National Board of Examinations - Journal of Medical Sciences
Volume 3, Issue 9, Pages 1026–1035, September 2025
DOI 10.61770/NBEJMS.2025.v03.i09.002

ORIGINAL ARTICLE

Association of Lipid Profile with Non-Alcoholic Fatty Liver Disease Diagnosed on Ultrasound

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Accepted: 2-July-2025 / Published Online: 9-September-2025

Abstract

Background: A global prevalence of 25.26% of non-alcoholic fatty liver disease has been reported and it constituted a wide spectrum of chronic hepatic disorders varying from simple steatosis to steato-hepatitis, fibrosis, and cirrhosis, without significant alcohol consumption. The diagnostic tests including MRI and liver biopsy are either costly or invasive as compared to ultrasonography for diagnosis and severity of NAFLD which is more convenient and non-invasive. Thus, the study investigates the association between lipid profiles and NAFLD severity diagnosed by ultrasonography. **Methods:** The study included 126 patients with NAFLD diagnosed on ultrasound, conducted between December 2022 and May 2024 at Government Multispecialty Hospital, Chandigarh. Data tools included socio-demographic information, clinical profile, HbA1c levels, lipid profiles, liver function tests, and ultrasound findings. Statistical analyses, including Chi-square, ANOVA, and multinomial logistic regression, were used to establish associations between variables. **Results:** More than half of the participants (55.6%) had grade I NAFLD, while 31.7% and 12.7% had grade II and III, respectively. Severity of NAFLD was significantly associated with increased levels of total cholesterol levels, TG, LDL, and reduced HDL. ALP and triglycerides were strong predictors of severity of NAFLD. **Conclusion:** The study found significant associations between lipid parameters and NAFLD severity, with ALP and triglycerides as key predictors. Factors like diabetes, gender, and BMI showed no significant correlations, suggesting a complex interplay of metabolic factors in NAFLD severity.

Keywords: Non-alcoholic fatty liver disease, Lipid profile, ultrasonography

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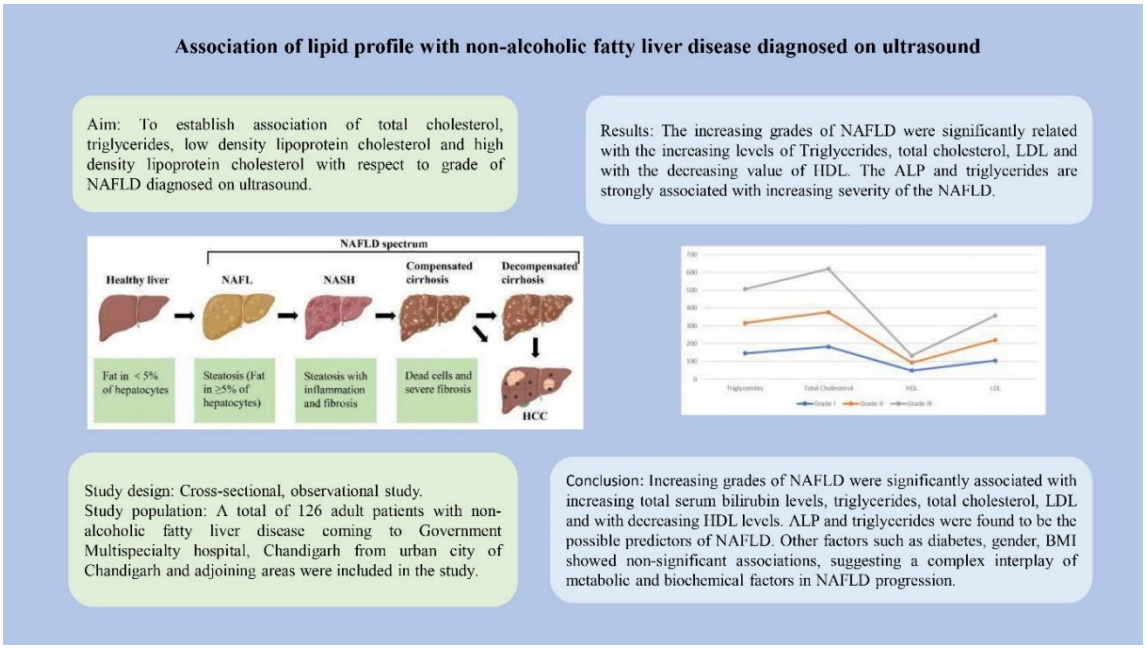
Graphical Abstract



