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Association of Lipid Profile and Liver Parameters with Different Grades of Non-alcoholic Fatty Liver Disease

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ABSTRACT

Background and aim: The present study aimed to determine lipid profile and liver function tests in patients with non-alcoholic fatty liver disease (NAFLD) and examine their possible association with various degrees of NAFLD.
Material and methods: Fifty patients with NAFLD were enrolled in this cross-sectional study, and their serum lipids and liver parameters were analyzed.
Results: Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels were increased in 54%, 68%, 42%, and 38% of NAFLD patients, respectively. Serum high-density lipoprotein (HDL) levels were decreased in 36% of NAFLD patients. Similarly, serum aspartate transaminase (AST), alanine transaminase (ALT), and total bilirubin were increased in 60%, 58%, and 52% of NAFLD patients, respectively whereas total protein levels were decreased in 38% of NAFLD patients. Increasing grades of NAFLD were substantially associated with increasing serum TC, TG, VLDL, AST, ALT, and total bilirubin. At the same time, HDL and total protein levels were decreasing.
Conclusions: A dyslipidemic pattern of lipid markers and abnormal liver function tests were observed in patients with NAFLD. Dyslipidemia and increased AST and ALT levels were also associated with increasing degrees of fatty liver in patients with NAFLD.

1. Introduction

The most prevalent type of liver disease, non-alcoholic fatty liver disease (NAFLD), is defined as hepatic fat buildup exceeding 5% of liver weight without a history of heavy alcohol consumption. It can advance to non-alcoholic steatohepatitis (NASH), cirrhosis, and even hepatocellular carcinoma.[1-4] Its prevalence differs depending on location. For instance, the prevalence of NAFLD is 27.37% in Asia and 24.13% in North America, with the Middle East having the highest prevalence (31.79%) and Africa having the lowest prevalence (13.48%).[5] According to estimates, NAFLD prevalence is rising across the board.[6, 7] A major comorbidity that is frequently observed in NAFLD patients is dyslipidemia, which is characterized by hypertriglyceridemia, decreases in high-density lipoprotein cholesterol (HDL-C), and increases in very low-density lipoprotein (VLDL) and low-density lipoprotein cholesterol (LDL-C).[8, 9] The most popular method for detecting fatty liver in the general population is liver ultrasonography.[10] Patients frequently seek care from gastroenterologists or hepatologists because of abnormally elevated aminotransferase levels. Because of this, abnormal aspartate transaminase (AST) and alanine transaminase (ALT) levels are used in several investigations to diagnose NAFLD.[11, 12] Measurement of aminotransferases, blood lipids, and insulin resistance (IR) are therefore commonly employed in clinical settings for NAFLD diagnosis. Lipid profile, AST, ALT, fasting blood sugar (FBS), CRP, and fasting insulin level play important roles in non-alcoholic fatty liver disease (NAFLD).[13, 14] These markers are useful alternatives to liver biopsies because they help doctors assess the severity and prognosis of the disease and implement treatment plans at an earlier stage.[15] This study aimed to determine lipid profile and liver function tests in patients with NAFLD and examine their possible association with various degrees of NAFLD.

2. Material and methods

This cross-sectional research occurred over a year in the Biochemistry Department of Shyam Shah Medical College in Rewa, India. The research was conducted with the approval of the institution's ethical review board (Ethical code number: IEC/MC/2020/460). Fifty patients of non-alcoholic fatty liver disease (NAFLD) of both sex, ages ranging from 18 to 60 years, were selected from the outpatient department of the medical ward at Shyam Shah Medical College and Affiliated Hospital Rewa, Madhya Pradesh, India. Following the completion of a thorough explanation of the study to each

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participant, written informed consent was collected from them. Coding and computer recording was used to protect the privacy of the subject data.

Exclusion criteria

Exclusion criteria are as follows: (a) subjects having a history of alcohol ingestion, (b) smokers, (c) patients with malignancy, (d) patients who have undergone gastrointestinal surgery previously, (e) patients with a history of the following drug intake: steroids, synthetic estrogens, heparin, calcium channel blockers, and (f) patients with other causes of liver diseases like viral and autoimmune liver diseases.

