Original Article

DOI: 10.7860/JCDR/2022/53485.16426

Relationship between Disorders of Lipid Profile and Features of Liver Cirrhosis- An Open Prospective and Comparative Analysis of Patients of Stavropol Territory in Russian Federation

Internal Medicine Section

TEMIRLAN RUSLANOVICH DUDOV[1], VIJAYA JAWAHAR SARITHALA[2], PAVEL VLADIMIROVICH KOROY[3], ALEXANDER VALENTINOVICH YAGODA[4]

ABSTRACT

Introduction: Liver plays an essential role in the metabolism, synthesis, transport and clearance of lipids and lipoproteins, therefore, changes in the lipid profile in liver pathology reflects the degree of its dysfunction. Leading role in the development of atherosclerosis belongs to lipid spectrum disorders in the form of hyperlipidaemia and dyslipidaemia, associated with an increased cardiovascular risk in liver cirrhosis.

Aim: To study the relationship between violations of lipid profile of blood with the features and the clinical picture of Liver Cirrhosis (LC).

Materials and Methods: The study was an open prospective and comparative analysis of patients with LC, conducted at Stavropol State Medical University (Stavropol State, Russia). Research was conducted from June till August 2020. In 108 patients with LC, blood concentration of total cholesterol, triglycerides, High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL) were studied in association with manifestations of the disease. Control group constituted of 45 healthy individuals, comparable in sex, age and ethnicity. Two sample student's t-test, Newman Keuls test, chi-square test with Yates's correction and Pearson's linear correlation coefficient (r) were calculated. Receiver Operating Characteristic (ROC) analysis was used, the Odds Ratio (OR) and its 95% Confidence Interval (CI), sensitivity, specificity, positive and negative predictive value and accuracy were determined. Differences were considered statistically significant at $p \leq 0.05$.

Results: Regardless of gender and age of patients, decrease of serum levels of total cholesterol ($p<0.05$), triglycerides ($p<0.05$), HDL ($p<0.05$) and LDL ($p<0.05$) were marked, associated with expression of portal hypertension and severity of liver cirrhosis. Parameters of LDL more than 2.16 mmol/L {OR 6.78-95% CI (2.74-16.78)} were connected with absence of oesophageal varices. Levels of triglycerides less than 0.83 mmol/L {OR 10.85-95% CI (2.86-41.19)} were associated with presence of oesophageal varices of grade III. Generally, hyperlipidaemia was observed in 17.6% of patients, and it was associated with alcoholic aetiology of liver cirrhosis ($\chi^2=3.7$; $p=0.053$). Hypocholesterolaemia (81.5% of cases) or hypotriglyceridaemia (48.1% of cases) was more commonly observed in patients with ascites ($\chi^2=8.8$; $p=0.003$), and classes B, C according Child-Pugh score ($\chi^2=4.0$; $p=0.045$).

Conclusion: In this study, it was found that, in liver cirrhosis, there is a decrease in the serum content of total cholesterol, triglycerides, HDL and LDL, regardless of gender and age of patients. The LDL values of more than 2.16 mmol/L are associated with an increased chance of absence of oesophageal varices in patients with liver cirrhosis.

Keywords: Dyslipidaemia, Dyslipidemia, High density lipoproteins, Low density lipoproteins, Oesophageal varices, Triglycerides

INTRODUCTION

Lipids and lipoproteins are important elements of the body that control cellular functions and homeostasis, including energy and upon contact with hepatotropic viruses, they reduce the toxic effects of viruses [1]. Liver plays an essential role in the metabolism, synthesis, transport and clearance of lipids and lipoproteins, therefore, changes in the lipid profile in liver pathology reflect the degree of its dysfunction [2].