Introduction

Non-alcoholic fatty liver disease (NAFLD) comprises of non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), and cirrhosis. Up to 15% to 30% of Indians have been found to have fatty liver. Usually, an incidental finding with no clinical manifestations, but few may have discomfort in right upper abdomen, malaise, fatigue, and hepatomegaly on examination. Liver biopsy is the confirmatory test for NAFLD but is an invasive procedure. Thus, ultrasonography being a more convenient and cost-effective method has been used for diagnosing of NAFLD [1]. Non-alcoholic Fatty Liver Disease has become the most common cause of chronic liver disease affecting 2-4% of people worldwide and 17-30% of people in Western nations. While in India, it has been shown to be as high as 15-30% [2-3].

Prevalence of obesity, diabetes, and metabolic syndrome is increasing globally, leading to an increase in the cases of NAFLD, dyslipidaemia being the common eitiology among these. Since the pathognomic feature of NAFLD is the accumulation of fat in the hepatocytes [4].

A diagnosis of fatty liver is made when two out of the following three criteria exist. 1) Liver echotexture is brighter than that of the spleen and kidney; 2) Portal and hepatic vein blending; 3) Decrease in the deep Echo-discontinuous diaphragm.

Grading of hepatic steatosis [5]

Grade 1: Raised hepatic echogenicity associated with normal peri-portal echogenicity and diaphragmatic echogenicity; Grade 2: Raised hepatic echogenicity associated with negligible peri-portal echogenicity and normal visualisation of diaphragm; Grade 3: Non-vsualisation of the diaphragm, undetectable periportal echogenicity, and increased liver echogenicity.

Risk factors for NAFLD

The various etiological factors that lead to NAFLD include genetic factors,

environmental factors, demographic factors, metabolic factors, and gut microbiota. The risk for fatty liver is higher among patients with T2DM, obesity, dyslipidaemia, metabolic syndrome and increasing age [6]. On the other hand, a term "lean NAFLD" has also been defined in literature as hepatic steatosis in people with body mass index (BMI) of less than 25kg/m^2 and around 20% patients of NAFLD are lean. However, lean NAFLD have a better metabolic and histological profile as compared to NAFLD associated with obesity [7].

Dyslipidaemia and NAFLD

The patients with cardiovascular diseases (CVD) risk factors, such as dyslipidaemia and type 2 diabetes mellitus, are more likely to develop NAFLD. Half of patients with type 1 diabetes were also reported to have NAFLD. A strong correlation was found between NAFLD and hypertriglyceridemia, in more than of the patients. The elevated low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), hypertriglyceridemia and reduced high density lipoprotein (HDL) levels are hallmark for NAFLD. The dyslipidaemia associated with NAFLD is pro-atherogenic and CVD is the leading cause of death in these patients. The NAFLD may be used as a one of the features of metabolic syndrome, insulin resistance and CVD risk assessment. Understanding and treating dyslipidaemia is crucial to the overall management of NAFLD patients, to avoid the cardiovascular diseases in these patients [8].

NAFLD and cardiovascular mortality

In recent years, the risk of cardiovascular diseases has increased In NAFLD patients owing to atherosclerosis. The atherogenic dyslipidaemia, systemic inflammation, poor metabolic profile in NAFLD patients increases the risk of atherosclerosis. Due to increasing frequency and severity of cardiovascular events in NAFLD patients, the atherosclerotic cardiovascular diseases risk may be predicted by NAFLD [9].

Among all the different modalities available, MRI is the best imaging for diagnosis of fatty liver. However, it is expensive making its use limited [7]. Ultrasonography (USG), with a sensitivity of 60% to 94% for diagnosing steatosis, is frequently used for population screening and the initial evaluation of patients with suspected NAFLD since it is widely accessible, simple to conduct, and less expensive [10]. The study was done to find out the association of lipid profile with non-alcoholic fatty liver disease diagnosed on ultrasound.

