Original Article

Serum Adiponectin and Tumor Necrosis Factor-alpha (TNF-α) as Non-Invasive Biomarkers for Early Detection and Risk Stratification of Liver Fibrosis in MASLD Patients

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Abstract

Background: Metabolic dysfunction associated steatotic liver disease (MASLD) is a progressive condition that may lead to fibrosis, cirrhosis, and hepatocellular carcinoma. This requires early identification of steatosis and fibrosis, which is best achieved with non-invasive measures such as FibroScan; however, liver biopsy is an invasive procedure that is not available in resource-limited settings. These difficulties suggest the necessity of low-cost, effective serum biomarkers. This paper compares serum adiponectin and TNF-α levels to evaluate fibrosis in MASLD patients from diverse population groups with varying socioeconomic and resource status. **Results:** Among the 113 patients with MASLD, male predominance was substantial ($\chi 2 = 24.03$, $p = 1.61e\text{-}06$). Liver metrics had sex-specific variations. Whereas females had more platelets ($p = 0.048$), males had higher ALT (alanine aminotransferase) than females ($p = 0.001$). There were no significant sex-based differences in the value of aspartate aminotransferase (AST) and fasting blood sugar (FBS). Fibrosis was predominantly distributed at early stages, with 43.4% of Fibrosis-Stage (F) 1 and 34.5% F2. Serum TNF-α levels increased progressively with fibrosis severity (F1: 7.91 ± 11.7 ng/mL to F4: 97.8 ± 39.1 ng/mL), whereas adiponectin levels decreased (F1: 5.99 ± 1.37 µg/mL to F4: 1.51 ± 0.16 µg/mL). TNF-α showed a positive correlation with LSM ($\rho = 0.663$, $p = 9.999e\text{-}14$), while adiponectin exhibited a strong negative correlation ($\rho = -0.845$, $p = 5.83e\text{-}32$). According to ROC evaluation (AUC = 0.971; threshold = less than 3.055 µg/mL; sensitivity = 95.8%, specificity = 95.9 proved to be impressive), adiponectin showed an impressive diagnostic value in distinguishing between mild (F1-F2) and severe (F3-F4) fibrosis. TNF-alpha was mediocre (AUC = 0.717) compared to AST, which has poor accuracy (AUC = 0.649). **Conclusion:** Non-invasive serum adiponectin and TNF-alpha levels are useful for assessing fibrosis severity in MASLD. TNF-α is the pro-inflammatory side of the fibrosis progression and augments the diagnostic accuracy of adiponectin. These indicators could help reduce invasive procedures by enabling early identification, risk assessment, and non-invasive monitoring of hepatic fibrosis.

Keywords: TNF-alpha, Metabolic dysfunction associated steatotic liver disease (MASLD), Adiponectin, Fibrosis, FibroScan.

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Introduction

Metabolic dysfunction associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), is the most common cause of chronic liver disease worldwide and affects about 25% of people globally [1] It is the primary contributor to hepatocellular carcinoma (HCC) and cirrhosis. It has contributed to liver-related mortality, making it one of the most rapidly growing indicators of liver transplantation across the world.[2] There are differences in diet, lifestyle, genetic susceptibility, and socioeconomic status that manifest as the prevalence of MASLD varies by region, ranging from 13.5% in Africa to 31.8% in the Middle East. The condition mainly affects individuals with type 2 diabetes (T2DM) (47.3–63.7%) and obesity (up to 80%), with a male-to-female ratio of approximately 9:1.[3]

Numerous conditions, such as cirrhosis, progressive fibrosis, non-alcoholic steatohepatitis (NASH), and simple steatosis, are associated with Metabolic dysfunction associated steatotic liver disease (MASLD).[4] Its natural history varies; according to biopsy investigations, 29% of patients experience regression, 34% experience stability, and 37% experience fibrosis advancement. The course of the disease is similar to that of cirrhosis from other causes once it manifests.[5] Among the many pathogenic drivers, obesity plays a central role by altering the adipokine milieu. Adipose tissue, beyond its role as an energy reservoir, functions as an endocrine organ that secretes bioactive peptides known as adipocytokines. These molecules exert either pro-inflammatory actions (e.g., leptin, TNF-α, interleukin-6 [IL-6]) or anti-inflammatory, anti-steatotic effects (e.g., adiponectin).

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Dysregulation of these adipocytokines promotes insulin resistance, inflammation, and hepatic injury.[5]

Adiponectin, an adipocyte complement-related protein of 30 kDa (ACRP30), is encoded by the apM1 gene. It shares structural homology with collagen and complements C1q, and its molecular structure resembles TNF-α, although their effects are functionally antagonistic.[6] Adiponectin exerts anti-inflammatory, anti-diabetic, and anti-obesity effects, partly by suppressing TNF-α activity.[7] In divergence, TNF-α, a key pro-inflammatory cytokine in MASLD pathogenesis, is secreted by Kupffer cells, infiltrating immune cells, and dysfunctional visceral adipose tissue. TNF-α signals through TNFR1 and TNFR2, activating the JNK and NF-κB pathways, thereby promoting inflammation, hepatocyte injury, and fibrogenesis.[8]

