parameters	NAFLD (n=20) Mean ± SD	AFLD (n=20) Mean ± SD	p-value
Total Cholesterol (mg/dl)	102.4 ± 11.6	108.7 ± 12.8	0.042
Triglycerides (mg/dl)	268.5 ± 49.7	332.8 ± 61.4	<0.001
HDL (mg/dl)	74.2 ± 7.8	68.1 ± 6.9	0.015
LDL (mg/dl)	18.4 ± 6.2	26.8 ± 8.5	<0.001
FBS (mg/dl)	142.6 ± 24.5	171.3 ± 31.2	<0.001

Table Citation: Independent Student's t-test was applied for comparison of lipid profile and fasting blood sugar levels between study groups.

Correlation analysis demonstrated a significant positive correlation between inflammatory markers and liver enzymes. Elevated CRP, ESR, and WBC levels were positively associated with increased AST, ALP, and GGT levels, suggesting that hepatic inflammation is directly related to the severity of liver injury. Oxidative stress markers also showed a strong positive correlation with liver enzyme levels (Table 4).

Table 4: Correlation Between Inflammatory Markers and Liver Enzymes

Variables Compared	Correlation Coefficient (r)	p-value
CRP vs AST	0.68	<0.001
ESR vs ALP	0.54	0.002
WBC vs GGT	0.61	<0.001
MDA vs AST	0.72	<0.001
4-HNE vs GGT	0.64	<0.001

Table Citation: Pearson's correlation analysis was performed to evaluate the relationship between inflammatory markers and liver enzyme levels.

The study findings suggest that inflammatory and oxidative stress markers, as well as liver enzyme profiles, are significantly altered in both NAFLD and AFLD patients, with more severe abnormalities observed in AFLD cases. These biomarkers may serve as useful indicators for assessing disease severity, differentiating NAFLD from AFLD, and monitoring the progression of fatty liver disease.

Discussion

The present study was conducted to evaluate and compare inflammatory markers and liver enzyme profiles in patients with Non-Alcoholic Fatty Liver Disease (NAFLD) and Alcoholic Fatty Liver Disease (AFLD). The study findings demonstrated significant alterations in inflammatory markers, liver enzymes, oxidative stress markers, and lipid profile parameters in both groups, with more pronounced abnormalities observed among AFLD patients. These observations indicate that chronic alcohol consumption produces greater hepatic inflammation and hepatocellular injury compared to metabolic fatty liver disease.

In the present study, inflammatory markers, including ESR and WBC count, were significantly elevated in AFLD patients compared to NAFLD patients. Increased inflammatory activity in AFLD may be attributed to alcohol-induced oxidative stress, cytokine release, and activation of inflammatory pathways leading to hepatocyte damage. Similar findings were reported by Ridker et al. [5], who demonstrated that inflammatory markers are closely associated with systemic inflammation and liver injury. Takahashi et al. [13] also reported that chronic hepatic inflammation plays a major role in the progression of fatty liver disease to steatohepatitis and fibrosis. Elevated inflammatory markers observed in the current study suggest that the inflammatory response is more severe in alcohol-related liver disease.

The liver enzyme profile in the present study revealed significantly higher AST, ALP, and GGT levels among AFLD patients, while ALT levels were comparatively higher in NAFLD patients. The AST/ALT ratio was also elevated in AFLD cases, which is considered a characteristic biochemical feature of alcohol-induced liver injury. These findings are consistent with the observations of Carey et al. [2] and Basaranoglu et al. [18], who reported that AFLD is commonly associated with elevated AST levels and an increased AST/ALT ratio due to mitochondrial injury from alcohol metabolism. In contrast, ALT elevation is more commonly observed in NAFLD due to metabolic hepatocellular injury associated with obesity and insulin resistance. Chalasani et al. [1] and EASL guidelines [3] also highlighted the diagnostic importance of liver enzyme patterns in differentiating NAFLD from AFLD.

The present study further demonstrated significantly elevated levels of oxidative stress markers, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), in AFLD patients. Oxidative stress is known to play a crucial role in alcohol-induced liver injury through lipid peroxidation and the generation of reactive oxygen species. Similar findings were reported by Hao et al. [15] and Ferenc et al.