Methods

The study was conducted after taking an approval from the Institutional Ethics committee of Government Medical College and Hospital, Sector 32, Chandigarh. The data were collected from the participants after taking informed consent, between December 2022 to May 2024 at Government Multispecialty Hospital, Sector 16, Chandigarh.

Study Population

126 adult patients of non-alcoholic fatty liver disease coming to Government Multispecialty hospital, Chandigarh from urban city of Chandigarh and adjoining areas were included in the study.

Study design

It was a cross-sectional, observational study.

Inclusion criteria

Those above the age of 18 years, diagnosed to have non-alcoholic fatty liver disease on ultrasound and presented with abdominal complaints in outpatient department or admitted in ward were included.

Exclusion criteria

Patients with fatty liver disease, with a history of significant alcohol consumption and with incomplete hospital records were excluded from the study. Patients taking somatogenic drugs like amiodarone, steroids, diltiazem, methotrexate etc, were also excluded from the study.

Data collection

Eligible patients were explained regarding scope, nature of the study and about the related risks in their own language. After taking an informed consent, the data were collected by detailed history, vital signs and clinical examination. Then they were subjected to ultrasonography using Siemen machine with 3.5-5 MHz transducer. Patients who were diagnosed with fatty liver on ultrasonography were subjected to biochemical testing. Fasting blood samples were taken and lipid profile was estimated in biochemistry lab on fully automated biochemistry analyser XL-1000. Patients were also subjected to other blood investigations like thyroid profile, HBA1c and complete hemogram.

Statistical analysis

Data were analysed using the software statistical package for social sciences (SPSS version 20.0, IBM Corp., Armonk, New York). Univariate analysis included mean (standard deviation) for quantitative variables and frequency (proportions) for qualitative variables. To find out the associations of various grades/severity of non-alcoholic fatty liver disease with socio-demographic details and laboratory investigations, Chi-square, and Analysis of variance (ANOVA) tests were used for qualitative and quantitative variables, respectively. A multinomial logistic regression analysis was done to identify the association of various risk factors with different severities of NAFLD categorised as mild, moderate, and severe. Associations with p-value<0.05 at 95% confidence level were taken as significant.

Results

A total of 126 patients who were diagnosed with non-alcoholic fatty liver disease were included in the study with the mean age of 51.8 (±10.4 SD) years and a range of 25 to 69 years. Approximately, equal number of females (n=65) and males (n=61) were included in the study. Baseline characteristics of clinical examination included blood pressures, body mass index, history of diabetes mellitus and grades of non-alcoholic fatty liver disease as shown on Table 1. Nearly half (48.4%) of the patients were obese and 21% were overweight. More than half (55.6%) of the participants had grade I NAFLD, whereas 31.75% and 12.7% had grades II and III, respectively. Approximately 44% of the participants in the study were diabetics. Mean fasting glucose (120 ± 64.8SD) mg/dl, glycated haemoglobin (HbA1c) (6.1 ± 1.6SD)% were calculated. Distribution of lipid parameters and liver function tests were observed for NAFLD grades (Figure 1).

Table 1. Baseline characteristics of the study participants (n=126).

Variables		Mean ± SD
Age (years)		51.8 ± 10.4
FBG (mg/dl)		120.9 ± 64.8
SBP (mm of Hg)		132.6 ± 16.6
DBP (mm of Hg)		76.3 ± 9.1
HbA1c (%)		6.1 ± 1.6
Variables		Frequency (%)
Gender	Male	65 (51.6)
	Female	61 (48.4
BMI (Kg./m^2)	Obese ≥30)	61 (48.41)
	Overweight (25-29.99)	27 (21.43)
	Normal (18.5-24.99)	37 (29.37)
	Underweight (<18.5)	1 (0.79)
Diabetes mellitus	Present	55 (43.7)
	Absent	71(56.3)
Grades of NAFLD	Grade I	70 (55.6)
	Grade II	40 (31.7)
	Grade III	16 (12.7)

