Lipid Profile and Ultrasonographic Grading in Alcoholic and Non Alcoholic Fatty Liver Patients

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ABSTRACT

Background

Fatty liver disease (FLD) is a common and major chronic liver disease. It has been implicated that patients have disorders of lipid metabolism and are involved in the pathogenesis of fatty liver. Hence, it was designed to observe the association between lipid profile and fatty liver disease.

Objective

This study was undertaken to evaluate the association of lipid profile status, hemoglobin and albumin levels with fatty liver disease patients diagnosed based on ultrasonography (USG).

Method

This Cross-sectional study was undertaken in the Department of Internal Medicine with the collaboration of the Department of Radiology and Department of Biochemistry, Universal College of Medical Sciences-Teaching Hospital (UCMS-TH), Bhairahawa, Nepal from March 2019 to February 2020 in a total of 100 patients diagnosed with fatty liver disease by ultrasonography. The fasting blood was collected for lipid profile and carried out in the automated analyzer following standard protocol.

Result

In 100 cases, the male to female ratio was 1.8:1. Fifty six percent of the total cases presented with alcoholic fatty liver disease (AFLD) while the remaining 44% with nonalcoholic fatty liver disease (NAFLD). The spectrum of lipid abnormality was observed with increased total cholesterol (TC), Low Density Lipoprotein (LDL), increased triglycerides (TG), Very Low Density Lipoprotein (VLDL) in alcoholic fatty liver disease cases as compared to nonalcoholic fatty liver disease cases. However, it has been observed that TG/HDL and Non-HDL/HDL were higher in nonalcoholic fatty liver disease as compared to alcoholic fatty liver disease. Moreover, a statistically significant difference was observed in HDL between AFLG2 and NAFLG2 (p-value: 0.012).

Conclusion

Dyslipidemia and decreased HDL have been implicated in fatty liver diseases. USG in conjunction with Non-HDL/HDL, TG/HDL, hemoglobin, and albumin can be useful in early screening and monitoring of dyslipidemia in fatty liver patients.

KEY WORDS

Alcoholic fatty liver, Dyslipidemia, Non-alcoholic fatty liver

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a condition defined by significant lipid accumulation (5-10%) in hepatic tissue in the absence of significant chronic alcoholic consumption.[1] It includes no more than 30 grams of alcohol per day in men and 20 grams per day in women.[2] Alcoholic fatty liver disease (AFLD), induced by excessive alcohol consumption, and nonalcoholic fatty liver disease (NAFLD), caused by obesity and insulin resistance are the most common diseases associated with hepatic steatosis.[3]

Though liver biopsy is the gold standard method for diagnosis of NAFLD, ultrasonography (USG) which is a non-invasive, simple tool, can be used for the early detection of NAFLD in asymptomatic patients.[4] NAFLD is a mild disease that affects both female and male. In the study conducted by Rao et al., the raised serum triglycerides (TG), total cholesterol (TC), and low density lipoprotein (LDL) were seen in 82.67%, 60% and 65.33% cases respectively, and significantly low HDL in 65.33% of NAFLD patients.[5]

Both ALD and NAFLD are frequently accompanied by extrahepatic complications, including cardiovascular disease and malignancy. The survival of patients with ALD and NAFLD depends on various disease-associated conditions.[6] Moreover, elevated LDL, TG or low HDL pattern is associated with NAFLD.[7]

The present study attempted to find out the spectrum of fatty liver patients who had visited tertiary care hospital UCMS-TH, in the southwestern region of Nepal. Since albumin is synthesized by liver as a part of liver function and hemoglobin being indicator of anemia in chronic liver patients, the association of fatty liver disease patients with the lipid profile, hemoglobin, and albumin has been observed.

METHODS

This cross-sectional study was carried out in the Department of Internal Medicine with the collaboration of the Department of Radiology and Department of Biochemistry, UCMS-TH, Bhairahawa, Nepal from March 2019 to February 2020. Patients who have been diagnosed with fatty liver disease based on USG finding on an ultrasound scanner (GE LOGIQ6 PRO) were included in the study. ALFD group was assigned based on excessive alcohol consumption 40 g/day in men (20 g/day in women), usually > 5 years, and/or ongoing daily alcohol consumption > 80 g in 2 weeks. NAFLD group was assigned based on no history of alcohol consumption or alcohol intake less than 140 g in men (70 g in women) on average per week in the past 12 months. The conversion formula of alcohol intake is: alcohol intake (g) = liquor consumed (mL) X alcohol content (%) × 0.8.[8,9] The Patients with other causes of liver disease like viral or alcoholic hepatitis, on drugs therapy or any chemotherapy and patient's age less than 1 year and > 80 yearswere excluded from this study. The ethical approval was taken with the institutional review committee registration number IRC/046/19, UCMS-TH.

