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A Prospective Cross-Sectional Study: Fatty Liver is a Risk Factor for Cardiovascular Events

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Abstract
Fatty liver is a silent killer which slowly progress and sometimes diagnosed accidentally on ultrasonography. Symptom of fatty liver appears when most of liver functions deranged. Most patients are asymptomatic and accidentally diagnosed to have fatty liver while undergoing routine radiological and biochemical test, because there is not any fixed biomarker for fatty liver. Cardiovascular risks are commonly linked with the chronic fatty liver. A prospective, cross-sectional study was conducted in the department of Biochemistry at GBCM and Subharti hospital, Dehradun comprising of 120 ultrasonographically confirmed fatty liver patients who were differentiated based on their grades of fatty liver by ultrasonography and alcoholic and non alcoholic based on alcohol consumption history given by patients. 5ml fasting blood sample was collected and analyzed for Liver and Lipid profile. A significant difference was observed on comparing them among their fatty liver grading and alcoholic status in fatty liver patients with significant difference of p-value(<0.05), using unpaired 't-test. TG: HDL ratio is found to be most significant on comparison in Alcoholic and Non alcoholics. The relative risk is evaluated for alcoholic fatty liver and cardiovascular risk (Atherogenic index-TG: HDL-C Ratio) and found to be 37%. We concluded that the cardiovascular risk is 37% more in the individuals who are alcoholic and having asymptomatic fatty liver.

INTRODUCTION

Fatty liver disease is a potentially fatal condition that advances slowly, often evading detection until incidentally discovered through ultrasonography. Manifestations of the disease typically emerge only after more than half of liver function has been compromised. Both alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) are prevalent culprits behind chronic liver disease (CLD), afflicting populations worldwide, whether in developing or developed nations. The escalating prevalence of these conditions can be attributed to the widespread availability of alcohol and the increasingly sedentary lifestyles of individuals. NAFLD encompasses a spectrum of conditions, ranging from benign fatty liver to more severe states such as steatohepatitis (NASH), indicating ongoing liver injury and ultimately liver cirrhosis. Similarly, ALD spans from simple steatosis to alcoholic hepatitis, cirrhosis and associated complications. There often exists considerable overlap between these diseases and the clinical presentation varies depending on the stage of liver disease[1]. A comprehensive assessment encompassing detailed clinical and dietary records of alcohol consumption, coupled with thorough examination of radiological, biochemical, and clinical data, provides the basis for diagnosing ALD. On the other hand, NAFLD frequently eludes detection until identified incidentally during routine checkups, with the majority of patients remaining asymptomatic. It is now recognized as a hepatic manifestation of metabolic syndrome[2]. Fatty liver disease is a prevalent condition characterized by the excessive build-up of fat within the liver. Both alcoholic and non-alcoholic fatty liver disease (NAFLD) are frequently identified causes of chronic liver disease (CLD) globally. Typically, a healthy liver maintains a modest level of fat, becoming problematic when fat accumulation reaches between 5-20% of the liver's weight. In Western populations, the prevalence of NAFLD ranges from 30-40% in men and 15-20% in women based on population-based studies[3]. Fatty liver has become one of the most common non-communicable diseases in India, with prevalence of 61.5% was observed[4]. Along the coastal regions of eastern India, approximately a quarter of the general population shows indications of fatty liver upon routine ultra sono graphic screening[5]. The majority of individuals with fatty liver are asymptomatic and typically diagnosed incidentally during routine radiological and biochemical examinations. The rising prevalence of this condition is attributed to sedentary lifestyles and the widespread availability of alcohol. Increased alcohol consumption globally has contributed to heightened morbidity and mortality rates, exacerbated by risk factors such as obesity, dyslipidemia and hypertension.

In recent times, emerging evidence has shed light on a fascinating association between liver diseases-specifically alcoholic fatty liver and non-alcoholic fatty liver-and a heightened risk of cardiovascular events. This correlation has piqued substantial interest among researchers and healthcare professionals alike. Understanding the relationship between liver fat accumulation and cardiovascular risk holds significant implications for patient management and preventive measures. The plasma parameter log (TG/HDL-C) serves as a widely utilized atherogenic index globally, while the fractional esterification rate of cholesterol and the triglycerides to HDL-cholesterol ratio stand out as potent predictors of positive outcomes on coronary angiography[6].