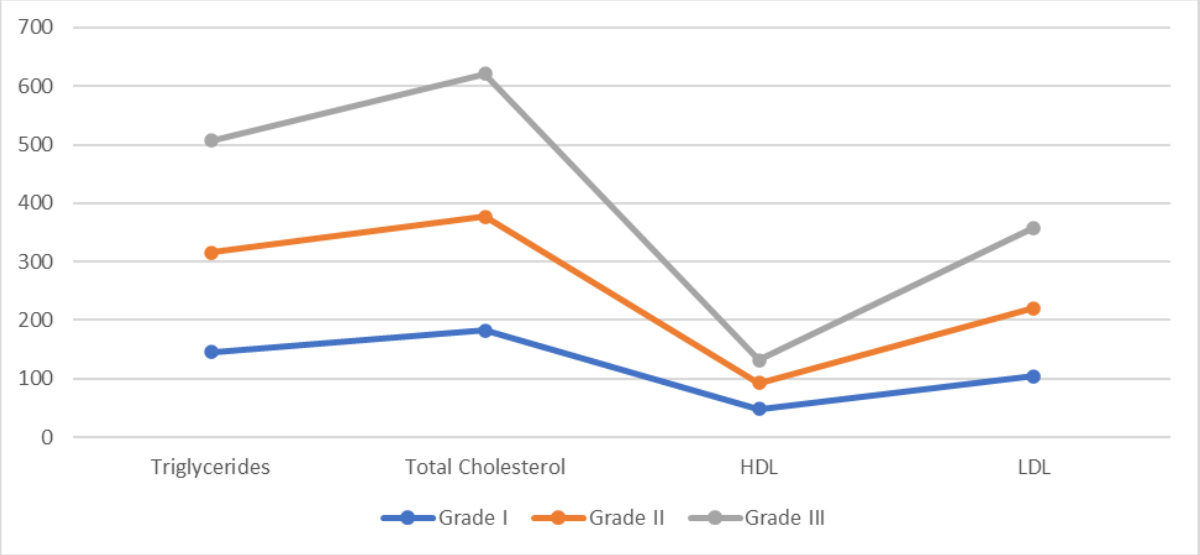


Figure 1. Correlation between NAFLD and serum lipid profile (n=126).

Socio-demographic characteristics and NAFLD

The mean age of the participants in years was 51.26 (10.94 SD), 52.4 (10.04 SD), 52.69 (9.39 SD), for grades I, II, III, of NAFLD, respectively. It was observed that the age of the participants had no association with the severity of NAFLD (p-value=0.847). Gender of the study participants had no association with the severity of disease (p-value=0.985). Likewise, severity of NAFLD was not associated with the body mass index of the participants (p-value=0.406) (Table 2).

Table 2. Association of NAFLD with clinical profile (n=126)

Variables		NAFLD grades [Mean (SD)]			p-value
		Grade I	Grade II	Grade III	
Age		51.26 (10.94)	52.4 (10.04)	52.69 (9.39)	0.847
Gender (%)	Male	34 (55.7)	19 (31.1)	8 (13.1)	0.985
	Female	36 (55.4)	21 (32.3)	8 (12.3)	
Body mass index (%)	Obese	38 (62.3)	19 (31.1)	4 (6.5)	0.406
	Overweight	13 (48.1)	8 (29.6)	6 (22.2)	
	Normal	18 (48.6)	13 (35.1)	6 (16.2)	
	Underweight	1 (100)	0	0	
DM (%)	Present	28 (50.9)	19 (34.5)	8 (14.5)	0.643
	Absent	42 (59.1)	21 (29.6)	8 (11.3)	
Lipid profile	Triglycerides (mg/dl)	145.03 (40.22)	170.53 (61.62)	191.56 (74.56)	**0.002**
	Total Cholesterol (mg/dl)	182.34 (50.39)	194.93 (61.02)	244.25 (107.29)	**0.003**
	HDL (mg/dl)	48.01 (9.43)	44.53 (11.18)	39.03 (11.4)	**0.006**
	LDL (mg/dl)	103.77 (39.99)	116.55 (45.6)	138.06 (54.55)	**0.013**
LFTs	Total serum bilirubin (mg/dl)	40.5 (0.28)	0.82 (0.31)	0.95 (0.52)	**<0.001**
	SGOT (IU/L)	40.5 (22.28)	54.5 (26.5)	59.34 (31.4)	**0.01**
	SGPT (IU/L)	31.43 (17.18)	38.24 (16.14)	32.45 (11.83)	0.099
	ALP (IU/L)	105 (38.65)	146 (69.15)	157 (101.09)	**<0.001**