The patients were examined with real-time USG, after 6-8 hours of the fasting period. Mostly supine and right anterior oblique views were obtained. The sagittal, transverse,

coronal, and subcostal oblique views were also performed using both a standard abdominal transducer and a higher frequency transducer. In few cases, intercostal views were also needed to be performed. The grading of Fatty liver was categorized as follows:

Mild (Grade 1)-Minimal diffuse increase in hepatic echogenicity with normal visualization of the diaphragm and intrahepatic vessel borders.

Moderate (Grade 2)-Moderate diffuse increase in hepatic echogenicity with slightly impaired visualization of intrahepatic vessels walls and diaphragm.

Severe (Grade 3)-Marked increase in echogenicity with poor penetration of posterior segment of the right lobe of liver and poor or no visualization of hepatic vessels and diaphragm.

The fasting blood sample was collected in plain vaccutainer and EDTA vial separately along with relevant clinical diagnosis and history. The serum was separated from plain vaccutainer and the tests were carried out on the same day. A lipid profile test was done which included TG analyzed by Glycerol Phosphate Oxidase-Phenol Antipyrine (GPO/PAP) method, TC and HDL by Cholesterol Oxidase-Phenol Antipyrine (CHOD-PAP) method, and calculated LDL derived from Friedwald's equation as LDL=TC-(HDL+VLDL) where VLDL level can be obtained by TG/5. Non-HDL: HDL ratio was derived from (VLDL+LDL)/HDL level and TG: HDL ratio was calculated from TG/HDL level. Non-HDL: HDL ratio and TG: HDL ratio was taken as risk indicator in FLD patients for discerning control over such risk condition. The other test carried out was albumin by Bromo-Cresol Green (BCG) dye binding method. All biochemical tests were carried out in a fully automated analyzer, Human XL-600 (Germany), where the EDTA vial blood was analyzed for hemoglobin level in hematology analyzer, Beckman coulter DxH 520.

Data were analyzed by Statistical Package for Social Service (SPSS) for Windows version 22, Inc., Chicago, IL. All the data were expressed in terms of percentage frequency, median and compared by non-parametric Chi-Square (χ^2) test, Kruskal Wallis-H (K W-H) test, Man Whitney U test, and Spearman's rho correlation. P-value < 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows that the median age of the studied subjects was 45 years with an interquartile range (IQR) of 20-80 maximum of the patients were between 20-40 years

followed by 41-60 years and least were more than 60 years. There was no statistical significance in age distribution in FLDG1 and FLDG2. Male to female ratio is 1.8:1 and observed no statistical significance difference in the two groups. The percentage of alcoholic and nonalcoholic patients with FLDG1 was 51.9% and 48.1% and FLDG2 were 73.7% and 26.3% respectively with no statistical significance difference.

Table 1. General Characteristics of the study subjects (N=100)

Characteristics	FLDG1 (n=81)	FLDG2 (n=19)	Total	p-value χ^2
Median Age (IQR) in years	44 (21-67)	45 (24-66)	45 (20-80)	
20-40 years n (%)	37 (45.7)	9 (47.5)	46	0.384
41-60 years n (%)	30 (76.9)	9 (47.4)	39	
> 60 years n (%)	14 (93.3)	1 (5.3)	15	
Gender (Male: Female)	1.8:1	5.3:1	1.94:1	
Male n (%)	50 (61.7)	16 (84.2)	66	0.063
Female n (%)	31 (38.3)	3 (15.8)	34	
Alcoholism				
Alcoholics n (%)	42 (51.9)	14 (73.7)	56	0.084
Non-Alcoholics n (%)	39 (48.1)	5 (26.3)	44	