Grading of non-alcoholic fatty liver on ultrasonography

Patients were examined with real-time USG after they had fasted for 6-8 hours. Right anterior oblique views and supine positions were mostly used. A standard abdominal transducer and a higher frequency transducer performed the sagittal, transverse, coronal, and subcostal oblique views. The grading of fatty liver was done as follows:

Grade I: Minimal diffuse increase in hepatic echogenicity with normal diaphragm and intrahepatic vessel borders visualization.

Grade II: Moderate diffuse increase in hepatic echogenicity with slightly impaired intrahepatic vessel walls and diaphragm visualization.

Grade III: Marked increase in echogenicity with poor penetration of the posterior segment of the right lobe of the liver and poor or no visualization of hepatic vessels and diaphragm.

Anthropometric measurements

The standard equipment measured height and weight with the subjects dressed in minimal clothing and barefoot. Calibrated electronic weighing scales were used for weight measurement, whereas height was measured to the nearest centimeter using a portable stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m2). The same individual performed all anthropometric measures.

Blood pressure measurements

Following a standard medical procedure, the systolic and diastolic blood pressures were assessed after 10 minutes of rest using a mercury sphygmomanometer.

Biochemical measurements

About 05 ml of fasting venous blood was collected under all aseptic precautions from patients, and samples were dispensed into plain tubes to analyze liver function tests and lipid parameters. Blood samples collected in plain vials were centrifuged at 3000 rpm for 10-15 minutes to get the serum. The biochemical parameters, viz. lipid parameters and liver function tests, were analyzed by standard methods using a Biosystem BA-400 chemistry analyzer. Low-density lipoprotein and very low-density lipoprotein cholesterol were calculated using Friedewald's equation.[16]

Statistical analysis

Statistical Package for Social Science version 20 (IBM, SPSS Statistics 20, Armonk, NY, USA) was used for all analyses. The categorization variables were expressed by number (%), whereas the numerical variables were expressed as mean and standard deviation (SD). One-way analysis of variance test (ANOVA) was used to calculate the p-value, and a p-value less than 0.05 was considered statistically significant.

3. Results

Out of 50 cases of NAFLD diagnosed on ultrasonography, grade I NAFLD cases were 20 (40%), grade II cases were 24 (48%), and grade III cases were 06 (12%). Table 1 shows the baseline characteristics of the studied subjects. The mean age of NAFLD patients was 43.64 years. Similarly, the mean BMI of the studied subjects was 27.15 Kg/m2. Systolic and diastolic blood pressures in NAFLD patients were 129.52 mmHg and 83.34 mmHg. The mean blood glucose level was within the normal range (110 mg/dl). Table 2 shows the distribution of patients showing abnormal lipid profiles in NAFLD patients.

Serum TC, TG, LDL, and VLDL levels were increased in 54%, 68%, 42%, and 38% of NAFLD patients, respectively. Serum HDL levels were decreased in 36% of NAFLD patients. Table 3 compares lipid changes in different grades of NAFLD by statistical analysis using analysis of variance (ANOVA). It was found that rising grades of NAFLD were substantially associated with increasing levels of serum TC, TG, and VLDL, while at the same time, HDL levels were reported to be decreasing. There was no statistically significant association between the serum levels of LDL and the rising grades of NAFLD. Table 4 shows the distribution of patients showing abnormal liver function tests in NAFLD. Serum AST, ALT, and total bilirubin were increased in 60%, 58%, and 52% of NAFLD patients, respectively, whereas total protein levels were decreased in 38% of NAFLD patients. Table 5 compares liver parameters between different grades of NAFLD by statistical analysis using analysis of variance (ANOVA). It was found that rising grades of NAFLD were substantially associated with increasing levels of serum AST, ALT, and total bilirubin. At the same time, total protein levels were reported to be decreasing. There was found to be no statistically significant association between the AST/ALT ratio and the rising grades of NAFLD.

Table 1. Baseline characteristics of studied subjects.

Variables	NAFLD (n=50)
Sex (M/F)	21/29
Age (years)	43.64±7.21
BMI (Kg/m2)	27.15±3.96
SBP (mmHg)	129.52±16.30
DBP (mmHg)	83.34±8.69
FBG (mg/dl)	110.00±37.73

BMI=Body mass index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; FBG= Fasting blood glucose.

Table 2. Distribution of patients showing abnormal lipid profile in NAFLD.