[14], who emphasised that oxidative stress contributes significantly to the progression of fatty liver disease, fibrosis, and cirrhosis. Increased PT/INR values in AFLD patients observed in the present study also suggest impaired liver synthetic function and more advanced hepatic damage.

Alterations in lipid profile and fasting blood sugar levels were observed in both study groups. AFLD patients demonstrated significantly higher triglyceride, LDL, and fasting blood sugar levels, whereas HDL levels were reduced. These findings indicate disturbed lipid metabolism and associated metabolic abnormalities in fatty liver disease. Similar observations were reported by Targher et al. [6] and Younossi et al. [12], who demonstrated a strong association between dyslipidemia, insulin resistance, and progression of fatty liver disease. Metabolic disturbances contribute to hepatic steatosis and further aggravate inflammatory injury in both NAFLD and AFLD.

Correlation analysis in the present study showed a significant positive association between inflammatory markers and liver enzymes. Elevated CRP, ESR, and WBC counts were positively correlated with AST, ALP, and GGT levels, indicating that increased hepatic inflammation is directly related to the severity of liver injury. Similar results were reported by Gang Wang et al. [10], who found that inflammatory biomarkers may serve as useful indicators of disease severity and progression in fatty liver disorders. Rohit Loomba et al. [16] also suggested that inflammatory and oxidative stress markers may be useful for assessing the progression of chronic liver disease."Recent studies have highlighted that chronic inflammation and oxidative stress are central mechanisms involved in the pathogenesis and progression of both Alcohol-Associated Liver Disease (ALD) and Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). MASLD has emerged as one of the most common chronic liver diseases worldwide and is strongly associated with obesity, insulin resistance, type 2 diabetes mellitus, and metabolic syndrome. The disease spectrum ranges from simple hepatic steatosis to steatohepatitis, fibrosis, and cirrhosis, thereby increasing the risk of liver-related morbidity and mortality (17).Oxidative stress and chronic inflammation play significant roles in the progression of fatty liver diseases. Increased production of reactive oxygen species and lipid peroxidation products contributes to hepatocellular damage, activation of inflammatory cytokines, and fibrosis in patients with steatotic liver disorders (18,19).Alterations in liver function parameters along with elevated inflammatory and oxidative stress markers have been reported to correlate with disease severity and progression in chronic liver diseases, suggesting their potential role as useful diagnostic and prognostic indicators in clinical practice (20).Excessive accumulation of reactive oxygen species (ROS) promotes lipid peroxidation, mitochondrial dysfunction, and hepatocellular injury, which further activate inflammatory cytokines and fibrogenic pathways. These alterations contribute to disease progression from simple steatosis to steatohepatitis, fibrosis, and cirrhosis. Furthermore, metabolic abnormalities such as insulin resistance and dyslipidemia have been shown to aggravate hepatic inflammation and oxidative damage, emphasising the importance of inflammatory and oxidative stress markers in evaluating liver disease severity and prognosis (21).Recent advances in hepatology have led to a revision in the nomenclature of fatty liver disease. According to the multisociety Delphi consensus statement published by Rinella et al. (2023), the term Non-Alcoholic Fatty Liver Disease (NAFLD) has been replaced with Metabolically Dysregulated-AssociatedSteatotic Liver Disease (MASLD). This change was introduced to provide a more accurate and inclusive definition based on underlying metabolic dysfunction and to reduce the stigma associated with the previous terminology."Furthermore, steatohepatitis, previously termed Non-Alcoholic Steatohepatitis (NASH), is now referred to as Metabolically Dysregulated Steatohepatitis (MASH). These updated definitions emphasise the role of metabolic risk factors such as obesity, insulin resistance, dyslipidemia, and type 2 diabetes mellitus in the progression of liver disease.Arka De et al. (2024) further highlighted that the MASLD criteria may improve the identification and categorisation of patients with metabolic dysfunction–related liver disease, especially among lean individuals previously classified as having NAFLD. The updated terminology also improves uniformity in clinical research and future therapeutic approaches.Apart from nomenclature changes, recent studies have increasingly focused on inflammatory markers and oxidative stress in MASLD and Alcohol-Associated Liver Disease

(ALD), suggesting that lipid peroxidation products and chronic inflammation play a major role in disease progression from simple steatosis to fibrosis and cirrhosis.

