Original Article

Association of Lipid Profile and Liver Parameters with Different Grades of NAFLD

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Abstract

Background: The exact mechanisms of Non-Alcholic Fatty Liver Disease (NAFLD), now called Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), are still unclear. Potential factors include genetic differences in the distribution of body fat and antioxidant systems. This study's objective was to evaluate lipid profiles and liver function tests in NAFLD patients and look into any possible associations with various NAFLD grades. Setting and design is observational Cross sectional study occurred over a year in the Department of Biochemistry and Department of Medicine. **Material and Methods:** Serum lipid levels and liver markers were examined in 62 NAFLD patients. **Results:** Our study found an increased patients' serum levels of TC, TG, LDL, and VLDL were found in 54.84%, 64.52%, 40.32%, and 46.77%, respectively, while decreased serum HDL levels were found in 51.61% of patients. Serum AST, ALT, and total bilirubin were increased in 62.9%, 58.1%, and 50% of NAFLD patients, respectively, in this current study, whereas decreased total protein levels were found in 41.9% of NAFLD patients. In a significant finding, of our study, abnormal values of ALT were found predominant among female patients of all Grades of NAFLD. **Conclusion:** Abdominal ultrasonography combined with routine monitoring of lipid parameters and liver function is key to early detection and tracking of disease progression among NAFLD patients.

Keywords: Non-Alcholic Fatty Liver Disease, Metabolic Dysfunction-Associated Steatotic Liver Disease, Lipid profile, Liver function tests, Ultrasonography.

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Introduction

Non-alcoholic fatty liver disease (NAFLD), the most common type of liver disease, is commonly described as hepatic fat accumulation greater than 5% of liver weight without a history of substantial alcohol use. NAFLD can also result in cirrhosis, hepatocellular cancer, or non-alcoholic steatohepatitis (NASH).[1] In 2019, there were 170,000 new NAFLD cases globally. Asia accounted for 48.3% of cases and 46.2% of deaths, while Middle-East and North Africa (MENA) accounted for 8.9% of cases and 8.6% of deaths. Asia had 2.08 million Disability-Adjusted Life Years (DALYs) due to NAFLD.[2]

Dyslipidemia is the most frequently observed comorbidity in non-alcoholic fatty liver disease (NAFLD) patients, further characterized by hypertriglyceridemia; which increases in low- density lipoprotein cholesterol (LDL-C) and decreases in high-density lipoprotein cholesterol (HDL-C).[3]

Liver ultrasonography (ultrasound) is indeed the most widely used technique for identifying hepatic steatosis, or fatty liver, in general populations because of its safety, low cost, and accessibility.[4] Patients with abnormally high aminotransferase levels often seek treatment from gastroenterologists or hepatologists. As a result, abnormal levels of alanine transaminase (ALT) and aspartate transaminase (AST) are employed in a number of examinations to identify NAFLD.[5]

As a result, measurements of blood lipids, insulin resistance (IR), and aminotransferases are frequently used in clinical settings to diagnose NAFLD. Important factors in non-alcoholic fatty liver disease (NAFLD) include the lipid profile, AST, ALT, fasting insulin level, CRP, and fasting blood sugar (FBS).[6] These markers are helpful substitutes for liver biopsies since they enable medical professionals to evaluate the disease's severity and prognosis and start treatment strategies earlier.[7]

The purpose of this study was to assess liver function tests and lipid profiles in NAFLD patients and investigate any potential correlations with different NAFLD grades. Primary objective of the study was to determine the point prevalence of NAFLD presenting with abnormal Lipid profile with liver dysfunction, while the secondary objective was to determine any association of NAFLD with severity of Liver disease.

Materials and Methods

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Biochemistry conducted an observational cross-sectional study over the course of a year. The institution's research ethics committee gave its clearance before the study was carried out. From the OPD of the Department of Medicine in the medical ward of Medical College and Hospital, sixty-two patients with non-alcoholic fatty liver disease (NAFLD) of both genders and ages ranging from 18 to 60 were chosen, during a period of 6-months. After a mandatory and thorough explanation of the study to each participant, each of them provided written informed consent. The subject data's privacy was safeguarded through computer recording and coding.

