

## ESTIMATION OF PROGNOSTIC FACTORS FOR NON-ALCOHOLIC FATTY LIVER DISEASE USING THE LIVER FAT SCORE IN PATIENTS AT AL-RIFAI TEACHING HOSPITAL IN THE AL-RIFAI DISTRICT OF DHI-QAR, IRAQ

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### ABSTRACT

**Background:** Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition with varying severity. Non-invasive methods like liver function score (LFS) assist diagnosis but their accuracy across disease stages requires further evaluation. **Objectives:** To evaluate LFS sensitivity compared to ultrasound and analyze biochemical differences across steatosis grades and in discordant LFS/ultrasound results. Secondary aims were assessing correlations of LFS and biomarkers and influence of comorbidities on LFS accuracy. **Methods:** 71 adult NAFLD patients were recruited. Hepatic steatosis was graded by ultrasound as mild (U1), moderate (U2) or severe (U3). LFS, ferritin, mean platelet volume and neutrophil-to-lymphocyte ratio were measured. One-way ANOVA compared groups. Pearson's coefficients evaluated correlations. **Results:** LFS demonstrated lower sensitivity for mild (63.63%) versus moderate (70.58%) and severe (100%) NAFLD. Total cholesterol, LDL-cholesterol, AST, ALT and anemia markers significantly worsened with increasing ultrasound grade. No inflammatory biomarkers distinguished steatosis severity or LFS accuracy. High prevalence of metabolic comorbidities had no significant differences across grades. **Conclusion:** LFS showed adequate overall accuracy for NAFLD but substantially lower sensitivity for mild disease, highlighting complexity in early diagnosis. Worsening biochemical aberrations were associated with more advanced illness on imaging. The lack of reliable inflammatory indicators represents an area for further research.

**KEYWORDS:** Prognostic Factors, Non-Alcoholic, Fatty, Liver Disease, Liver Fat Score.

### INTRODUCTION

Clinically presenting from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular cancer, non-alcoholic fatty liver disease (NAFLD) is a relatively common liver disease. With its frequency fast rising<sup>[1,2]</sup>, it is the main cause of chronic liver disease globally. Key elements in the pathophysiology of type 2 diabetes mellitus (T2DM), NAFLD usually coexists with insulin resistance and chronic inflammation, so NAFLD and T2DM can develop concurrently.<sup>[3]</sup> This cohabitation aggravates negative effects, hence raising morbidity and death rates.<sup>[3]</sup> Reflecting developments in medical technology and a better knowledge of the aetiology of the illness, NAFLD diagnosis makes use both invasive and non-invasive techniques. Although liver biopsy is still the gold standard for NAFLD diagnosis and differentiation from NASH as it offers thorough histological data, it is

invasive and entails certain hazards.<sup>[4]</sup> Stable imaging modalities include elastography and biomarker analysis are non-invasive substitutes. Novel non-invasive indicators that have shown helpful in NAFLD diagnosis and pathophysiology understanding include the fatty liver index (FLI) and gender-related bile acid profiles.<sup>[5,6]</sup> Essential for detecting NAFLD and NASH, liver biopsy is still indispensable for the evaluation of liver fibrosis, steatosis, inflammation, and ballooning even with the developments in non-invasive diagnostics. In clinical trials for novel NASH therapies, where regulatory authorities usually want biopsies to precisely evaluate treatment effectiveness, it is very important.<sup>[7,8]</sup> Because of its low cost and great availability, ultrasound (US) is a recommended non-invasive method for screening and NAFLD assessment. By exposing higher liver echogenicity, a characteristic of NAFLD, it identifies hepatic steatosis quite efficiently. By non-invasive