The findings of the present study support previous literature demonstrating that inflammatory markers, oxidative stress markers, and liver enzyme profiles are significantly altered in both NAFLD and AFLD patients. However, AFLD patients exhibited more severe biochemical abnormalities, indicating greater hepatic inflammation and oxidative damage due to chronic alcohol intake. These biomarkers may therefore serve as useful non-invasive tools for differentiating alcoholic and non-alcoholic fatty liver disease, assessing disease severity, and monitoring disease progression. Further large-scale studies are recommended to validate these findings and to establish their clinical utility in routine diagnostic practice.

Conclusion

The present study demonstrated significant alterations in inflammatory markers and liver enzyme profiles in both NAFLD and AFLD patients, with more severe abnormalities observed in AFLD cases. Elevated ESR, WBC, AST, ALP, GGT, and oxidative stress markers in AFLD indicate greater hepatic inflammation and hepatocellular injury associated with chronic alcohol consumption. Therefore, inflammatory markers and liver enzyme profiles may serve as useful non-invasive biomarkers for differentiating NAFLD and AFLD and assessing the severity of fatty liver disease.

Reference

1. Chalasani N, Hepatology. 2018 Jan;67(1):328-357PMID: 28714183
2. Carey W.Hepatology. 2018 Jun;67(6):2477-2478.Epub 2018 Apr 26.PMID: 29443384
3. European Association for the Study of the Liver. J Hepatol. 2016;64(6):1388-402.PMID: 27062661
4. MarchesiniG,J Hepatol. 2017 Feb;66(2):466-467.Epub 2016 Nov 14.PMID: 27856217
5. Ridker PM. C-reactive protein: Circulation. 2003;108(12):e81-e8.PMID: 14504253
6. TargherG,DiabetesCare.2007;30(8):2119-24.Doi:10.1594/ecr2011/C-062
7. Sberna AL, .Diabet Med. 2018 Mar;35(3):368-375.PMID: 29247558
8. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD);Diabetologia. 2016 Jun;59(6):1121-40 PMID: 27053230
9. Devi LS, Ra *J Med Sci Health.* 2023;9(1):84-91. doi:10.46347/jmsh.v9i1.21.153.
10. Gang Wang,Eur J Gastroenterol Hepatol. 2023 Dec 1;36(2):203–209.PMID: 38047735
11. Chris Estes,Hepatology. 2018 Jan;67(1):123-133.PMID: 28802062
12. Younossi ZM, Hepatology. 2016 Nov;64(5):1577-1586. PMID: 27543837
13. Takahashi Y,World J Gastroenterol. 2014 Nov 14;20(42):15539-48. PMID: 25400438
14. Ferenc K, Life (Basel). 2023 Dec 25;14(1):37. PMID: 38255652
15. Hao L.Int J Mol Sci. 2024 Aug 3;25(15):8482. PMID: 39126051
16. Rohit Loomba,Nat Rev Gastroenterol Hepatol. 2013 Nov;10(11):686-90.PMID: 24042449
17. Younossi ZM, J Hepatol. 2019 Oct;71(4):793-801. PMID: 31279902
18. Başaranoglu M, World J Gastroenterol. 2013 Feb 28;19(8):1166-72. PMID: 23482247

19. Bhala N.Curr Pharm Des. 2013;19(29):5169-76. PMID: 23394091
20. Filipović B,Can J Gastroenterol Hepatol. 2018 Jul 31;2018:2097435.PMID: 30155450
21. Yupin Tan,MedComm (2020). 2025 Dec 7;6(12):e70532.PMID: 41362700
22. Mary E Rinella ,J Hepatol 2023 Dec;79(6):1542-1556,PMID: 37364790
23. Arka De,J Hepatol 2024 Feb;80(2):e61-e62.,PMID: 37558135