Several biochemical markers have been studied to assess chronic alcohol consumption in patients with alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), including serum GGT, AST/ALT ratio and carbohydrate deficient transferrin (CDT), among others. Additionally, individuals with NAFLD are prone to experiencing numerous extra-hepatic chronic complications, such as atherosclerosis and elevated cardiovascular risk[7]. Cardiovascular disease emerges as the primary cause of mortality among individuals with non-alcoholic fatty liver disease (NAFLD), with NAFLD itself serving as an independent contributor to the elevated risk of heart disease[8]. As of now, liver biopsy remains the gold standard for diagnosing non-alcoholic steatohepatitis (NASH), despite being an invasive procedure with various limitations[9,10]. The research is going on for the search of biomarker of FLD, so we planned to conduct this research work in the state of Uttarakhand where alcohol consumption is more. And to evaluate the Cardiovascular risk, ratio of triglycerides to HDL-cholesterol is utilized.

Aims and Objectives:

- Estimation of Liver Profile in alcoholic and non-alcoholic fatty liver patients
- Estimation of Lipid Profile in alcoholic and non-alcoholic fatty liver patients
- Comparison of Liver and Lipid Profile among the AFLD and NAFLD
- Comparison of Liver and Lipid Profile on the basis of Grading of FLD
- Evaluation of Cardiovascular risk in ALD and NAFLD

MATERIAL AND METHODS

Study Design: A prospective observational cross-sectional study was conducted in the biochemistry department at GBCM and KKBM Subharti Hospital, Jhajra, Dehradun, following ethical clearance from the institutional ethical committee (IEC) with registration number GBCM/IEC/2023/07-03 dated 25/07/2023.

Sample Size and Sampling: Upon obtaining informed or written consent, 120 patients with confirmed fatty liver disease (FLD) via ultrasonography were recruited. They were categorized into alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD) based on their self-reported alcohol consumption history. Blood samples of 5ml were collected in serum separation test tubes (SST) from patients visiting the medicine and surgery outpatient departments (OPD) of KKBM Subharti Hospital between June and December 2023.

Study Procedure: Liver profile parameters including AST[11], ALT[12], total bilirubin[13], direct bilirubin[14], ALP, GGT, protein[15], albumin[16] and lipid profile parameters such as triglycerides[17], total cholesterol[18] and HDL[19] were assessed using an EM-200 fully automated clinical chemistry analyzer. Data analysis was performed using Microsoft Excel, Graph Pad InStat and SPSS. Results were presented as Mean±SD. Statistical analysis was conducted using the 't' test, with a $p<0.05$ considered significant. The relative risk for cardiovascular disease was evaluated in AFLD and NAFLD patients using contingency tables.

Inclusion Criteria: Patients aged 20-80 years with ultrasonographically confirmed fatty liver disease were included in the study.

Exclusion Criteria: Patients with diabetes mellitus, hypertension, age less than 20 years or more than 80 years and those with confirmed endocrinopathies were excluded from the study.

RESULTS AND DISCUSSIONS

Out of 120 fatty liver patients 77.50% (93 patients) were grade 1 fatty liver and 20.85% (25 patients) and 1.65% (2 patients) were grade 2 and 3 respectively (Fig. 1).