Liver fibrosis, a common consequence of chronic liver injury, is characterized by excessive extracellular matrix (ECM) deposition and structural distortion of the liver, ultimately progressing to cirrhosis, liver failure, or HCC.[9] In MASLD, fibrosis progression is driven by multiple mechanisms, including insulin resistance, oxidative stress, gut dysbiosis, and adipocytokine imbalance.[10] The primary process in fibrogenesis is the activation of hepatic stellate cells (HSCs), which is triggered by signals from Kupffer cells, free fatty acids, cytokines, and adipokines like TNF-α and adiponectin.[11,12]

There is a pressing need for trustworthy, non-invasive biomarkers to evaluate fibrosis because of the rapidly rising incidence of MASLD and the drawbacks of liver biopsy as an invasive and expensive technique. As opposing arms of the inflammatory axis, adiponectin and TNF-α are important in the pathophysiology of disease and could be easily obtained serum-based biomarkers. Nevertheless, there is still little and conflicting data on their combined association with fibrosis severity, especially across diverse communities like those in Jorhat, Assam. To identify easy, affordable, and patient-friendly non-invasive markers that can help with early detection and risk stratification, this study will assess the relationship between serum adiponectin and TNF-α levels and the degree of hepatic fibrosis in patients with MASLD.

Materials and Methods

Study Design and Setting: This hospital-based cross-sectional study was conducted at the Department of Biochemistry in collaboration with the Department of General Medicine and the Multidisciplinary Research Unit (MRU), Jorhat Medical College and Hospital (JMCH), Assam, between December 2023 and November 2024. Ethical clearance was obtained from the Institutional Ethics Committee (Human), and written informed consent was obtained from all participants before enrolment.

Study Population and Sample Size: Consecutive sampling was used to recruit 113 patients aged 18 to 50 with elevated C-reactive protein (CRP) levels and ultrasonographically confirmed MASLD. Cochran's formula was used to determine the sample size, assuming a 95% confidence level, an 8% margin of error, and a 25% disease prevalence (13,14). Included were patients who had an ultrasound diagnosis of MASLD. People with chronic viral hepatitis, drug-induced liver injury, chronic renal failure, ischaemic heart disease, cancer, pregnancy, and substantial alcohol consumption (>140 g/week for men and >70 g/week for women) (15) were not included. Additionally, patients on platelet-modifying or lipid-lowering drugs were not included.

Fibrosis Assessment by Elastography: S-Shearwave elastography was used in transient elastography (FibroScan; Samsung RS80A with Prestige) to measure liver stiffness (LSM), which is expressed in kilopascals (kPa). Each patient had 10 valid measurements, and the median was considered indicative of the patient's degree of fibrosis.[16]

Clinical and Biochemical Assessment: Biochemical tests and a thorough clinical evaluation were performed on each participant. Aseptic procedures were followed during the collection of 5 mL of fasting venous blood. Sodium fluoride vials were used to estimate fasting blood sugar (FBS), EDTA vials were used to determine platelet counts, and plain vials were used for liver function tests and cytokine assays. Blood was then transferred into the proper collection tubes. Serum was separated by centrifugation and stored at -80°C until analysis. FBS was estimated using the glucose oxidase-peroxidase method, while serum AST and ALT were determined using the enzymatic rate method on a Vitros 5600 Biochemistry Analyzer (Ortho Clinical Diagnostics, USA). Platelet counts were measured using a Sysmex XN-350 haematology analyser (Transasia Biomedical Ltd., India). All biochemical analyses were performed under strict internal quality control using two levels of control materials analyzed daily, and results were accepted only when control values fell within the manufacturer-specified range.

The degree of hepatic fibrosis was estimated non-invasively using the FIB-4 index, a validated biomarker-based scoring system. A FIB-4 score < 1.30 indicated no or minimal fibrosis (F0–F1), whereas a score > 2.67 suggested advanced fibrosis (≥ F3). Intermediate values (1.30–2.67) were considered indeterminate and required additional evaluation.[17]

Estimation of Serum TNF-α and Adiponectin: Serum TNF-α and adiponectin concentrations were quantified in duplicate using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (ABclonal, Wuhan, China) according to the manufacturer's protocol. ELISA assays were performed using an Ebra Mannheim ELISA reader and quantified at 450 nm with a LisaScan EM Microplate Reader (Erba Mannheim, India). Concentrations were calculated using a four-parameter logistic regression curve.[18]

Statistical Analysis: Statistical analyses were conducted using R software (v.4.5.1, R Foundation for Statistical Computing, Vienna, Austria) in RStudio (v.2025.05.1). Continuous variables were presented as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution, which was assessed using the Shapiro-Wilk test. Comparisons between males and females were performed using the Mann-Whitney U test (Wilcoxon rank-sum test), with the U statistic, p-value, and rank-biserial correlation reported. Categorical variables were expressed as percentages and compared using the Chi-square test. The Kruskal-Wallis rank-sum test was considered to compare several groups of people with different stages of fibrosis (F1-F4). The Relationship between continuous

variables, including serum TNF-α, adiponectin, AST, and FibroScan results, was evaluated using Spearman's rank correlation; p-values < 0.05 were considered statistically significant.