Table 2 shows the spectrum of lipid profile variables with different types and grades of FLD. The maximum AFLD had increased TG in 32 cases (57.1%), decreased HDL in 30 cases (68.2%), increased TC in 14 cases (31.8%), increased LDL in 9 cases (20.5%) as compared to NAFLD with increased TG in 32 cases (72.7%), decreased HDL in 31 cases (55.4%), increased TC in 15 cases (26.8%), increased LDL in 6 cases (10.7%) respectively. The FLDG2 were found maximum with increased TG in 13 cases (68.4%), increased TC in 6 cases (31.6%), increased LDL in 4 cases (21.1%) as compared to FLDG1 with TG in 51 cases (63%), increased LDL in 11 cases (13.6%) and increased TC in 23 cases (28.4%) respectively. On contrary, the decreased HDL was maximum in FLDG1 with frequency of 52 cases (64.2%) as compared to FLDG2 with 9 cases (47.4%). However, there was no statistically significant association in the frequency of TC, TG, VLDL, HDL and LDL with types and grades of FLD.

The multivariate analysis of non parametric Kruskel Wallace-H (KW-H) among overall FLD cases show no statistically significant association in Lipid profile variables. However, Man Whitney U test shows statistically significant difference in HDL level between AFLDG2 and NAFLDG2 (p-value: 0.012). The ratio of non (N)-HDL to HDL as well as TG to HDL were increased in NAFLD as compared to AFLD.

Table 4 shows that the association of Lipid profile was non-significant with hemoglobin level, and albumin level with TC, HDL, LDL, and TG. However, decreased HDL was observed in maximum of 45 cases (62.5%) in ≤ 10 g/dl hemoglobin level as compared to 16 cases (57.1%) in > 10 g/dl hemoglobin level. Similarly, increased TG was observed maximum in 51 cases in ≤ 3.5 g/dl as compared to 13 cases in > 3.5 g/dl albumin level.

Table 2. Spectrum of Lipid profile in the FLD subjects (N=100)

Lipid profile	Status	Total case	FLD Type		p-value	FLD Grade		p-value
n(%)		(n=100)	AFLD	NAFLD		FLDG1 (n=81)	FLDG2 (n=19)	χ^2
TC	Normal	71	30 (68.2)	41 (73.2)	0.5	58 (71.6)	13 (68.4)	0.7
	High	29	14 (31.8)	15 (26.8)		23 (28.4)	6 (31.6)	
TG, VLDL	Normal	36	12 (27.3)	24 (42.9)	0.1	30 (37.0)	6 (31.6)	0.6
	High	64	32 (57.1)	32 (72.7)		51 (63.0)	13 (68.4)	
HDL	Normal	39	14 (31.8)	25 (44.6)	0.1	29 (35.8)	10 (52.6)	0.1
	Low	61	30 (68.2)	31 (55.4)		52 (64.2)	9 (47.4)	
LDL	Normal	85	35 (79.5)	50 (89.3)	0.1	70 (86.4)	15 (78.9)	0.4
	High	15	9 (20.5)	6 (10.7)		11 (13.6)	4 (21.1)	

Table 3. Association of Median (IQR) Lipid profile value with FLD types and grades

Lipid Median (IQR)	Total Cases (N=100)	FLD Types and Grades				p-value
		AFLDG1 (n=42)	AFLDG2 (n=14)	NAFLDG1 (n=39)	NAFLDG2 (n=5)	KW-H
TC (mg/dl)	172.0	165.0	187.0	172.0	146	0.646
	(130-208.75)	(129-197)	(127-213)	(127-223)	(130.5-208)	
TG (mg/dl)	191.0	161	173.5	197.0	238.0	0.583
	(113.5-290.75)	(90-288.25)	(120.25-305)	(118-258)	(156-385)	
VLDL (mg/dl)	38.1	32.2	34.7	39.4	47.6	0.583
	(22.7-58.15)	(18-57.65)	(25.05-61)	(23.6-51.6)	(31.2-77)	
HDL (mg/dl)	39.0	37.5	42*a	39.0	36.0*a	0.107
	(34.25-39.0)	(30.75-45)	(39.75-49.75)	(32-48.1)	(35.35-39)	
LDL (mg/dl)	84.5	82.8	88.1	91.6	69.0	0.697
	(63.2-113.85)	(67-106.6)	(62.45-124)	(61-127)	(35.95-116.7)	
N-HDL/ HDL	3.33	3.12	3.24	3.46	3.08	0.838
	(2.49-4.18)	(2.31-4.19)	(1.97-3.95)	(2.62-3.46)	(2.53-3.08)	
TG/HDL	4.56	4.19	4.25	5.07	6.80	0.506
	(2.75-7.33)	(2.35-7.5)	(2.12-7.46)	(3.51-7.11)	(4.22-6.8)	