Clinical profile and NAFLD

Systolic and diastolic blood pressures of the patient were also compared for severity of NAFLD. However, no significant association was observed among the two (p-value= 0.806 and 0.068, respectively). Majority of the diabetic patients, had grade I NAFLD, although the association was not statistically significant (p-value=0.643) Higher mean of parameters of lipid profile was significantly associated with higher grade of NAFLD except HDL levels, where higher level of HDL was associated with lower grade of NAFLD as shown in Figure 1 and Table 2. On comparing the liver function tests with severity of NAFLD, higher mean of the parameters was significantly associated with higher grades of NAFLD except serum glutamic pyruvic transaminase (SGPT) levels (Table 2).

Predictors of non-alcoholic fatty liver disease

Factors significant in bivariate analysis and based on literature, were all included in the multinominal logistic regression (Table 3). Of all these factors, alkaline phosphatase, and triglyceride levels were the significant predictors of moderate (grade II) NAFLD, in which for every unit increase in ALP, the odds of moderate NAFLD compared to mild (grade I) NAFLD increased by 1.017 (95% CI: 1.004-1.031). Similarly, triglycerides also exhibited a significant association indicating a slight increase in the likelihood of moderate NAFLD with rising triglyceride levels with and odds of 1.013 (95% CI: 1.002-1.026). Alkaline phosphatase (ALP) also predicted the likelihood of NAFLD, with higher odds of 1.023 (95% CI: 1.008-1.039) for severe (grade III) NAFLD when compared to mild NAFLD.

Table 3. Predictors for non-alcoholic fatty liver disease among the study participants (n=126)

NAFLD	Predictor	Odds ratio	95% CI
Moderate – mild (Ref.)	SGOT	1.013	0.982 – 1.045
	SGPT	1.004	0.964 – 1.046
	ALP	**1.017**	**1.004 – 1.030**
	Cholesterol	0.995	0.983 – 1.005
	LDL	1.001	0.987 – 1.014
	Triglycerides	**1.013**	**1.002 – 1.025**
	HDL	1.002	0.951 – 1.057
	HbA1c	1.333	0.794 – 2.237
	BMI	0.856	0.676 – 1.083
	Gender	0.782	0.305 – 2.002
	Diabetes Mellitus	0.418	0.305 – 2.002
	Obese-normal weight	1.076	0.165 – 6.991
	Overweight-Normal weight	0.775	0.180 – 3.338
Severe-mild (Ref.)	SGOT	1.024	0.984 – 1.064
	SGPT	0.947	0.881 – 1.016
	ALP	**1.023**	**1.007 – 1.038**
	Cholesterol	1.010	0.994 – 1.024
	LDL	1.007	0.986 – 1.026
	Triglycerides	1.006	0.988 – 1.024
	HDL	0.996	0.929 – 1.066
	HbA1c	1.015	0.481 – 2.142
	BMI	1.089	0.865 – 1.370
	Gender	0.754	0.178 – 3.193
	Diabetes Mellitus	1.170	0.112 – 12.172
	Obese-normal weight	0.124	0.012 – 1.230
	Overweight-Normal weight	1.215	0.208 – 7.087

Model	Deviance	AIC	R^2	X^2	df	P
1	185	245	0.287	54.9	28	**0.002**

Discussion

Non-alcoholic fatty liver disease (NAFLD) along with Non-alcoholic steatohepatitis (NASH) have become one of the major causes of mortality and morbidity in the world. This study was an observational cross-sectional design including 126 participants who had NAFLD diagnosed on the ultrasound, with various grades of the liver damage. The objective was to understand the relationship between lipid parameters and the severity of NAFLD. Majority of the population was in the 5th and the 6th decade of their lives, with a mean age of 51.3 years. Sharma S et al [11] and Mahaling et al [1] showed a mean age of 48.3 years and 49.1 years, respectively, showing the distribution in the same age brackets as the current study. Another study by Bhusal et al [12] also had a similar mean age of 45.39±11.99 years, where most of the study population was in 4th and 5 the decades of life. The mean age in the western studies have been between 41-45 years.