Receiver Operating Characteristic (ROC) Curve Analysis:

To determine the diagnostic value of serum biomarkers in mild (F1-F2) and severe fibrotic (F3-F4), the ROC curve analysis was conducted. The pROC package of R was used to produce ROC curves, and the area under the curve (AUC) was obtained to quantify the discriminative ability of a given biomarker. Interpretation of AUC was done in reference to classical thresholds of 0.90 excellent, 0.80-0.89 good, 0.70-0.79 fair, 0.60-0.69 poor, and below 0.60 failed to measure (19). The ggplot2 was used to plot ROCs, each curve of which was annotated with its AUC. The Youden index was used to check the most suitable cutoffs (maximizing). The sum of sensitivity and specificity provides clinically relevant thresholds for risk stratification in MASLD patients.

Results

Participant Characteristics

A male-to-female ratio of 2.65:1 was observed among 113 patients with Metabolic dysfunction associated steatotic liver disease (MASLD), with 82 males (72.6%) and 31 females (27.4%). This clearly shows a male predominance. Chi-square analysis confirmed that this gender distribution was statistically significant ($\chi^2 = 24.03$, p = 1.61e-06) [Figure 1]. The mean age of the cohort was 41.50 ± 7.49 years, ranging from 18 to 50 years, with a median of 42 years. Females had a slightly higher mean age (43.32 ± 7.25 years) than males (40.80 ± 7.50 years), although the difference was not statistically significant.

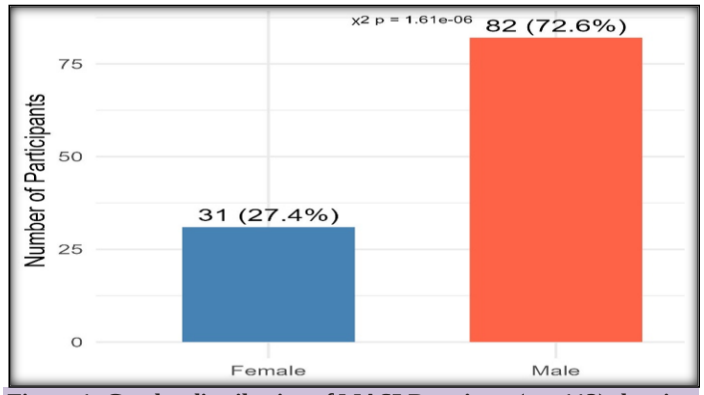

Figure 1: Gender distribution of MASLD patients (n = 113) showing male predominance. Chi-square analysis indicates a statistically significant difference ($\chi^2 = 24.03$, p =1.61e-06).

Biochemical and Hematological Parameters

Table 1: Baseline biochemical and hematological parameters of MASLD patients

Parameter	Mean ± SD	Range	Median
AST (U/L)	52.13 ± 60.28	13 - 450	36.0
ALT (U/L)	56.15 ± 55.99	9 - 405	42.0
Platelet (×10³/µL)	155.43 ± 62.19	55 - 389	147.0
FBS (mg/dL)	114.10 ± 41.64	61 - 269	98.0

The overall biochemical and hematological profile is summarized in Table 1. Mean serum AST and ALT levels were 52.13 ± 60.28 U/L and 56.15 ± 55.99 U/L, respectively. Platelet counts ranged from 55–389 ×10³/µL (mean = 155.43 ± 62.19 ×10³/µL), and FBS ranged from 61-269 mg/dL (mean = 114.10 ± 41.64 mg/dL), reflecting a heterogeneous metabolic profile among participants.

Gender-Based Comparison of Clinical Parameters

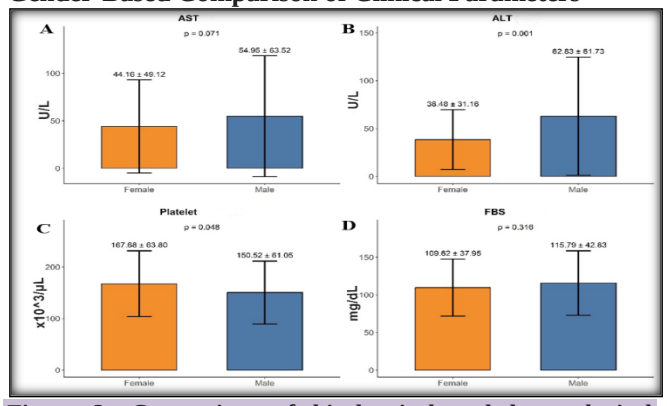

Figure 2: Comparison of biochemical and hematological parameters between male and female participants. Bars represent mean ± SD, with p-values from the Mann-Whitney U test indicated above each comparison. (A) AST, aspartate aminotransferase; (B) ALT, alanine aminotransferase; (C) Platelet count (×10³/µL); (D) FBS, fasting blood sugar (mg/dL).

Fibrosis Assessment

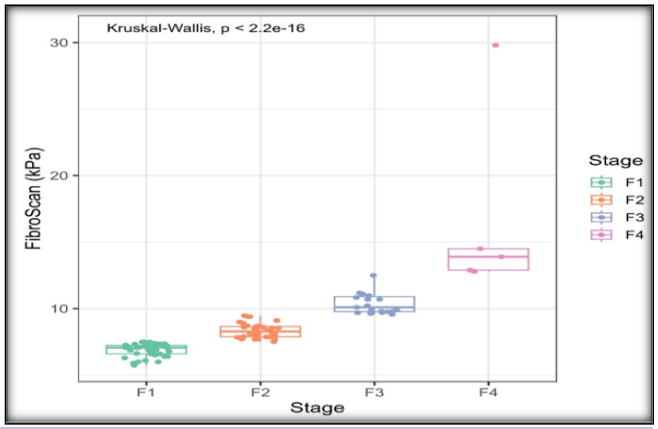

Figure 3: FibroScan categories and liver stiffness measurements across fibrosis stages. (A) The boxplots illustrate the distribution of Fibroscan (kPa) measurements across fibrosis stages F1-F4, showing the median, interquartile range (IQR), and potential outliers for each stage.