Calculation of Sample Size: A reference study conducted previously have shown that more than 35% of NAFLD patients tend to show deranged lipid profile/ TG/LDL/Total Cholestrol levels.[8] Assuming level of confidence (z) as 90%, expected prevalence (P) to be 35%, and margin of error (d) as 10%, sample size N was calculated using the formula: N= z2 P(1-P)/d2 as 62.

Inclusion Criteria

Every individual with non-alcoholic fatty liver disease (NAFLD), regardless of gender, between the ages of 18 and 60; Patients attending Medicine OPD/ IPD of GMC Jammu

Exclusion criteria

All such patients who consumed alcohol or smoked, had cancer, had gastrointestinal surgery, had viral or autoimmune liver diseases and all such patients who have previously used heparin, calcium channel blockers, steroids, or synthetic estrogens.

After six to eight hours of fasting, patients were assessed using real-time USG. The most common positions employed were supine and right anterior oblique views. Images from sagittal, transverse, coronal, and subcostal oblique views were taken during the ultrasound examination using a regular abdomen transducer and a higher frequency transducer. The following criteria were used to determine a fatty liver's final grade:

Grade I: The liver showed a slight increase in echogenicity, but the diaphragm and intrahepatic vessel borders were still visible and normal.

Grade II: The liver had a moderate increase in echogenicity, slightly obscuring the intrahepatic vessel walls and diaphragm.

Grade III: The liver showed a notable rise in echogenicity, making it difficult to see the right lobe's posterior segment, and the hepatic vessels and diaphragm were barely or not visible.

Blood pressure (systolic and diastolic) was measured using a mercury sphygmomanometer after the patient rested for 10 minutes.

Approximately 5 ml of fasting venous blood was collected from patients under aseptic conditions and transferred to plain tubes for lipid profile and liver function tests. To extract the serum, blood samples obtained in simple vials were centrifuged for 10 to 15 minutes at 3000 rpm. Friedewald's equation was used to compute low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, while a Biosystem BA-400 chemistry analyzer was used to assess lipid parameters and liver function tests in accordance with conventional procedures.[9]

Statistical analysis: Data analysis was done using SPSS Statistics 20, the Statistical Package for Social Science. The numerical variables were expressed as mean ± standard deviation (SD), while the categorization variables were expressed as numbers (%). The p- value was calculated using one-way ANOVA, with $p < 0.01$ indicating statistical significance.

Results

Out Of sixty-two NAFLD cases identified by USG, twenty-three (37%) were grade I, twenty-eight (45%) were grade II, and eleven (18%) were grade III. [Table 1] displays the patients' baseline characteristics. The average age of patients with NAFLD was 44.75 ± 6.55 years. The diastolic and systolic blood pressures of NAFLD patients were 80.44 ± 9.23 mmHg and 126.38 ± 15.45 mmHg, respectively, which were marginally higher than usual. At 108.05 ± 39.18 mg/dL, the mean fasting blood glucose level was within the normal range.

[Table 2] shows lipid changes across NAFLD grades. Higher NAFLD grades correlated with increased TC, TG, and VLDL, but decreased HDL. LDL levels didn't show a significant association with NAFLD grade.

[Table 3] shows distribution of aberrant lipid profiles in people with NAFLD. Here, increased Serum TC, TG, LDL, and VLDL levels were found in 54.84%, 64.52%, 40.32%, and 46.77% of patients, respectively, while decreased serum HDL levels were found in 51.61% of patients.

[Table 4] shows liver parameters across NAFLD grades. Higher NAFLD grades correlated with increased AST, ALT, and total bilirubin, but decreased total protein. The escalating grades of NAFLD were not shown to be statistically correlated with the AST/ALT ratio.

[Table 5] shows the distribution of patients with NAFLD who have abnormal liver function tests. While 41.9% of NAFLD patients had lower total protein levels, 62.9%, 58.1%, and 50% of NAFLD patients had elevated serum AST, ALT, and total bilirubin.

[Table 6] shows gender-wise distribution of two specific liver function tests, i.e. AST and ALT in NAFLD patients. Abnormal Serum AST was found more in Females across Grade I and II of NAFLD, whereas abnormal values of ALT were found predominant among female patients of all Grades of NAFLD.