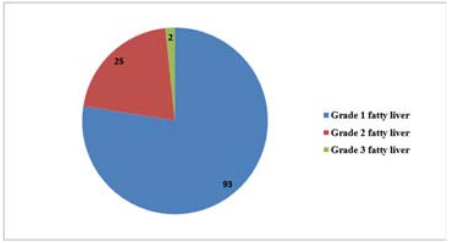

Fig. 1: Frequency of fatty liver patients on the basis of grading

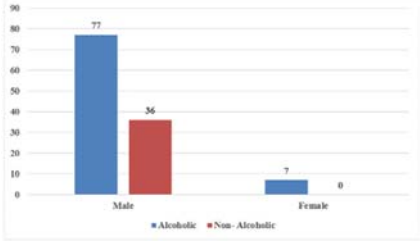

Fig. 2: Frequency of FLD on the basis of Gender

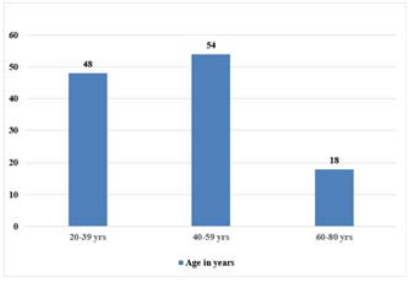

Fig. 3: Frequency of FLD on the basis of Age

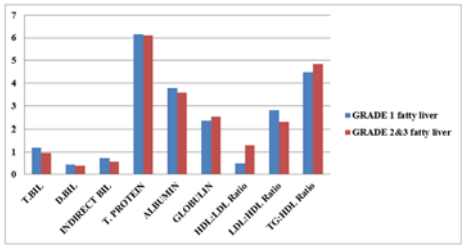

Fig. 4: Comparison according to Grading of fatty liver

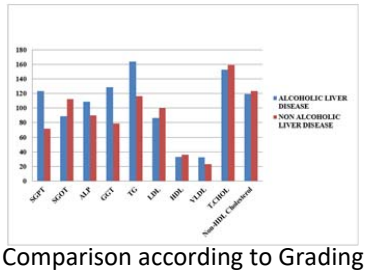

Fig. 5: Comparison according to Grading of fatty liver

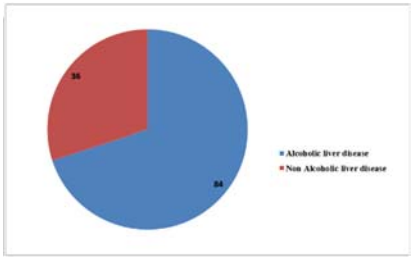

Fig. 6: Frequency of fatty liver patients on the basis of alcohol consumption

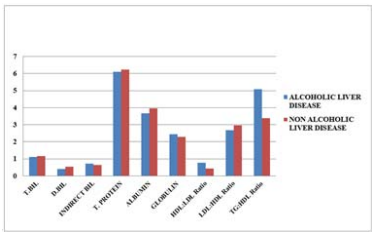

Fig. 7: Comparison of alcoholic and non alcoholic fatty liver disease

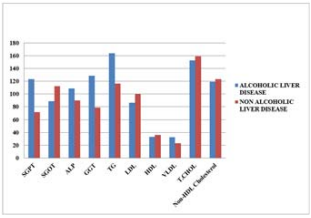

Fig. 8: Comparison of alcoholic and non alcoholic fatty liver disease

Table 1: Comparison among liver profile and lipid profile of grade 1 fatty liver patients with grade 2 and 3 fatty liver patients

PARAMETERS	GRADE 1 (mean± S.D) n = 93	GRADE 2 and 3 (mean± S.D) n = 27	p-value	Significance
T.BIL (mg/dl)	1.18±1.73	0.96±1.05	0.53	NS
D.BIL (mg/dl)	0.45±0.73	0.39±0.53	0.69	NS
INDIRECT BIL (mg/dl)	0.73±1.15	0.56±0.55	0.45	NS
SGPT (U/L)	117±223.26	124.48±215.24	0.87	NS
SGOT (U/L)	87.6±109.5	78.74±97.07	0.70	NS
ALP (U/L)	100±82.08	114.78±126.81	0.47	NS
GGT (U/L)	111.35±201.5	123.15±179.20	0.78	NS
T. PROTEIN (g/dl)	6.15±0.68	6.10±0.62	0.73	NS
ALBUMIN (g/dl)	3.80±0.48	3.57±0.58	0.03	S
GLOBULIN (g/dl)	2.35±0.47	2.53±0.58	0.09	NS
TG (mg/dl)	148.5±114.7	154.96±123.26	0.80	NS
LDL (mg/dl)	95.26±44.3	74.30±36.02	0.02	S
HDL (mg/dl)	34.6±11.03	30.7±10.3	0.03	S
VLDL (mg/dl)	29.72±22.9	30.9±24.65	0.81	NS
T. CHOL (mg/dl)	159.58±50.8	139±40	0.05	S
Non-HDL Cholesterol (mg/dl)	124±46.12	105±34	0.04	S
HDL: LDL Ratio	0.48±0.40	1.29±3.95	0.05	S
LDL: HDL Ratio	2.8±1.45	2.30±1.07	0.09	NS
TG: HDL Ratio	4.49±3.27	4.85±3.30	0.53	NS

P value= <0.05 is considered as significant. (NS= non-significant, S= significant)

Table 2: Comparison among liver profile and lipid profile of alcoholic and non-alcoholic fatty liver patients