Leading role in the development of atherosclerosis belongs to lipid spectrum disorders in the form of hyper and dyslipidaemia, associated with an increased cardiovascular risk in LC. Until now, there is discussion about the relationship between liver cirrhosis and Ischaemic Heart Disease (IHD) [3]. The incidence of coronary artery disease in patients with liver cirrhosis ranges from 2.5-5.1% to 21.7-36.8% and higher (up to 77%) [4-8]. The disputable data may be based on the heterogeneity of patients, differences in the influence of aetiology of the disease, risk factors, etc., [4,6,9]. A number of factors are considered as cardioprotective in patients with LC, including a decrease in the content of lipids and lipoproteins in blood, coagulopathy, thrombocytopenia, systemic vasodilation, malnutrition and hyperketonaemia [10]. Thus, with LC, dyslipidaemia was detected less frequently than in the general population and the phenomenon of hypolipidaemia prevailed in a large number of patients, which may explain the low cardiovascular risk [11-13].

On the other hand, a decrease in the serum lipid content is responsible for the development of complications of LC, such as infections, trophological insufficiency, adrenal dysfunction, anaemia, etc., [14]. Hypocholesterolaemia was a predictor of survival in patients with LC [14].

Despite the presence in LC, reduced serum levels of total cholesterol, Very Low-Density lipoprotein (VLDL), LDL and HDL and triglycerides, there is a point of view about the absence of changes in the lipid spectrum or their shift towards dyslipidaemia [6,15-19]. In addition, the possible relationship of blood lipid profile with the clinical components of LC, such as the duration of the disease, activity, manifestations of portal hypertension, the severity and prognosis of the disease has not yet been fully clarified [13,17,20]. There is no clear evidence of the existence of an association between lipid imbalance and manifestations of portal hypertension in LC. At the same time, a decrease in total cholesterol was a predictor of the development of future hepatic complications (especially oesophageal varices) in non alcoholic fatty liver disease patients with severe fibrosis or Child-Pugh class A cirrhosis [21].

The aim of the research was to study the relationship between violations of the lipid profile of the blood with the features and the clinical picture of LC.

MATERIALS AND METHODS

The present study was of an open prospective and comparative analysis of patients, which followed the STROBE guidelines, and was conducted from June till August 2020. The research was corresponded with the Helsinki declaration of World Medical Association on ethical principles for medical research involving human subjects. The study was approved by the Ethics Committee of the Stavropol State Medical University, Russia, Protocol No 100, from 17 June 2021. Patients and individuals in the control group had given their informed consent to participate in the study.

Total 108 patients with LC (52 men, 56 women, mean age 55.91±0.75 years) were examined, who were under observation and undergoing treatment at Department of Gastroenterology at Stavropol regional clinical hospital, Stavropol state, Russia.

Sample size calculation: Sample size was calculated using formula $n=(Z^2pq)/\Delta^2$, with Standard Error (SE) 4.3%, CL 90%, N=153.

Inclusion criteria: The study included patients with alcoholic or viral LC in the age group of 18 years or older.

Exclusion criteria: Patients aged less than 18 years; LC of non viral and non alcoholic aetiology; diabetes mellitus, chronic renal failure, malignant neoplasms, acute pancreatitis, thyroid dysfunction; gastrointestinal bleeding; intake of lipid or glucose regulating drugs; parenteral nutrition. The choice of exclusion criteria is associated with the maximum exclusion of diseases and conditions that affect lipid metabolism.

Study Procedure

The patients underwent clinical, biochemical and instrumental examination, including general blood analysis, biochemical analysis of blood, liver function tests, immunological analysis, Polymerase Chain Reaction (PCR) diagnostics, ultrasound examination of abdomen, fibroelastometry, endogastroduodenoscopy. The serum content of total cholesterol, HDL, LDL, triglycerides was studied on an automatic Siemens ADVIA 1800 analyser (USA).

Aetiology of the disease was obtained using anamnestic and PCR data for viral aetiology. Activity of the disease was evaluated using the levels of Alanine Transaminase (ALT). Using the ultrasound findings presence of ascites and hypersplenism were found. By the results of endogastroduodenoscopy, the presence of oesophageal varices was noticed. Grading of oesophageal varices was done in accordance with Soehendra classification of oesophageal varices [22]. Severity of LC was estimated using Child-Pugh score [23].