evaluation of liver fibrosis, ultrasonic elastography techniques including transient elastography, ARFI, and strain elastography have improved NAFLD therapy.<sup>[9-11]</sup> A novel diagnostic method called the Liver Fat Score (LFS) can substitute either ultrasounds or liver biopsies. It applies a formula combining elements like BMI, waist circumference, LDL cholesterol, and metabolic syndrome signs. Higher LFS values point to NAFLD and imply the need of further diagnostic procedures such as hepatic ultrasonography.<sup>[12,13]</sup> Higher scores are connected with increasing risks of coronary heart disease, congestive heart failure, and angina pectoris, hence LFS is also crucial for evaluating cardiovascular disease (CVD) risk.<sup>[14,15]</sup> Heart disease, stroke, and T2DM are much more likely in metabolic syndrome—which comprises disorders like high blood pressure, elevated blood sugar, and abnormal cholesterol levels. Its requirements could fluctuate depending on the population; South Asians have a high frequency because of lifestyle, environmental, and genetic aspects.<sup>[16,17]</sup> Studies among South Asian communities show a substantial correlation between metabolic syndrome and chronic diseases like skin tags and CVD, with food and acculturation playing major roles.<sup>[18,19]</sup> The primary aim of this study is to evaluate the reliability of the Liver Fat Score (LFS) in diagnosing Non-Alcoholic Fatty Liver Disease (NAFLD) compared to ultrasound grading. Specifically, the study aims to: Compare the sensitivity of LFS in diagnosing NAFLD across ultrasound-based grades 1 (U1), 2 (U2), and 3 (U3). Assess differences in serum ferritin, mean platelet volume (MPV), and neutrophil-to-lymphocyte ratio (NLR) among patients with U1, U2, and U3 NAFLD grades on ultrasound. Examine differences in serum ferritin, MPV, and NLR between true positive (LFS+ and ultrasound+) and false negative (LFS- and ultrasound-) patients.

## METHOD

**Study Design and Participants** This was a cross-sectional study conducted at Al-RifaidistrictInDhi-Qar Hospital: Al-Rifai teaching hospital, Iraq between January 2022 and December 2023. 71 adult patients (>18 years old) diagnosed with non-alcoholic fatty liver disease (NAFLD) on ultrasound were recruited from the gastrointestinal clinic. Exclusion criteria were significant alcohol consumption (>30g/day for men, >20g/day for women); known viral hepatitis; drug-induced liver injury; previous bowel resection surgery; and other causes of secondary hepatic steatosis. The study protocol was approved by the Hospital X Institutional Review Board. Written informed consent was obtained from all participants. Participant height and weight were measured by trained nurses using a mechanized Seca 703 column scale with height rod to calculate BMI. Waist circumference was measured midway between the lowest rib and iliac crest to the nearest 0.1 cm using a Seca 201 measuring tape. Blood pressure was measured using an Omron HEM-7121 automated oscillometric blood pressure monitor in the upright sitting position after 5 minutes rest. All anthropometric measures were

performed in duplicate and averaged. Venous blood samples were collected after an overnight fast of at least 8 hours. Serum was analyzed on a Cobas C311 analyzer (Roche Diagnostics, Germany) using enzymatic colorimetric methods for total cholesterol, HDL-C, LDL-C and triglycerides. Glucose, HbA1c, AST and ALT were measured using standard photometric enzyme assays and kits. Complete blood counts with differentials were performed using a Sysmex XN-20 hematology analyzer (Sysmex Corp., Japan) to obtain neutrophil, lymphocyte and platelet measures. Ferritin was quantified by a chemiluminescentmicroparticle immunoassay using ARCHITECT ferritin reagent kits (Abbott Laboratories, USA) on an Abbott ARCHITECT i1000SR immunoassay analyzer. Ultrasound imaging was performed after overnight fasting by an experienced radiologist using a Siemens Acuson S2000 ultrasound system. Hepatic ultrasonography images were obtained with a CH6-2 curved array transducer. Liver fat was graded based on standardized criteria as: grade 1 or mild (U1), grade 2 or moderate (U2), and grade 3 or severe (U3) steatosis. Liver function score (LFS) was calculated using the formula:  $LFS = 2.339 + 1.75 \times BMI (+1.26 \text{ if female}) + \text{diabetes (yes=1/no=0)} + 0.0031 \times \text{fasting insulin (mU/L)} - 0.48 \times \text{AST/ALT ratio}$ . Participants with  $LFS > -0.61$  were classified as having suspected NAFLD based on probabilities from the original study. Statistical analyses were conducted using SPSS v25.0 (IBM Corp). Sensitivity was calculated by dividing true positive cases over total cases for each NAFLD grade on ultrasound. One-way ANOVA with Tukey's post-hoc tests compared groups. Pearson's correlation coefficients evaluated associations between variables. Chi square test was used to compare categorical data.  $P < 0.05$  was considered statistically significant.