Parameters	Alcoholic liver disease (mean±sd) N = 84	Non alcoholic liver disease (mean±sd) N = 36	P-value	Significance
T.BIL (mg/dl)	1.11±1.63	1.17±1.57	0.85	NS
D.BIL (mg/dl)	0.40±0.49	0.53±1.01	0.34	NS
INDIRECT BIL (mg/dl)	0.72±1.02	0.64±0.58	0.70	NS
SGPT (U/L)	123.75±211.10	72.58±115.20	0.17	NS
SGOT (U/L)	88.96±104.04	112.36±242.36	0.45	NS
ALP (U/L)	109±103.7	90.19±63.24	0.30	NS
GGT (U/L)	128.79±225.19	79.03±93.24	0.20	NS
T. PROTEIN (g/dl)	6.10±0.7	6.24±0.58	0.29	NS
ALBUMIN (g/dl)	3.67±0.54	3.95±0.34	0.004	ES
GLOBULIN (g/dl)	2.43±0.52	2.29±0.45	0.16	NS
TG (mg/dl)	164.38±127.98	116.53±73.81	0.03	S
LDL (mg/dl)	86.46±43.47	100.08±42.10	0.11	NS
HDL (mg/dl)	33.65±10.75	36.14±10.97	0.25	NS
VLDL (mg/dl)	32.8±25.5	23.31±14.76	0.03	S
T. CHOL (mg/dl)	152.9±5.86	159.53±45.40	0.50	NS
Non-HDL Cholesterol (mg/dl)	119.33±44.78	123.39±43.78	0.64	NS
HDL: LDL Ratio	0.76±2.28	0.42±0.21	0.37	NS
LDL: HDL Ratio	2.67±1.38	2.96±1.41	0.29	NS
TG: HDL Ratio	5.07±3.58	3.39±1.96	0.009	ES

P value= <0.05 is considered as significant. (NS= non-significant, S= significant, ES= extremely significant)

Table 3: Relative risk of cardiovascular diseases among afld and nafld patients

PARAMETERS	TG: HDL(n=83) (>3 = MORE CARDIOVASCULAR RISK)	TG: HDL (n=37) (<3 = LESS CARDIOVASCULAR RISK)	
Fatty liver with			
Alcohol exposure (n = 84)	83	1	Positive predictive value=100%
Fatty liver with out alcohol exposure (n=36)	0 36		Negative predictive value=97.29%
	Sensitivity= 98.80%	Specificity=100%	RELATIVE RISK =37%

Authors' contributions-

Name of Author	Contributing Role
Dr. Abhinav Manish	Project Supervision, Data Analysis and Data Validation, Project Guidance, Manuscript Preparation and Correspondence, Trial Registry and Report preparation.
Dr. Anuradha Bharosay	Project supervision and Project report analysis
Ms. Kanchan Negi	Sample collection and Data Collection.
Mr. Ritesh Srivastava	Data Collection

Out of 120 patients 113 were male and 7 were female (Fig. 2) and all the 7 females was alcoholic.

Most patients belong to the age group of fourth and fifth decade of life (Fig. 3). The mean age was found to be (47±14 yrs). On comparison of (Grade 1) FLD patients with grade 2 and 3 FLD, there was a significant difference found between the **Albumin, LDL, HDL, Total Cholesterol, Non-HDL Cholesterol, HDL: LDL ratio** with significance value of <0.05 tabulated in (Table 1) and represented in (Fig. 4 and Fig. 5).

The results are represented graphically and tabulated below.

Out of 120 fatty liver patients 70% (84) were AFLD and 30% (36) were NAFLD (Fig. 6).

There was a significant difference found between the **Albumin, Triglyceride, VLDL and TG: HDL ratio** with extremely significance value of <0.05 tabulated below in (Table 2). And graphically represented in Fig. 7 and Fig. 8). The results are represented graphically and tabulated below.

The two-sided p<0.0001, considered extremely significant evaluated using Fisher's Exact Test in Graph Pad Instat. The row/column association is statistically significant. Fatty liver patients are on increased risk of getting cardiovascular disorders on exposure to alcohol with relative risk of 37% tabulated below in (Table 3).

Excessive alcohol consumption poses a significant global healthcare challenge, carrying immense social, economic and clinical repercussions. According to the World Health Organization (2014), it contributed to 3.3 million deaths in 2012. Prolonged alcohol abuse inflicts damage on nearly every organ of the body.