Man Whitney U test, p-value: *a0.012 (AFLDG2 vs NAFLDG2)

Table 4. Association of Lipid profile in FLD with Hemoglobin (Hb) and Albumin (Alb)

Lipid profile	status	Hb level (g/dl): IQR (3.3-17.7)		p-value	Alb level (g/dl): IQR (3.2- 3.5)		p-value
		>10 (n=28)	≤ 10 (n=72)		> 3.5 (n=22)	≤ 3.5 (n=78)	χ^2
TC	Normal	19 (67.9)	52 (72.2)	0.66	15 (68.2)	56 (71.8)	0.7
	High	9 (32.1)	20 (27.8)		7 (31.8)	22 (28.2)	
HDL	Normal	12 (42.9)	27 (37.5)	0.62	5 (22.7)	34 (43.6)	0.07
	Low	16 (57.1)	45 (62.5)		17 (77.3)	44 (56.4)	
LDL	Normal	23 (82.1)	62 (86.1)	0.61	19 (86.4)	66 (84.6)	0.83
	High	5 (17.9)	10 (13.9)		3 (13.6)	12 (15.4)	
TG	Normal	8 (28.6)	28 (38.9)	0.33	9 (40.9)	27 (34.6)	0.5
	High	20 (71.4)	44 (61.1)		13 (59.1)	51 (65.4)	

DISCUSSION

AFLD represents a broad range of histological changes ranging from simple steatosis to heavier forms of liver injury, including alcoholic hepatitis, cirrhosis, or the parallel development of hepatocellular carcinoma.[10] NAFLD is emerging as the most common chronic liver condition in the Western world. It is associated with insulin resistance and frequently occurs with features of metabolic syndrome.

Paik et al. included a total of 186 patients in their study, out of that 106 cases were NAFLD and 80 were AFLD. There was no significant difference between the NAFLD and ALFD groups (p=0.635).[11] In our study, a total of 100 patients included 44 cases with NAFLD and 56 cases with AFLD. Similar to their study no significant difference between the NAFLD and AFLD groups (p-value = 0.8) was observed.

Mahaling et al. in their study out of 70 cases that were diagnosed as NAFLD on USG, NAFLDG1 cases were 47.15%, NAFLDG2 were 42.85% and NAFLDG3 was 10%.[4] The mean age of the patients was found to be 49.14 years. The male to female ratio was 3:4. Serum TG, TC, LDL and VLDL levels were raised in 67.14%, 45.71% 34.28%, 25.71% of cases respectively. Low serum HDL levels were seen in 62.85% of patients. Their study has shown increasing grades of NAFLD were significantly associated with increasing values of TC.[4] In our study, NAFLDG3, and AFLDG3 cases were not present. The male to female ratio was 1.8:1. The median age of the patients was found to be 45.90 years which is similar to their study. The TC, TG, VLDL, and LDL were raised in 29%, 64% and 15% of the FLD cases where 26.8%, 57.1%, and 10.7% were of NAFLD cases only. Similar to their study, low serum HDL levels were seen in 55.4% of the NAFLD cases. The significant association was only observed in the median HDL level between AFLDG2 and NAFLDG2 (p-value: 0.012). This result implicates the decrease median HDL level in NAFLDG2 than AFLDG2 showing diseases severity and progression as grading increases based on USG finding. Unlike their study, no significant association was seen between other lipid profiles except HDL with FLD.

Pradhan et al. in their study which included a total of 1,500 patients found that 447 patients had AFLD. Chronic liver disease (CLD) was detected in 144 patients (9.6%).[12] On multivariate analysis, they found the following variables to be significantly associated with CLD: male sex (odds ratio [OR]: 1.81; 95% confidence interval [CI]: 1.12-2.94; P=0.02).[12] Similar to the study, male predominance was found in our study as well. Moreover TC, TG, and LDL were raised in 29%, 64% and 15% of the FLD cases where 31.8%, 72.7% and 20.5% were with only AFLD. Similar to their study, low HDL level were seen in 68.2% of the AFLD cases.