Table 1: Baseline Characteristics of Patients

Variables	NAFLD (n=62)
Age (Years)	44.75 ± 6.55
Gender (M/F)	28/34
DBP (mmHg)	80.44 ± 9.23
FBG (mm/dL)	108.05 ± 39.18

SBP=Systolic blood pressure; DBP=Diastolic blood pressure; FBG= Fasting blood glucose.

Table 2: Lipid Profile with Different Grades of NAFLD

Ultrasound Grades	Grade I n= 23		Grade II n= 28		Grade III n= 11		P-value
Lipid Profile (mg/dL)	Mean	SD	Mean	SD	Mean	SD	
TC	182.91	±49.42	208.07	±50.06	248.32	±50.94	0.009*
TG	157.22	±54.09	211.14	±97.68	266.17	±102.42	0.009*
HDL-C	46.78	±6.01	40.33	±5.58	30.51	±4.06	< 0.0001*
LDL-C	112.54	±45.96	143.56	±54.62	148.83	±57.13	0.284
VLDL-C	31.90	±9.03	45.41	±13.77	57.25	±24.96	0.009*

*p-value<0.01 = Significant; TC=Total cholesterol; TG=Triglyceride; HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol; VLDL-C=Very low-density lipoprotein cholesterol.

Table 3: Distribution of patients having abnormal lipid profile.

Fatty Liver Grade	Grade I n= 23		Grade II n= 28		Grade III n= 11		Total n= 62		Total (%)	
Lipid Profile	N	A	N	A	N	A	N	A	N	A
TC	15	8	10	18	3	8	28	34	45.16	54.84
TG	13	10	5	23	4	7	22	40	35.48	64.52
HDL-C	12	11	13	15	5	6	30	32	48.39	51.61
LDL-C	15	8	15	13	7	4	37	25	59.68	40.32
VLDL-C	15	8	13	15	5	6	33	29	53.23	46.77

N=Normal; A=Abnormal; TC=Total cholesterol; TG=Triglyceride; HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol; VLDL-C=Very low density lipoprotein cholesterol.

Table 4: Liver Parameters with Different Grades of NAFLD

Ultrasound Grades	Grade I n= 23		Grade II n= 28		Grade III n= 11		p-value
Liver Function Tests	Mean	SD	Mean	SD	Mean	SD	
AST (U/L)	34.56	±6.02	38.48	±8.75	45.26	±11.84	0.0021*
ALT (U/L)	38.42	±7.41	45.23	±9.01	58.83	±16.37	< 0.0001*
Total Bilirubin (mg/dl)	1.03	±0.31	1.06	±0.32	1.54	±0.41	0.0078*
Total Protein (gm/dl)	7.05	±0.64	5.86	±0.95	5.56	±0.94	0.009*
AST/ALT	0.88	±0.10	0.86	±0.19	0.87	±0.16	0.865

*p-value<0.01 = Significant; †AST: Aspartate transaminase; ‡ALT: Alanine transaminase.

Table 5: Distribution of patients showing abnormal liver function tests in NAFLD

Fatty Liver Grades	Grade I n= 23		Grade II n= 28		Grade III n= 11		Total n= 62		Total (%)	
Liver Function Tests	N	A	N	A	N	A	N	A	N	A
AST	10	13	11	17	2	9	23	39	37.1	62.9
ALT	12	11	10	18	4	7	26	36	41.9	58.1
Total Bilirubin	13	10	14	14	4	7	31	31	50.0	50.0
Total Protein	17	6	15	13	4	7	36	26	58.1	41.9

N= Normal; A= Abnormal; AST: Aspartate transaminase; ALT: Alanine transaminase.

Table 6: Gender-wise distribution of patients showing AST and ALT tests in NAFLD

Fatty Liver Grades		Grade I n= 23		Grade II n= 28		Grade III n= 11	
Liver Function Tests		N	A	N	A	N	A
AST	Male	6	4	5	7	1	5
	Female	4	9	6	10	1	4
ALT	Male	6	3	6	7	3	3
	Female	6	8	4	11	1	4

N= Normal; A= Abnormal; AST: Aspartate transaminase; ALT: Alanine transaminase.