Comparisons between males and females revealed sex-specific differences [Figure 2]. AST levels were slightly higher in males (54.95 ± 63.52 U/L) than in females (44.16 ± 49.12 U/L), but the difference was not significant (U = 990, p = 0.071). ALT levels were significantly higher in males (62.83 ± 61.73 U/L) than in females (38.48 ± 31.16 U/L; U =

770, p = 0.001), indicating sex-dependent variation in liver enzyme activity. Platelet counts were higher in females (167.68 ± 63.80 ×10³/μL) than in males (150.52 ± 61.05 ×10³/μL, U = 1579, p = 0.048). FBS levels were comparable between sexes (p = 0.812). Overall, ALT and platelet counts showed significant sex-specific differences, whereas AST and FBS did not differ significantly between males and females.

The mean FIB-4 index was 2.20 ± 1.85 (range: 0.447-11.172). Based on established cutoffs, 39 patients (34.5%) were low risk (<1.30), 45 (39.8%) intermediate risk (1.30-2.67), and 29 (25.7%) high risk (>2.67). Fibrosis staging indicated that most participants were in F1 (43.4%), followed by F2 (34.5%), F3 (17.7%), and F4 (4.4%), suggesting predominance of early to moderate fibrosis. Liver stiffness measurements (LSM) increased progressively with fibrosis stage, ranging from 5.74 to 29.8 kPa (mean = 8.46 ± 2.65 kPa; median = 7.79 kPa), with no significant sex-related differences (p = 0.131). Mean age also increased with fibrosis severity, from 41.29 ± 7.75 years in F1 to 44.4 ± 5.13 years in F4, indicating that advanced fibrosis was more common in slightly older individuals. Kruskal-Wallis analysis confirmed significant differences in LSM across fibrosis stages (χ^2 = 85.66, df = 3, p< 2.2e-16) [Figure 3].

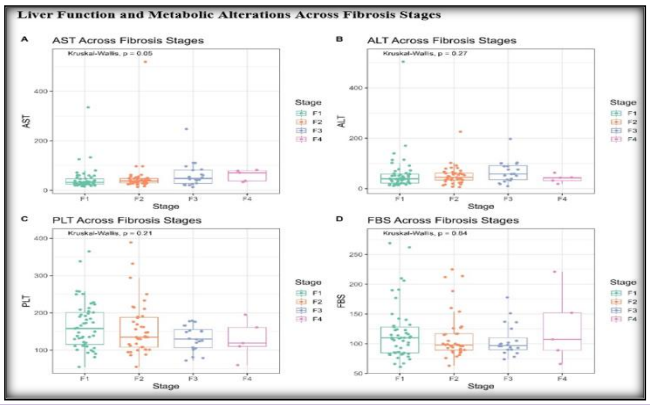

Figure 4. Liver function and metabolic parameters across fibrosis stages (F1-F4).

Boxplots depict (A) AST (U/L), (B) ALT (U/L), (C) platelet count (PLT, 10 3 / uL), and (D) fasting blood sugar (FBS, mg/dL) at various stages of fibrosis. Each point represents an individual patient. The interquartile range with median is marked by boxes, and the minimum and maximum values are marked together by a single box. The Kruskal-Wallis test was used to assess differences between the stages, and the p-values were reported in the text for each plot.

AST levels increased progressively from F1 (45.71 ± 48.57 U/L) to F3 (63.35 ± 52.62 U/L), followed by a slight decline in F4 (60.8 ± 23.04 U/L) (p = 0.049) (Figure 4A). The same trends were observed with respect to the ALT levels, though not significantly different (p = 0.273) (Figure 4B). Although FBS remained relatively unchanged across the stages (p = 0.840) (Figure 4D), the number of platelets steadily declined during F1 to F4 (165.94 x10 3/ul to 129 x10 3/ul, respectively) (Figure 4C). The findings lead to rising fibrosis, progressive liver damage, and metabolic dysregulation.

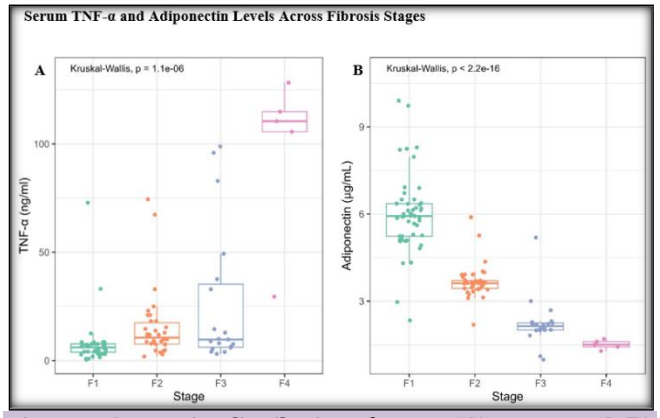

Figure 5. Stage-wise distribution of serum (A) TNF-α and (B) adiponectin levels across fibrosis stages F1-F4. In boxplots, the median and the interquartile range (IQR) are displayed; the whiskers stretch up to 1.5X IQR, and the points that lie outside this are drawn as outliers. The Kruskal-Wallis test was used to evaluate differences between stages, and p-values are indicated above each plot.