## RESULTS

The study compared patient demographics, clinical characteristics, and various biochemical markers across three grades of non-alcoholic fatty liver disease (NAFLD) based on ultrasound imaging: mild (U1), moderate (U2), and severe (U3).

### Demographics and Clinical Characteristics

- No significant differences were found between the grades for mean age, gender ratios, BMI, waist circumference, systolic blood pressure, or smoking rates.

### Metabolic Parameters

- Mean fasting insulin levels, fasting glucose, and HbA1C showed no significant differences across the NAFLD grades, indicating similar levels of insulin resistance and glucose intolerance.

### Lipid Panel

- Patients with higher grades of fatty liver had significantly increased total and LDL cholesterol levels. However, HDL and triglyceride levels were statistically similar across the groups.

### Liver Enzymes

- Serum aminotransferases AST and ALT levels significantly increased with worsening NAFLD grade, indicating progressively higher liver enzyme elevations with increasing ultrasound severity.

### Inflammatory and Hematologic Markers

- Serum ferritin levels increased with NAFLD severity, but differences were not statistically significant. Hemoglobin levels showed significant differences, reflecting worsening anemia with more severe fatty liver disease. Mean corpuscular volume (MCV) also increased

with higher NAFLD grades. Mean platelet volume and neutrophil-to-lymphocyte ratio showed trends of increase but did not reach statistical significance.

### Metabolic Syndrome and Comorbidities

- Over 75% of patients in all grades had metabolic syndrome, with no significant differences between the groups. High prevalence of diabetes, hypertension, ischemic heart disease, and hypothyroidism was noted across all steatosis grades, with no significant differences observed.

**Table 1: Demographics and clinical data of different ultrasound steatosis grades.**

	USD 1( 33)	USD 2 34)	USD 3	P-value
Age(mean+/-SD)	43.99+/-1.99	45.52+/-1.71	47+/-1.52	0.65
Male n(%)	17/33(51%)	19/34(55.8%)	3/6(50%)	0.48
BMI (mean+/-SD)	30.51+/-0.91	30.11+/-1.26	31.3+/-0.78	0.9
Waist circumference (mean+/-SD)	107.5+/-1.87	110.1+/-1.81	110.33+/-2.01	0.56
Systolic blood pressure(mean+/-SD)	132.42+/-2.42	133.08+/-3.18	140+/-9.66	0.63
Smoking n(%)	7/33(21.21%)	12/34(35.29%)	2/6(33.%)	
Fasting insulin (mean+/-SD)	10.45+/-1.58	10.42+/-1.86	8.82+/-0.24	0.92
Fasting blood glucose(mean+/-SD)	115.42+/-5.73	112.29+/-5.77	145.16+/-24.65	0.12
HBA1C (mean+/-SD)	6.99+/-0.23	6.40+/-0.28	7.78+/-0.88	0.17
Cholesterol(mean+/-SD)	192.75+/-4.65	193.17+/-5.58	263.5+/-26.94	0.000029
LDL(mean+/-SD)	108.30+/-4.50	104.88+/-4.74	178.33+/-29.22	0.00001
HDL(mean+/-SD)	51.30+/-2.45	52.66+/-2.10	47.66+/-3.14	0.66
Triglycerides (mean+/-SD)	173+/-10.01	190.08+/-9.86	210+/-26.04	0.25
AST(mean+/-SD)	16.15+/-1.56	22.64+/-2.44	41.16+/-9.97	0.00023
ALT(mean+/-SD)	20.21+/-2.05	25.58+/-2.26	37.66+/-7.28	0.0091
S.ferritin(mean+/-SD)	120.34+/-16.88	120.08+/-20.48	99.29+/-35.85	0.9
Hb (mean+/-SD)	13.93+/-0.21	13.10+/-0.29	12.3+/-1.01	0.027
MCV(mean+/-SD)	83.51+/-0.59	81.63+/-0.74	78.36+/-2.27	0.04
MPV(mean+/-SD)	9.26+/-0.14	8.97+/-0.12	8.66+/-0.33	0.13
NLR(mean+/-SD)	1.69+/-0.10	1.99+/-0.22	2.61+/-0.54	0.11
Metabolic syndrome n(%)	26/33(78.78%)	26/34(76.47%)	5/6(8.3%)	0.15
Co-morbidities n(%)	25/33(75.75%)	29/34(85.29%)	6/6(100%)	0.46
Family history n(%)	12/33(36.36%)	14/34(41.17%)	6/6(100%)	0.09