Discussion

Ultrasonography and physical examination were used to diagnose 62 individuals with varied degrees of NAFLD in our hospital-based observational cross-sectional study. Further, criteria as established by AGA, American Gastroenterology Association, were used for classification of the patients.[4] An attempt has been made to characterize the abnormalities of liver function tests and cholesterol levels in NAFLD patients in a North Indian context. In a recent study conducted by Baghel et al,[10] in 2023revealed that 68% of NAFLD patients had higher blood TG levels, 54% had higher total cholesterol, 42% had higher LDL, 38% had higher VLDL, and 36% had lower HDL levels. Serum triglyceride, total cholesterol, and LDL levels were elevated in 26.6%, 27.5%, and 1.8% of NAFLD patients, respectively, according to a different study by Khanal et al,[11] 20.2% of NAFLD patients had low serum HDL levels, which were substantially correlated with increased serum LDL and total cholesterol; our findings are consistent with the above. Additionally, the researchers found no statistically significant connection between declining NAFLD grades as assessed by sonography and blood lipid or HDL levels. In our investigation, rising fatty liver grades in NAFLD patients were linked to blood LDL levels, however this relationship was not statistically significant. According to a study cited here, individuals with NAFLD had elevated levels of total cholesterol,

triacylglycerol, LDL, and VLDL in 58%, 61%, 49%, and 39% of cases, respectively. However, it was also noted that 49% of NAFLD patients had a drop in HDL. Additionally, they found a strong correlation between rising NAFLD grades and lower HDL and higher levels of LDL, VLDL, triacylglycerol, and total cholesterol.[8] Our study found an increased Serum TC, TG, LDL, and VLDL levels in 54.84%, 64.52%, 40.32%, and 46.77% of patients, respectively, while decreased serum HDL levels were found in 51.61% of patients, our finding were concurrent to the above study, except that of VLDL levels which stood higher for 46.77% of patients.

In this study conducted by Khalil et al,[8] 58 males (58%) and 42 females (42%) make up the majority of the population. According to the same study, there is a statistically significant positive link between age and NAFLD grades (p-value 0.000). As people age, their steatosis grade increases. In a different study, Tanwani et al,[12] found that 203 (67.7%) of the participants were men and 97 (32.3%) were women.

Other similar studies by Mahmoud et al,[13] also reported increased prevalence of NAFLD with advancement of age, however our study was not conducted on those lines, the average age in our case was found to be 44.75 ± 6.55 years. This is similar to another study by Sen et al,[14] where the subjects' mean age was determined to be 46.65 ± 15.06 years. Mansour-Ghanaei et al,[15] state that NAFLD patients exhibit a distinctive dyslipidemic pattern. Compared to people without NAFLD, NAFLD patients had greater total cholesterol, lower HDL, and a higher ratio of total cholesterol to HDL. TG levels strongly correlated with NAFLD, but LDL didn't show a correlation. However, Studies conducted by Pardhe et al,[16] and Jain et al,[7] came to similar conclusions about the pattern of lipid parameters in NAFLD and no relation could be found between lipid profile and the grades of fatty liver.

NAFLD is now called MASLD (Metabolic Dysfunction-Associated Steatotic Liver Disease) as of 2023, highlighting its metabolic roots. The exact mechanisms are still unclear. Potential factors include genetic differences in the distribution of body fat and antioxidant systems. Lipid buildup in liver cells, mainly as triglycerides, is a key step in MASLD development. Insulin resistance is a major contributor, but the exact metabolic pathways involved are not well understood, involving changes in lipid absorption, synthesis, breakdown, or secretion.[17]