Serum TNF-α concentrations increased progressively with fibrosis stage, from 7.91 ± 11.7 ng/mL in F1 to 97.8 ± 39.1 ng/mL in F4 (p = 1.1e-06) (Figure 5A). On the other hand, adiponectin levels declined with increasing fibrosis, from 5.99 +/- 1.37 μg/mL in F1 to 1.51 +/- 0.16 μg/mL in F4 (p=2.2e-16), indicating that adiponectin and fibrosis severity have a very strong negative relationship.

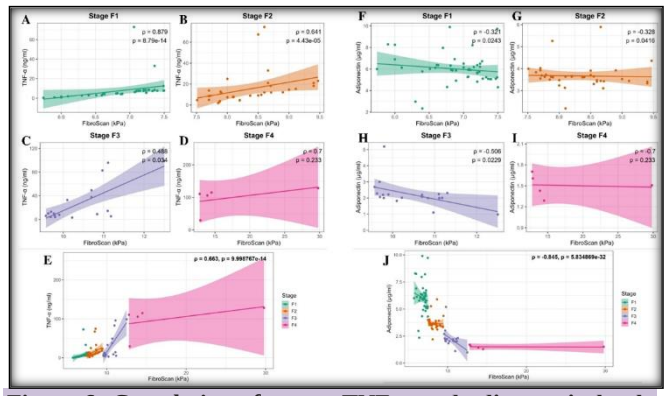

Figure 6: Correlation of serum TNF-α and adiponectin levels with FibroScan liver stiffness (LSM, kPa) across fibrosis stages. A-D: Stage-specific scatter plots illustrating the Spearman correlation between serum TNF-α levels and LSM in fibrosis stages F1-F4. Strong positive correlations were observed in early stages (F1: ρ = 0.879, p = 8.79e-14; F2: ρ = 0.641, p = 4.43e-05), a moderate correlation in F3 (ρ = 0.488, p = 0.034), and no significant correlation in F4 (ρ = 0.700, p = 0.233). E: Overall TNF-α levels correlation across all stages shows a significant positive association (ρ = 0.663, p = 9.998e-14). The shaded region represents the 95% confidence interval. F-I: Stage-specific scatter plots showing the negative correlation between serum adiponectin and LSM in stages F1-F4. Weak to moderate negative correlations were observed in F1-F3 (F1: ρ = -0.321, p = 0.024; F2: ρ = -0.328, p = 0.042; F3: ρ = -0.506, p = 0.023), with no significant correlation in F4 (ρ = -0.7, p = 0.233). J: Combined adiponectin levels analysis, across all stages demonstrates a strong negative correlation (ρ = -0.845, p= 5.83e-32). Shaded areas indicate 95% confidence intervals.

Correlation of Biomarkers with Liver Stiffness: Spearman correlation revealed a significant positive correlation between TNF-α and LSM (ρ = 0.663, p= 9.998e-14), strongest in early fibrosis (F1: ρ = 0.879, p = 8.79e-14; F2: ρ = 0.641, p = 4.43e-05). Correlation weakened in F3 (ρ = 0.488, p = 0.034) and F4 (ρ = 0.700, p = 0.233) due to small sample size (Figure 6A-E). On the other hand, LSM showed a pronounced negative relationship with adiponectin at all stages (ρ= -0.845, p = 5.83e-32), and in early stages and in high-variability F4 (Figure 6F-J). The levels of AST were slightly positively correlated with LSM (r = 0.252, p = 0.012), with higher levels as fibrosis progressed.

Receiver Operating Characteristic (ROC) Curve Analysis

The ROC analysis revealed that adiponectin was a strong discriminator, distinguishing mild (F1-F2) from severe fibrosis (F3-F4) with an optimal cutoff of <3.055 µg/mL, yielding 95.8% sensitivity and 95.9% specificity (Table 2, Figure 7). TNF-α showed good specificity (93.2%) and poor sensitivity (45.8%), yielding moderate diagnostic performance (AUC = 0.717), whereas AST showed poor to fair diagnostic performance (AUC = 0.649). These findings indicate that adiponectin is a potent independent biomarker, and TNF-alpha can serve as a supplementary biomarker indicative of an inflammatory process. Coupled measurement of biomarkers could enhance early diagnosis and risk stratification of fibrosis in MASLD.