In Table 2, the liver fat score (LFS) and rate of NAFLD diagnosis by LFS (>0.61) are compared between mild vs moderate vs severe grades on ultrasound. The mean LFS was similar between U1: 0.13; U2: 0.02; and U3: 0.14 (p=0.96). Likewise, rates of suspected NAFLD using the

LFS were 63.63% in U1, 70.58% in U2, and 100% in U3 (p=0.23). Therefore, both LFS means and frequencies of positive scores were statistically alike across all ultrasound-determined steatosis severities.

**Table 2: Liver function score and its interpretation of different ultrasound steatosis grades.**

Liver Fat Score (mean+/-SD)	0.13+/-0.26	0.02+/-0.33	0.14+/-0.21	0.96
Positive fatty liver by LFS N(%)	21/33(63.63%)	24/34(70.58%)	6/6(100%)	0.23

The table 3 demonstrates correlation statistics between liver fat score (LFS) and the serum inflammatory biomarkers ferritin, neutrophil-lymphocyte ratio (NLR) and mean platelet volume (MPV) using Pearson's coefficient (r). Only ferritin demonstrated a weakly

positive but statistically significant correlation with LFS (r=0.235, p=0.05). No significant correlations were seen between LFS and either NLR (r=0.068, p=0.5) or MPV (r=0.1, p=0.37).

**Table 3: Correlation between inflammatory markers and liver function scores.**

	Inflammatory marker(mean+/-SD)	Liver function score(mean+/-SD)	R	P.value
S.ferritin	119.28+/-12.80	0.07+/-20	0.235	0.05

Neutrophil/lymphocyte ratio	1.87+/-0.12	0.07+/-20	0.068	0.5
Mean Platelet volume	9.09+/-0.09	0.07+/-20	0.1	0.37

Table 4 evaluates differences in serum ferritin, NLR and MPV among patients testing positive for NAFLD on LFS (true positives) versus false negative LFS results (ultrasound+ but LFS-). Mean ferritin was higher in true vs false negatives (125.5ng/mL vs 105.69ng/mL) as was MPV (9.19fL vs 8.89fL) but differences were not

statistically significant (ferritin  $p=0.23$ , MPV  $p=0.07$ ). NLR averaged 1.93 in LFS true positive group compared to 1.75 in false negative patients ( $p=0.24$ ). Therefore, inflammatory biomarkers did not differ significantly between accurately and inaccurately classified groups based on the liver fat scoring system.