Liver function is often assessed using AST and ALT enzymes. Previous studies have linked ALT to NAFLD. ALT values within the normal reference range have also been associated with an increased risk of developing NAFLD.[5] In a recent study conducted by Baghel et al,[10] in 2023, Serum AST, ALT, and total bilirubin levels were high in 60%, 58%, and 52% of NAFLD patients, respectively. Comparatively, lower amounts of total protein were found in 38% of NAFLD patients. There was a significant correlation between the severity of fatty liver disease and the mean values of AST and ALT, which were also significantly higher than reference ranges. Serum levels of total bilirubin, ALT, and AST were elevated in 62.9%, 58.1%, and 50% of NAFLD patients, respectively, in this current study, whereas decreased total protein levels were found in 41.9% of NAFLD patients. Mansour-Ghanaei and colleagues [15] similarly reported similar findings, noting that NAFLD patients had significantly higher ALT and AST values than non-NAFLD patients. They also found a connection between the severity of the illness and serum ALT and AST. The severity of fatty liver disease was found to be directly correlated with elevated AST and ALT values by Namoos et al. [18]. There is other research too to support this and have unequivocally demonstrated NAFLD, and liver enzymes have a significant relationship. Zakeri et al,[19] further suggested that elevated levels of ALT and dyslipidemia may cause development and progression of NAFLD.

Other studies like those conducted by Mahaling et al,[20] in 2013 found that HDL decreased in 62.85% of cases and that total cholesterol, TG, LDL, and VLDL were elevated in 45.71%, 67.14%, 34.28%, and 25.71% of cases, respectively. In contrast, Tanwani et al.12 found that HDL decreased in 51.7% of cases and that total cholesterol, TG, LDL, and VLDL were elevated in 62%, 50.7%, 39%, and 39% of cases, respectively. Serum total cholesterol (P-value 0.005), TG (P-value 0.002), LDL (P-value 0.001), and VLDL (P-value 0.003) levels were shown to significantly rise with increasing NAFLD grade, while HDL levels significantly decreased (P-value 0.001). This is consistent with the findings of previous research on the subject.[12,13,20]

The two most significant risk factors for NAFLD are obesity and metabolic syndrome. In a reference research, 37% of 100 individuals had diabetes mellitus, 18% had hypertension, and 7% had both conditions.[8] However, the current study's mean fasting blood glucose level of 108.05 ± 39.18 mg/dL was within the normal range, while Systolic and diastolic blood pressures were found slightly above normal in most of the patients. In a significant finding, of our study, abnormal values of ALT were found predominant among female patients of all Grades of NAFLD.

Liver enzymes aren't a definitive diagnostic tool for fatty liver disease due to their high variability. However, recent studies suggest AST and ALT can help predict NAFLD and its severity, highlighting their potential utility in diagnosis.[15]

Serum Bilirubin is a key component of a liver function test (LFT) panel. It measures the level of bilirubin (a byproduct of red blood cell breakdown) in the blood to evaluate liver health, detect damage, and diagnose jaundice or bile duct blockages. This study also found a relation of advancing NAFLD grades correlated with increased total bilirubin and decreased total protein levels. However, the AST/ALT ratio didn't show a significant link with fatty liver progression. Further studies are needed to clarify the relationship between NAFLD and these biomarkers. Liver biopsy remains the gold standard for diagnosing NAFLD due to its high accuracy. However, it's not feasible for widespread use given the large patient population, high costs, and risks associated with the invasive procedure, such as sampling errors, pain, and potentially life-threatening complications like bleeding.[21]

The two most significant risk factors for NAFLD are obesity and metabolic syndrome. In a reference research, 37% of 100 individuals had diabetes mellitus, 18% had hypertension, and 7% had both conditions.[8] However, the current study's mean fasting blood glucose level of 108.05 ± 39.18 mg/dL was within the normal range.

The major finding of the study is that most of NAFLD patients

typically show a dyslipidemic lipid profile and abnormal liver function, thus making abdominal ultrasonography combined with routine monitoring of lipid parameters and liver function key to early detection and tracking of disease progression.

Conclusion

The current study's conclusions may be constrained by a small sample size from a single institution and the absence of a control group, limiting the generalization of the results. However, the current study data clearly highlights that NAFLD is frequently associated with impaired liver function and abnormal lipid profiles. Therefore, integrating abdominal ultrasonography with routine metabolic and hepatic monitoring offers a viable, non-invasive strategy for early detection and long-term management of the disease.

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Conflicts of interest

There are no conflicts of interest.

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