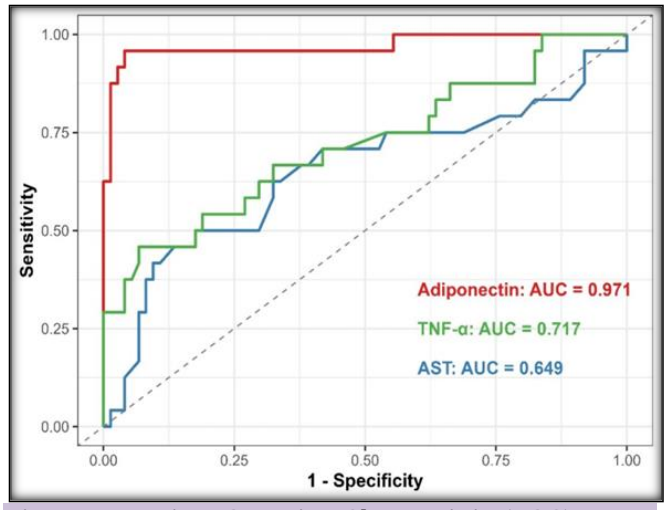

Figure 7: Receiver Operating Characteristic (ROC) curves depicting the diagnostic performance of serum adiponectin, TNF-α, and AST levels in differentiating mild (F1-F2) and severe (F3-F4) stages of hepatic fibrosis in patients with MASLD.

Table 2: Optimal cutoff values, sensitivity, and specificity of serum biomarkers for differentiating mild and severe fibrosis in MASLD patients.

Biomarker	Cutoff Value	Sensitivity	Specificity	AUC Value	Diagnostic value
Adiponectin (µg/mL)	≤ 3.055	95.8%	95.9%	0.971	Strong predictor of fibrosis severity
TNF-α (ng/mL)	≥ 27.259	45.8%	93.2%	0.717	Supportive marker; not strong alone
AST (U/L)	≥ 53.5	50.0%	82.4%	0.649	Poor

Color-coded red, green, and blue marks adiponectin, TNF-alpha, and AST, respectively. The diagonal line is dashed; it shows the reference line (AUC = 0.5), indicating no discrimination. The relative diagnostic accuracy of each biomarker is annotated on the curves with AUC values.

Discussion

One of the global medical issues is Metabolic dysfunction associated steatotic liver disease (MASLD), including both simple steatosis and non-alcoholic steatohepatitis (NASH) that may follow the path of cirrhosis and hepatocellular carcinoma.[20] The prevalence of MASLD has risen over time, from 26 percent in research conducted before 2005 to 38 percent in research conducted after 2016.[21] This is in line with the rising rates of type 2 diabetes and obesity, two significant risk factors for the disease's progression.[22]

The current study clearly demonstrated a male predominance, with 72.6% of participants being men and a male-to-female ratio of 2.65:1. This finding is consistent with previous studies that found a higher incidence of MASLD in men, most likely as a result of increased visceral fat accumulation and de novo lipogenesis (20, 23, 24). The average age of the participants was 41.5 ± 7.49 years, with females having a slightly higher mean age than males. This is in line with global trends that show MASLD is more prevalent in younger men but is increasing in older women.[25,26]

Liver damage is frequently evaluated using biochemical indicators, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[27,28] Males had higher mean ALT and AST levels than females in our cohort. While ALT showed inconsistent trends across stages, AST levels gradually increased with fibrosis stage, peaking at F3. These results corroborate earlier findings that transaminases reflect hepatocellular injury, but their reliability as stand-alone markers is limited, as they do not consistently correlate with fibrosis severity.[29–31]

Fibrosis risk was non-invasively stratified using the Fibrosis-4 (FIB-4) index, which included age, ALT, AST, and platelet counts. 25.7% of individuals were high risk, 39.8% were intermediate risk, and 34.5% were low risk. This distribution emphasizes the usefulness of FIB-4 for identifying individuals who may need further testing, such as elastography or biopsy, to accurately stage fibrosis.[32] These results were supported by FibroScan's liver stiffness measurement (LSM), which showed a progressive increase with increasing fibrosis severity. In line with worldwide findings that early fibrosis predominates in MASLD populations, the majority of patients were in stage F1, with a lesser percentage in advanced stages (F3–F4).[33,34]

In this investigation, the anti-inflammatory and insulin-sensitizing adipokine adiponectin demonstrated a strong inverse correlation with the degree of fibrosis. The consistent drop in serum levels from F1 to F4 and the strong negative correlation

between all stages suggest that hypoadiponectinemia may play a role in the development of fibrosis. These results are in line with previous studies that connected reduced adiponectin to increased necroinflammation and fibrogenesis in MASLD.[35–37] Conversely, the pro-inflammatory cytokine TNF-α increased as fibrosis progressed, showing a significant positive correlation in early fibrosis (F1-F2) that decreased in F3 and F4. This pattern suggests a major role for TNF-α in the onset and early progression of fibrosis, consistent with other research linking TNF-α to hepatic inflammation and stellate cell activation.[38-40] Their opposing tendencies highlight the hepatoprotective effect of adiponectin and TNF-α's fibrogenesis-promoting role in the pathogenesis of MASLD.

Receiver operating characteristic (ROC) analysis revealed that adiponectin is a dependable non-invasive biomarker for distinguishing between mild (F1-F2) and severe (F3-F4) fibrosis, with an AUC of 0.971. The optimal cutoff of 3.055 µg/mL produced excellent sensitivity (95.8%) and specificity (95.9%). TNF-α's potential as a supplementary marker was highlighted by its intermediate diagnostic performance (AUC = 0.717), good specificity (93.2%), and low sensitivity (45.8%). In contrast, AST showed low discriminatory capacity (AUC = 0.649), consistent with prior research indicating that it has poor predictive value for fibrosis in MASLD (41,42). These results highlight the potential benefits of integrating TNF-α and adiponectin measures for non-invasive fibrosis assessment and risk categorization.