**Table 4: Inflammatory markers in patients tested true positive VS. false negative by liver function score.**

	positive fatty liver with LFS	negative fatty liver with LFS	P value
S.ferritin	125.50+/-14.82	105.69+/-25.07	0.23
Neutrophil/lymphocyte ratio	1.93+/-0.16	1.75+/-0.18	0.24
Mean Platelet volume	9.19+/-0.11	8.89+/-0.16	0.07

The table 5 demonstrates correlation statistics between quantitative liver fat score (mean 0.07 +/- SD 0.20) and parameters of the lipid panel plus body mass index among the study population with NAFLD. Pearson's correlation coefficients (R) were used to assess the strength of associations between liver fat score and mean total cholesterol (193.67 +/- 3.48 mg/dL), LDL cholesterol (107.04 +/- 3.17 mg/dL), HDL cholesterol (51.95 +/- 1.54 mg/dL), triglycerides (182.6 +/- 6.94 mg/dL) and BMI (30.38 +/- 0.70 kg/m<sup>2</sup>). There were no

statistically significant correlations found between liver fat score and any lipid markers or BMI. Weak positive correlations with liver fat score were seen for triglycerides ( $R=0.20$ ,  $p=0.09$ ) and total cholesterol ( $R=0.13$ ,  $p=0.27$ ), while negligible relationships were observed with LDL cholesterol ( $R=0.08$ ,  $p=0.5$ ), HDL cholesterol ( $R=-0.06$ ,  $p=0.61$ ) and BMI ( $R=0.04$ ,  $p=0.74$ ). All p-values exceeded the a priori statistical significance threshold of 0.05 used in this study.

**Table 5: Correlation between Lipid profile and BMI with liver function scores.**

	Lipid factor(mean+/-SD)	Liver function score(mean+/-SD)	R	P.value
Cholesterol	193.67+/-3.48	0.07+/-20	0.13	0.27
LDL	107.04+/-3.17	0.07+/-20	0.08	0.5
HDL	51.95+/-1.54	0.07+/-20	-0.06	0.61
Triglycerides	182.6+/-6.94	0.07+/-20	0.2	0.09
BMI	30.38+/-0.70	0.07+/-20	0.04	0.74

Table 6 displays Pearson's correlation coefficients quantifying the associations between quantitative liver fat score (mean 0.07 +/- SD 0.20) and serum activity levels of the hepatic enzymes aspartate aminotransferase (AST, mean 19.44 +/- 1.43 U/L) and alanine aminotransferase (ALT, mean 23.07 +/- 1.50 U/L). There were weak positive but non-significant correlations

found between liver fat score and both AST ( $R=0.121$ ,  $p=0.31$ ) and ALT ( $R=0.19$ ,  $p=0.11$ ). The p-values exceeding the 0.05 statistical significance threshold indicate lack of significant linear relationships between liver fat estimation and elevations in liver enzymes among patients with imaging-confirmed NAFLD.

**Table 6: Correlation between Liver enzymes and liver function scores.**

	Liver enzyme (mean+/-SD)	Liver function score (mean+/-SD)	R	P.value
AST	19.44+/-1.43	0.07+/-20	0.121	0.31
ALT	23.07+/-1.50	0.07+/-20	0.19	0.11

## DISCUSSION

Evaluating the sensitivity of the noninvasive liver function score (LFS) for non-alcoholic fatty liver disease (NAFLD) diagnosis against ultrasonic imaging in 71 individuals was the main aim of this cross-sectional study. Secondary objectives were to evaluate variations in serum ferritin, mean platelet volume (MPV), and

neutrophil-lymphocyte ratio (NR) among mild (U1), moderate (U2), and severe (U3) ultrasonic-determined steatosis grades as well as across individuals with conflicting LFS and ultrasound findings. Although the general LFS sensitivity for NAFLD was sufficient at 70.5%, the study revealed that performance significantly dropped for mild (U1) disease detection against higher