Most of the cases, 55.6% were in grade I of liver damage, followed by grade II in 31.7% of the population and grade III being the least common in the study population, only in 12.7%. Other studies have also shown a similar pattern. One study [12] also showed (n= 100) that 83% had mild fatty liver, and 17% had moderate, while none were having severe fatty liver amongst his study population. Mahaling et al [1] (n= 70) showed that 48.15% of the population had grade I liver

damage, 41.85% had grade II liver damage, while only 10% of the population had grade III liver damage. Sen et al [13] (n= 385) also showed that 83.4% of the study population had grade 0 liver damage, followed by grade I with 11.7%, grade II with 3.1% and the least with grade III, 1.8%. In a study by Baghel et al [14] (n= 50), they showed that there were more cases of grade II (48%) than grade I (40%) and grade III (12%).

In the current study it was found that the serum triglycerides (TG), total cholesterol and LDL were increased in 59.5%, 48.4%and 41.3%, respectively, while HDL was decreased in 58.7% of the study population. Similar findings were seen in the study by Baghelet al [14], and Mahaling et al [1]. Sen et al [13] showed similar trend, but the mean values for the lipid parameters were even higher than seen in the current study. Liver enzymes like alanine transaminase (ALT) and aspartate transaminase (AST) determine the liver function in general. In this study it was observed that the values of total serum Bilirubin, ALP, AST, ALT, were increasing with increase in the severity of the liver disease. Further, the increase in the values of total serum bilirubin (TSB), AST, and ALP had a significant positive correlation with increasing severity of the liver disease ($p< 0.001$, for all), while the change in the values of ALT had no significant correlation with the severity of the disease ($p= 0.099$). In other studies, like Baghel et al [14], Mansour-Ghanaeiet al [15], also found that higher values of lipid parameters were more likely associated with the severity of the liver disease. In another study, Ramesh et al [16] found that the increase serum ALT and dyslipidaemia maybe directly responsible for increase in NAFLD and also supported by Zakeri et al, who reported that elevated ALT and dyslipidaemia may play a role in the development and progression of NAFLD [17]. In this study it was noticed that increased total serum bilirubin was directly and significantly related with the increase in NAFLD chances. The patients included in this study were all diagnosed with NAFLD during the routine ultrasonography subjected to those who presented to opd with complaints such as pain abdomen, anorexia, weight loss, weight gain and patients admitted in wards.

The regression analysis highlighted the importance of ALP and triglycerides as significant predictors of NAFLD severity. ALP consistently demonstrated a stronger association with increasing severity of NAFLD, underscoring its relevance as a marker for disease progression. While triglycerides were associated with moderate NAFLD, their impact on severe NAFLD was not significant. Other factors such as diabetes, sex and BMI showed non-significant associations, suggesting a complex interplay of metabolic and biochemical factors in NAFLD progression.

Study Limitations

The study has several limitations inherent to its design. As a hospital-based, cross-sectional study, there is a possibility of selection bias, and the findings may not be fully generalizable to the broader community. The absence of a control group limits comparative analysis. Additionally, the lack of longitudinal follow-up prevents evaluation of disease progression over time. The relatively short study duration and sample drawn from a single urban setting may also restrict the representativeness of the results.

Conclusion

The study findings revealed that over half of the participants were diagnosed with grade I non-alcoholic fatty liver disease. An upward trend in lipid profile parameters, including low-density lipoprotein, triglycerides, and total cholesterol, was observed with increasing severity of NAFLD. A statistically significant association was found between higher NAFLD grades and elevated levels of total serum bilirubin, triglycerides, total cholesterol, LDL, as well as reduced high-density lipoprotein levels. Among the biochemical markers, alkaline phosphatase and triglycerides emerged as potential risk factors for disease progression. Conversely, variables such as diabetes, gender, and body mass index did not show significant associations, indicating that the development and advancement of NAFLD likely involve a multifaceted interaction of metabolic and biochemical factors.

Statements and Declarations:

Conflicts of interest

The authors declare they do not have conflict of interest.

Funding

No funding was received for conducting this study.

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