Limitations

Limitations of the study include its cross-sectional design, small sample size, and absence of longitudinal follow-up. To validate and improve non-invasive fibrosis assessment, future research should include larger, multicenter cohorts, longitudinal evaluations, and the integration of new biomarkers with liver histology. Translation into routine clinical practice will be facilitated by establishing standardized cutoff values and investigating the molecular mechanisms underlying TNF-α and adiponectin in the progression of fibrosis.

Conclusion

All things considered, our data show that MASLD is more common in middle-aged males in our group and is associated with metabolic changes, including decreased platelet counts and elevated liver enzymes. While TNF-α reflects the inflammatory milieu associated with disease development, adiponectin shows great promise as a biomarker for the non-invasive diagnosis of progressive fibrosis. By incorporating these indicators into clinical practice, it may be possible to enhance early detection, lessen the need for invasive procedures, and enable individualised therapy plans.

Declaration

Ethics approval and consent to participate: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Jorhat Medical College and Hospital (approval number: SMEJ/JMCH/MEU/841/pt-2/2011/5024). Written informed consent was obtained from all participants prior to inclusion.

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Conflicts of interest

There are no conflicts of interest.

References

1. Lindenmeyer CC, McCullough AJ. The Natural History of Nonalcoholic Fatty Liver Disease-An Evolving View. Clin Liver Dis. 2018 Feb;22(1):11–21.
2. Powell EE, Wong VWS, Rinella M. Non-alcoholic fatty liver disease. Lancet Lond Engl. 2021 Jun 5;397(10290):2212–24.
3. Toshikuni N, Tsutsumi M, Arisawa T. Clinical differences between alcoholic liver disease and nonalcoholic fatty liver disease. World J Gastroenterol. 2014 Jul 14;20(26):8393–406.
4. Björnsson E, Angulo P. Non-alcoholic fatty liver disease. Scand J Gastroenterol. 2007 Sep;42(9):1023–30.
5. Mcavoy NC, Ferguson JW, Campbell IW, Hayes PC. Review: Non-alcoholic fatty liver disease: natural history, pathogenesis and treatment. Br J Diabetes Vasc Dis. 2006 Nov 1;6(6):251–60.
6. Dalamaga M, Diakopoulos KN, Mantzoros CS. The Role of Adiponectin in Cancer: A Review of Current Evidence. Endocr Rev. 2012 Aug;33(4):547–94.
7. Aygun C, Senturk O, Hulagu S, Uraz S, Celebi A, Konduk T, et al. Serum levels of hepatoprotective peptide adiponectin in non-alcoholic fatty liver disease. Eur J Gastroenterol Hepatol. 2006 Feb;18(2):175–80.
8. Polyzos SA, Kountouras J, Zavos C, Tsiaousi E. The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. Diabetes Obes Metab. 2010 May;12(5):365–83.
9. Elpek GÖ. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol WJG. 2014 Jun 21;20(23):7260–76.
10. Bashir A, Duseja A, De A, Mehta M, Tiwari P. Non-alcoholic fatty liver disease development: A multifactorial pathogenic phenomena. Liver Res. 2022 Jun 1;6(2):72–83.
11. Basaranoglu M, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: A tale of "second hit." World J Gastroenterol WJG. 2013 Feb 28;19(8):1158–65.
12. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425–56.
13. Ahmed SK. How to choose a sampling technique and determine sample size for research: A simplified guide for researchers. Oral Oncol Rep. 2024 Dec 1;12:100662.
14. Cochran WG. Sampling Techniques. John Wiley & Sons; 1977. 460 p.
15. Puneem US, Nagasubramanian VR, Sindgi VM, Ramakrishnan SMR, Pelluri R. Health-related quality of life and its determinants among South Indian type 2 diabetes patients with and without non-alcoholic fatty liver disease. Egypt Liver J. 2023 Oct 12;13(1):57.
16. Ferraioli G, Wong VWS, Castera L, Berzigotti A, Sporea I, Dietrich CF, et al. Liver Ultrasound Elastography: An Update to the World Federation for Ultrasound in Medicine and Biology Guidelines and Recommendations. Ultrasound Med Biol. 2018 Dec;44(12):2419–40.

17. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Use of the fib4 index for non-invasive evaluation of fibrosis in non-alcoholic fatty liver disease. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2009 Oct;7(10):1104–12.
18. Findlay JWA, Dillard RF. Appropriate calibration curve fitting in ligand binding assays. AAPS J. 2007 Jun;9(2):E260–7.
19. Çorbacıoğlu ŞK, Aksel G. Receiver operating characteristic curve analysis in diagnostic accuracy studies: A guide to interpreting the area under the curve value. Turk J Emerg Med. 2023 Oct 3;23(4):195–8.
20. Maurice J, Manousou P. Non-alcoholic fatty liver disease. Clin Med. 2018 Jun 1;18(3):245–50.
21. Teng ML, Ng CH, Huang DQ, Chan KE, Tan DJ, Lim WH, et al. Global incidence and prevalence of nonalcoholic fatty liver disease. Clin Mol Hepatol. 2023 Feb;29(Suppl):S32–42.
22. Roh E, Jung HN, Heo JH, Han K, Kang JG, Lee SJ, et al. Impact of type 2 diabetes on the risk of cardiovascular disease and cardiovascular mortality in patients with non-alcoholic fatty liver disease: a nationwide population-based study. BMJ Public Health. 2025 Jun 18;3(1):e002020.
23. Balakrishnan M, Patel P, Dunn-Valadez S, Dao C, Khan V, Ali H, et al. Women have Lower Risk of Nonalcoholic Fatty Liver Disease but Higher Risk of Progression vs Men: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2021 Jan;19(1):61-71.e15.
24. Makri E, Goulas A, Polyzos SA. Epidemiology, Pathogenesis, Diagnosis and Emerging Treatment of Nonalcoholic Fatty Liver Disease. Arch Med Res. 2021 Jan 1;52(1):25–37.
25. Arshad T, Golabi P, Paik J, Mishra A, Younossi ZM. Prevalence of Nonalcoholic Fatty Liver Disease in the Female Population. Hepatol Commun. 2018 Nov 27;3(1):74–83.
26. Nagral A, Bangar M, Menezes S, Bhatia S, Butt N, Ghosh J, et al. Gender Differences in Nonalcoholic Fatty Liver Disease. Euroasian J Hepato-Gastroenterol. 2022 Jul;12(Suppl 1):S19–25.
27. Thakur S, Kumar V, Das R, Sharma V, Mehta DK. Biomarkers of Hepatic Toxicity: An Overview. Curr Ther Res. 2024 Jan 1;100:100737.
28. Thapa BR, Walia A. Liver function tests and their interpretation. Indian J Pediatr. 2007 Jul 1;74(7):663–71.
29. Mera JR, Dickson B, Feldman M. Influence of gender on the ratio of serum aspartate aminotransferase (AST) to alanine aminotransferase (ALT) in patients with and without hyperbilirubinemia. Dig Dis Sci. 2008 Mar;53(3):799–802.
30. Moriles KE, Zubair M, Azer SA. Alanine Aminotransferase (ALT) Test. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Oct 10]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK559278/
31. Qiu J, Kuang M, He S, Yu C, Wang C, Huang X, et al. Gender perspective on the association between liver enzyme markers and non-alcoholic fatty liver disease: insights from the general population. Front Endocrinol. 2023 Dec 6;14:1302322.
32. Xu X lan, Jiang L shun, Wu C si, Pan L ya, Lou Z qi, Peng C ting, et al. The role of fibrosis index FIB-4 in predicting liver fibrosis stage and clinical prognosis: A diagnostic or screening tool? J Formos Med Assoc. 2022 Feb 1;121(2):454–66.
33. Halaoui AF, Ali AH, Habib SG, Kanso M, Daniel F, Mukherji DM, et al. Gender differences in liver fibrosis among patients younger than 50 years: A retrospective cohort study. Clin Res Hepatol Gastroenterol. 2020 Oct 1;44(5):733–8.
34. Liu J, Tian Y, Fu X, Mu C, Yao M, Ni Y, et al. Estimating global prevalence, incidence, and outcomes of non-alcoholic fatty liver disease from 2000 to 2021: systematic review and meta-analysis. Chin Med J (Engl). 2022 Jul 20;135(14):1682–91.
35. Boutari C, Perakakis N, Mantzoros CS. Association of Adipokines with Development and Progression of Nonalcoholic Fatty Liver Disease. Endocrinol Metab. 2018 Mar 21;33(1):33–43.
36. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatol Baltim Md. 2004 Jul;40(1):46–54.
37. Mohamed MS, Youssef TM, Abdullah EE, Ahmed AE. Correlation between adiponectin level and the degree of fibrosis in patients with non-alcoholic fatty liver disease. Egypt Liver J. 2021 Sep 21;11(1):78.
38. Ajmal MR, Yaccha M, Malik MA, Rabbani MU, Ahmad I, Isalm N, et al. Prevalence of nonalcoholic fatty liver disease (MASLD) in patients of cardiovascular diseases and its association with hs-CRP and TNF-α. Indian Heart J. 2014 Nov 1;66(6):574–9.
39. Seo YY, Cho YK, Bae JC, Seo MH, Park SE, Rhee EJ, et al. Tumor Necrosis Factor-α as a Predictor for the Development of Nonalcoholic Fatty Liver Disease: A 4-Year Follow-Up Study. Endocrinol Metab. 2013 Mar;28(1):41–5.
40. Vachliotis ID, Polyzos SA. The Role of Tumor Necrosis Factor-Alpha in the Pathogenesis and Treatment of Nonalcoholic Fatty Liver Disease. Curr Obes Rep. 2023;12(3):191–206.
41. Bao B, Xu S, Sun P, Zheng L. Neutrophil to albumin ratio: a biomarker in non-alcoholic fatty liver disease and with liver fibrosis. Front Nutr [Internet]. 2024 Apr 8 [cited 2025 Oct 11];11. Available from: https://www.frontiersin.org/journals/nutrition/articles/10.3389/fnut.2024.1368459/full
42. Thong VD, Quynh BTH. Correlation of Serum Transaminase Levels with Liver Fibrosis Assessed by Transient Elastography in Vietnamese Patients with Nonalcoholic Fatty Liver Disease. Int J Gen Med. 2021 Apr 16;14:1349–55.