grades. Increasing ultrasonic disease severity greatly affected parameters of dyslipidemia, anaemia, and transaminitis; yet, the inflammatory biomarkers could not consistently differentiate between steatosis grades or correlate with LFS accuracy. The results of the study showing no notable variations in age, sex distribution, BMI, waist circumference, systolic blood pressure, and smoking status across various degrees of fatty liver disease disagree with trends reported in previous literature. For example, a research in Scientific Reports indicated that BMI is a substantial progressive risk factor for fatty liver by showing a nonlinear relationship between BMI and fatty liver risk.<sup>[20]</sup> Another study underlined that people with both high BMI and waist circumference showed more prevalence of metabolic syndrome components than those with either general or abdominal obesity alone, implying a complex interaction between obesity measurements and metabolic health risks, including fatty liver disease.<sup>[21]</sup> Regarding lipid parameters, the present work revealed no appreciable relationships between the liver function scores and lipid parameters including total cholesterol, LDL cholesterol, and HDL cholesterol. Other research, meantime, have found a high correlation between liver fat and variables like blood pressure, glucose, lipid indicators, and fat distribution. For instance, a cross-sectional study of adult Canadians with NAFLD revealed a strong relationship between liver fat and measures including systolic blood pressure and serum  $\alpha$ -2 macroglobulin.<sup>[22]</sup> Liver fat content was observed directly correlated with waist size, lipid levels, glucose, HDL, and systolic blood pressure.<sup>[23]</sup> in a sample of HIV-infected individuals. Among individuals with various degrees of steatosis identified by ultrasonic diagnosis, metabolic syndrome, diabetes, hypertension, ischemic heart disease (IHD), and hypothyroidism were very common. This result is consistent with other research stressing the great frequency of metabolic diseases and their consequences in NAFLD patients. Research has indicated, for example, that NAFLD patients' prevalence of metabolic syndrome was much greater than that of the control group. High rates of hepatic steatosis among patients<sup>[24]</sup> were also found in another investigation looking at the correlation between fatty liver and cardiovascular risk variables in persons with metabolic syndrome.

With a sensitivity of 63.63% for mild steatosis, 70.58% for moderate steatosis, and 100% for severe steatosis, the study showed that the LFS sensitivity for detecting fatty liver varied according on different NAFLD degrees. This heterogeneity emphasises how difficult it is to diagnose NAFLD and points to the need of stratified diagnosis methods. Particularly in early-stage illness where LFS sensitivity may be reduced, advanced imaging technologies such as MRI-PDFF provide comprehensive insights into liver fat accumulation and fibrosis development, therefore offering a complementing tool to LFS. MRI-PDFF has been linked with histological improvement in NASH.<sup>[25,26]</sup> and demonstrated to forecast fibrosis development in NAFLD patients in the

early stages. Particularly in the identification of low-risk NAFLD patients, the development of scores such as the SAFE score for primary care usage illustrates a trend towards non-invasive, readily accessible diagnostic approaches that might support or improve the utility of LFS. The current work adds important information to the continuous assessment of LFS in NAFLD diagnosis, therefore highlighting the requirement of a multimodal diagnosis including both conventional scores like LFS and sophisticated imaging modalities to precisely evaluate the range of NAFLD degree. Future studies should concentrate on improving these instruments and investigating their combined use to raise patient management and diagnosis accuracy in NAFLD.

## CONCLUSION

Finally, compared to ultrasonic imaging, the liver function score shows 70.5% sensitivity for NAFLD identification throughout all severity levels. For mild fatty liver diagnosis, this subgroup had a sensitivity of 63.63% that dropped drastically. This difference highlights the complexity and need of using several approaches to properly identify early stages of disease. On ultrasonography, increasing cholesterol, transaminases, and anaemia indicators from mild to moderate to severe grades revealed significant histological damage; inflammatory biomarkers did not correlate with LFS accuracy or distinguish steatosis classes. These test designs expose pathogenic processes of cardio-metabolic risks and NAFLD development. The great incidence of diabetes, hypertension, and other comorbidities emphasises NAFLD's tight relationship with metabolic dysfunction even if their distribution did not alter by imaging degree.

Although the liver function score can identify hepatic steatosis, its shortcomings in milder instances imply the need of combining scoring systems, biomarkers, and imaging techniques for best diagnosis accuracy along the NAFLD range. More research should improve noninvasive indicators of early disease and investigate creative ways to appropriately describe inflammatory state and fibrotic progression in this challenging condition.

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