Fatty Liver Grade		Grade I		Grade II		Grade III		Total		Total (%)	
Lipid Profile		N	A	N	A	N	A	N	A	N	A
TC		13	7	8	16	2	4	23	27	46	54
TG		12	8	4	20	0	6	16	34	32	68
HDL-C	Male	9	0	9	1	1	1	32	18	64	36
	Female	8	3	5	9	0	4				
LDL-C		13	7	12	12	4	2	29	21	58	42
VLDL-C		15	5	13	11	03	03	31	19	62	38

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 3. Comparison of lipid changes in different grades of NAFLD.

Ultrasound Grades	Grade I		Grade II		Grade III		P-value
Lipid Profile (mg/dl)	Mean	SD	Mean	SD	Mean	SD	
TC	185.10	46.76	213.50	47.16	241.00	48.60	0.027*
TG	161.15	46.30	209.13	90.19	279.17	129.00	.0087*
HDL-C	47.12	6.86	40.56	5.45	32.33	3.83	< .00001*
LDL-C	105.75	45.96	131.11	44.79	152.83	57.86	0.063NS
VLDL-C	32.23	9.26	41.83	18.04	55.83	25.80	0.009*

NS: Not significant; *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 4. Distribution of patients showing abnormal liver function tests in NAFLD.

Fatty Liver Grades		Grade I		Grade II		Grade III		Total		Total (%)	
Liver Function Tests		N	A	N	A	N	A	N	A	N	A
AST	Male	7	2	4	6	0	2	20	30	40	60
	Female	3	8	5	9	1	3				
ALT	Male	7	2	6	4	1	1	21	29	42	58
	Female	4	7	2	12	1	3				
Total Bilirubin		12	8	11	13	1	5	24	26	48	52
Total Protein		16	4	13	11	2	4	31	19	62	38

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

Table 5. Comparison of liver function tests between different grades of NAFLD.

Ultrasound Grades	Grade I		Grade II		Grade III		P-value
Liver Function Tests	Mean	SD	Mean	SD	Mean	SD	
AST (U/L)	34.00	5.78	37.08	8.63	48.50	12.24	0.0016*
ALT (U/L)	39.05	7.77	43.96	8.39	57.67	18.46	0.0007*
Total Bilirubin (mg/dl)	1.01	0.38	1.04	0.22	1.41	0.34	0.0212*
Total Protein (gm/dl)	6.99	0.74	5.95	1.42	5.93	0.83	0.0103*
AST/ALT	0.88	0.10	0.86	0.19	0.87	0.16	0.8659NS

Not significant; *Significant; AST: Aspartate transaminase; ALT: Alanine transaminase.

4. Discussion

This hospital-based observational cross-sectional study included patients with varying degrees of NAFLD. In the current study, an attempt was made to describe the abnormality of lipid levels and liver function tests among patients with NAFLD in a central Indian setting. Our study showed that 68% of patients with NAFLD had elevated serum triglyceride levels, 54% had elevated total cholesterol, 42% had increased LDL, and 38% had increased VLDL. 36% of NAFLD patients had low HDL values in their serum. According to Khanal and colleagues' study, 26.6%, 27.5%, and 1.8% of patients with NAFLD, respectively, had high levels of serum triglycerides, total cholesterol, and LDL. Low serum HDL values were seen in 20.2% of patients with NAFLD patients.[17] Our findings are consistent with those of Khanal et al.,[17] who discovered that increasing grades of NAFLD were significantly associated with rising serum total cholesterol and LDL. In contrast, the researchers' examination revealed no statistically significant link between blood triglyceride or HDL levels and worsening NAFLD grades as determined by sonography. Although increasing grades of fatty liver in NAFLD patients were associated with serum LDL levels in our study, the association was statistically insignificant. Similarly, in a study by Khalil et al., total cholesterol, triacylglycerol, LDL, and VLDL were increased in 58%, 61%, 49%, and 39% of NAFLD patients, respectively. However, their study decreased HDL in 49% of NAFLD patients. They also observed a significant association between increasing grades of NAFLD with elevated total cholesterol, triacylglycerol, LDL, and VLDL and decreased HDL.[18] According to Mansour-Ghanaei et al.,[19] patients with NAFLD have a characteristic dyslipidemic pattern. Those with NAFLD had lower HDL levels, higher total cholesterol levels, and greater total cholesterol to HDL ratio than those without NAFLD. Furthermore, a strong correlation was observed between TG and the NAFLD group, although no correlation was seen between LDL and the NAFLD group. However, they could not find any correlation between the lipid profile and the degree of fatty liver. Studies conducted by Pardhe et al.[20] and Jain et al.[15] came to the same conclusions about the pattern of lipid parameters in NAFLD. A similar study was carried out in Nepal by Bhusal et al.[21] who found a significant positive association between the presence of NAFLD and increasing levels of serum total cholesterol, LDL, and triglyceride, as well as a significant drop in HDL in NAFLD patients. However, they did not find a significant positive link between the grade of fatty liver and the level of the various components of the lipid profile. This could be explained by the small sample size and the inability to include patients with severe NAFLD. Since the disease was first described, the pathogenesis of NAFLD has remained a mystery. Because the process or mechanisms are still being figured out, much current thought is still purely hypothetical. Potential causes include variations in body-fat distribution and antioxidant systems, which may have a hereditary basis. A prerequisite for developing non-alcoholic fatty liver disease is the buildup of lipids in the hepatocytes, primarily in triglycerides. The main metabolic abnormalities that cause fat accumulation are not well understood. However, they may involve changes to the hepatic lipid metabolism's absorption, synthesis, breakdown, or secretion routes. These changes could be caused by insulin resistance, which is the most reproducible factor in the development of NAFLD.[22]