Bhusal et al. had shown mild NAFLD in 83%, moderate in 17% and severe in none of the participants which is a similar finding in our study.[13] Age of the participants ranged from 26 to 79 years with the mean being 45±11.99 years. Similar to their study, the present study has shown increased TG, TC, and LDL levels however HDL level was decreased in NAFLD cases. Similarly, TG, TC, and LDL levels were raised in 72.7%, 31.8%, 20.5% of the AFLD cases respectively and High density lipoprotein level was decreased in 68.2% of cases. In contrast to their study NAFLDG1 was observed in 48.1% and 26.3% in NAFLDG2. We studied patients with AFLD too in whom 51.9% were having FLDG1 and 73.7% had FLDG2. This indicates alcoholism and chronicity progresses with the FLD grade. The increasing grades of non-alcoholic fatty liver disease weren't significantly associated with increased levels of lipid abnormalities.[13] Unlike their study, we found no association between the presence of dyslipidemia and either type of fatty liver disease. Similar to their study, no significant association of different grades of FLD was observed with increasing lipid abnormalities.

Khalil et al. in their study, found that the largest group of patients (38%) was in the fifth decade of life followed by 30% in the sixth decade of life. As the grade of NAFLD increased, there was an associated significant increase in levels of serum TC (p-value 0.005), TG (p-value 0.002) LDL (p-value 0.001), VLDL (p-value 0.003) and associated significant decrease in HDL (p-value 0.001).[14] Unlike their study, most of the patients were in their third to the fourth decade of life (46%) followed by fifth to the sixth decade (39%) and lowest number of patients were older than 60 years of age (15%) and there was no association between dyslipidemia and FLD except decrease in HDL level between AFLD and NAFLD.

Khanal et al. had found that the mean age of fatty liver in males was 44.3 years and in females was 51.9 years.[15] 22.9% of patients with NAFLD had increased liver size. Significant association with increasing grades of fatty liver was found with increasing levels of TC (p = 0.028), LDL (p = 0.017), liver size (p = 0.001), and body mass index (BMI) (p

= 0.045) in patients diagnosed with NAFLD. No significant association with increasing grades of FLD was found with increasing levels of TG (p = 0.32) and high density lipoprotein (p = 0.25).[15,16] In contrast our study has shown a strong association with median HDL level and there was no significant association with TG, TC, and LDL. This difference in the lipid abnormality spectrum might arise due to the wide distribution of lipid level value and pattern of alcohol consumptions.

The median hemoglobin concentration is 10 with an IQR of 3.3-17.7 g/dl overall cases. Among the studied cases, 72% were anemic (Hb ≤ 10 g/dl) and 28% were having normal hemoglobin concentration (Hb > 10 g/dl). FLDG2 was observed anemic with a frequency 88.2% whereas FLDG1 with 69.1%. The median serum albumin level was 3.2 with IQR of 3.2-3.5 g/dl. In total 78% of the cases were having a lower level of serum albumin while 22% had normal level. FLDG2 was observed a low albumin level with frequency 84.2% whereas FLDG1 was observed low albumin with 76.1%. However, there was no statistical significance in the distribution of hemoglobin level and serum albumin with different FLDG1 and FLDG2. In our study, the status of hemoglobin and albumin as an indicator of anemia and liver function respectively has not shown significant changes in the lipid profile in FLD cases. The potential area of investigation can be done in large population to identify the protective mechanism of hemoglobin and albumin level in deranged lipid profile status in FLD patients.

The study has not used the liver biopsy which is the gold standard method to identify the AFLD and NAFLD. The large sample size with multicentric study is needed to validate the lipid profile as a predictor of the FLD.

CONCLUSION

The spectrum of dyslipidemia with decreased HDL has been implicated in fatty liver diseases. USG in conjunction with Non-HDL/HDL, TG/HDL, hemoglobin, and albumin may be useful risk indicators in early screening and monitoring dyslipidemic fatty liver patients to prevent adverse effect of this disease.

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