In the hilly state of Uttarakhand, India, alcohol consumption is widespread, with ready availability even in departmental stores. This accessibility, combined with sedentary lifestyles and metabolic disturbances, heightens the risk of developing fatty liver. Despite being largely asymptomatic, fatty liver poses a substantial health concern. The liver, being the primary site of ethanol metabolism, endures the earliest and most severe tissue damage from excessive alcohol intake[20,21]. Intensive ethanol consumption leads to a broad array of hepatic abnormalities, with the most notable ones being fatty liver (statuses), hepatitis, and fibrosis/cirrhosis[22]. In animal models and in vitro studies, alcohol triggers metabolic, toxic, and inflammatory damage, resulting in mitochondrial dysfunction, the production of reactive oxygen species (ROS), Bax translocation to mitochondria, cytochrome c release and the activation of caspases[23-25]. Alcohol metabolism significantly contributes to alcohol-induced mitochondrial and endoplasmic reticulum (ER) stress, leading to apoptotic cell death[26]. Alcohol and its metabolites, such as acetaldehyde, are highly reactive and can lead to increased production of reactive oxygen species (ROS) and accumulation of misfolded proteins, thus triggering endoplasmic reticulum (ER) stress.

Clinical symptoms associated with fatty liver disease are not extensively documented. In our study, most patients visiting the medicine outpatient department (OPD) presented with complaints such as stomach upset, symptoms of inflammatory bowel disease (IBD), mild tenderness in the hypogastria and fatigue. Although the precise association of these symptoms with fatty liver disease remains unclear, it is plausible that changes in gut microbiota may contribute to symptoms such as IBD and stomach upset. Hepatitis could be the reason for the tenderness. Uslusoy HS, Nak SG *et al*.[27] demonstrated in a Turkish study that the aminotransferases level was normal, instead of patient is suffering from the NAFLD. In our study, also the transaminases were normal in accordance with their study. Our finding affirms that the transaminases alone are not a reliable marker of Fatty liver disease. Present study showed fatty liver to be a disease of middle-aged men in fourth and fifth decade (47±14 years) life at presentation and more than three-fourth being male. These results are in accordance with the Mohan *et al*.[28] and Pinidiyapathirage *et al*.[29] who also demonstrated male predominance of fatty liver disease specifically NASH. On the opposite side Fernandes *et al*.[30] showed that the females are more prone to have NAFLD. Spruss *et al*.[31] also demonstrated that female mice were more susceptible to FLD compare to male mice in an animal study. In our study we found that the Lipid Profile is deranged with the degree of fatty liver in exponential manner, up to Grade 1 the lipid Profile is mildly deranged and with time as the fatty liver progress the Lipid profile derangement increases. Our results are in accordance with the Drozdz *et al*.[32] who demonstrated the deranged lipid profile specifically increased cholesterol level in the serum of FLD patients. In our study we found that the TG: HDL ratio is a very good predictor of the cardiovascular risk assessment with good sensitivity of 98.81% and specificity of 100%. The relative risk calculated is 37% which clarify that the risk of getting cardiovascular disorders is more in patients who are exposure to alcohol. Our results are in accordance with the studies of Komas *et al*.[33] demonstrated Increased plasma TG and decreased HDL-C levels have been associated with CVD and their ratio, TG/HDL-C, has been proposed as a novel biomarker for predicting the risk of both clinical entities. Hajian-Tilaki *et al*.[34] also demonstrated that both TG/HDL-C and LDL-C/HDL-C ratios comparably predict CVD risk. Martínez-Marroquín *et al*.[35] demonstrated in Mexican study that the TG/HDL-C ratio exhibited a linear correlation with the Lindavista Score and a heightened risk of cardiovascular disorders. These findings suggest that the TG/HDL-C ratio serves as a convenient, readily available and cost-effective tool for stratifying cardiovascular risk among asymptomatic fatty liver patients.

CONCLUSION

The fatty liver is a disease which represents lately or accidentally diagnosed on ultrasonography. And the risk of getting cardiovascular disorders is more in these patients on exposure to alcohol. Hence, it is important to diagnose fatty liver early. TG/HDL-C ratio is a practical, easy and economical instrument to categorize cardiovascular risk in asymptomatic fatty liver diseases in remote settings on the basis of biochemical lipid profile.

Limitation of the Study: Study group is confined to the Uttarakhand region only, so geographical variation could be the limitation, which requires more external validity, also similar multi centric studies with large cohort groups would bring more light to it.

Declarations Section:

- Ethics approval and consent to participate- Ethical clearance from the institutional ethical

committee (IEC) with registration number GBCM/IEC/2023/07-03 dated 25/07/2023

- Consent for publication-Consent for publication was taken from the participants of the trial.
- Availability of data and material-Data will be provided whenever asked
- Competing Interest: There are no financial and non–financial competing interests for this trial
- Funding- Financial support for sample analysis was provided by the Gautam Buddha Chikitsa Mahavidyalaya, Jhajra Dehradun, Uttarakhand

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