The quality of liver function is frequently determined by the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). It has been established that ALT and NAFLD are related in earlier investigations. The risk of developing NAFLD has been linked to even ALT readings within the normal reference range.[5, 11] In the current study, patients with NAFLD had elevated serum levels of AST, ALT, and total bilirubin in 60%, 58%, and 52% of the cases, respectively. In comparison, 38% of NAFLD patients had lower total protein levels. Mean AST and ALT levels in patients with NAFLD were also considerably higher than reference ranges. Additionally, a substantial association was found between AST and ALT levels and fatty liver disease severity. Similar results were reported by Mansour-Ghanaei and colleagues,[19] who found that NAFLD patients had considerably higher ALT and AST levels than non-NAFLD patients. Additionally, they revealed a strong link between serum levels of ALT and AST and the severity of fatty liver disease. Similar results were obtained by Namoos et al.,[23] who observed a direct correlation between high AST and ALT levels and the severity of fatty liver disease. The vast majority of research done in the past has demonstrated that there is a substantial connection between NAFLD and liver enzymes.[12, 20, 24] Zakeri et al., suggest that elevated ALT and dyslipidemia may play a role in the development and progression of NAFLD.[24] Ramesh et al. found that patients with NAFLD had slightly higher levels of ALT and AST than controls, albeit the differences were not statistically significant.[22] It appears liver enzymes do not definitively diagnose fatty liver disease. This is due to the high degree of variability exhibited by liver enzymes. In contrast, multiple studies conducted in recent years have demonstrated that liver enzymes such as AST and ALT can aid in diagnosing or predicting NAFLD and its severity.[19, 25] In this investigation, we also noticed that increasing grades of NAFLD were significantly linked with rising serum total bilirubin levels, while total protein levels were reportedly declining. No statistically significant relationship was established between the AST/ALT ratio and the progression of fatty liver in NAFLD patients. additional research is required

to understand better the connection between serum bilirubin and total protein and NAFLD. However, our research is limited by the following factors.

1. Because our study was conducted in a hospital, there was an inherent bias in the selection of patients.

2. The period for the study was also very brief.

3. This study also lacks the control subjects to examine the possibility of NAFLD.

4. The NAFLD was detected using ultrasonography. The liver biopsy is considered the gold standard for identifying fatty liver disease; however, due to the invasive nature of the procedure, the possibility of complications, and the high cost, it is not advised for the general public.

5. Due to the limited number of people analyzed and the fact that the research was conducted at a single hospital centre, the findings of the study cannot be extrapolated to the entire population.

5. Conclusion

Patients with NAFLD had a dyslipidemic pattern of lipid markers and abnormal liver function tests. Grades of fatty liver in those with NAFLD were also correlated with dyslipidemia and elevated AST and ALT. Therefore, individuals with non-alcoholic fatty liver disease should undergo lipid parameters and liver function testing to track disease progression.

Conflict of Interest

The authors declared that there is no conflict of interest.

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