Hypocholesterolaemia was determined when total cholesterol values were less than 2.59 mmol/L (100 mg/dL) and/or LDL cholesterol was less than 1.81 mmol/L (70 mg/dL) and/or HDL cholesterol was less than 1.03 mmol/L (40 mg/dL). Hypotriglyceridaemia was diagnosed with triglyceride values less than 0.79 mmol/L (70 mg/dL) [12,13]. Hyperlipidaemia was indicated by levels of total cholesterol ≥5.17 mmol/L (200 mg/dL), LDL ≥4.14 mmol/L (160 mg/dL), or triglycerides ≥1.7 mmol/L (150 mg/dL) [4].

The control group consisted of 45 healthy individuals (control size was calculated in accordance with samples), recruited from students and staff of medical university and blood donors, matched by sex (21 men, 24 women), age (average age 52.18±2.51 years), ethnicity and having normal blood lipid profile [24].

STATISTICAL ANALYSIS

The data obtained were statistically processed using Microsoft office excel 2010, IBM Statistical Package for the Social Sciences (SPSS) statistics version 21.0. Quantitative values with normal distribution are presented in the form of mean±standard error of the mean. Two sample student's t-test, Newman-Keuls test, χ^2 test with Yates' correction were calculated. The relationship between the traits was identified using the Pearson's linear correlation coefficient (r). To assess the predictive role of the trait, ROC analysis was used and the OR and its 95% CI were determined. The diagnostic value was characterised by Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV) and Negative Predictive Value (NPV), Accuracy (Ac), expressed as a percentage. Differences were considered statistically significant at $p\leq0.05$.

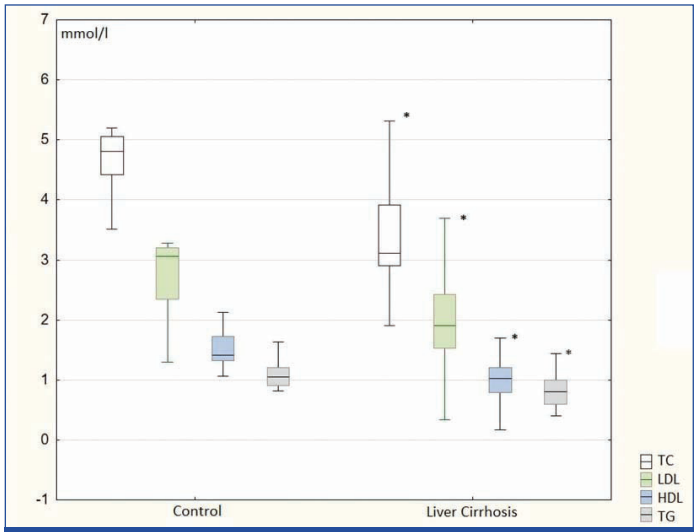
RESULTS

In the present study, total 108 patients with LC were included, of which, 52 were males and 56 were females, with a mean age of 55.91±0.75 years [Table/Fig-1]. Alcoholic LC was observed in 55 (50.9%) of the patients, LC of viral aetiology was noticed in 53 (49.1%) of individuals Hepatitis B Virus (HBV) infection was detected in 14 (26.4%) of cases, Hepatitis C Virus (HCV) was detected in 39 (73.6%) of patients. Minimal, moderate and high activity of LC was observed in 39 (80.6%), 16 (14.8%) and 5 (4.6%) of patients, respectively. Viral and alcoholic aetiology of LC was distributed equally. With viral LC, HCV was detected in most cases. Most of patients had minimal disease activity, hypersplenism, oesophageal varices, ascites, as well as compensated (39.8%) and sub compensated (45.4%) variants of the disease. The average ALT and Aspartate Aminotransferase (AST) values reached 77.62±7.75 U/L and 49.94±4.10 U/L, respectively.

Variables	n (%)
Sex	
Female	56 (51.9%)
Male	52 (48.1%)
Age	
Middle-age (40-59 years)	58 (53.7%)
Old (≥60 years)	50 (46.3%)
Aetiology of LC	
Viral LC	53 (49.1%)
Alcoholic LC	55 (50.9%)
Type of virus	
HBV	14 (26.4%)
HCV	39 (73.6%)
Activity of LC	
Minimal	39 (80.6%)
Moderate	16 (14.8%)
High	5 (4.6%)
Hypersplenism	
Present	78 (72.2%)
Absent	30 (27.8%)
Ascites	
Present	63 (58.3%)
Absent	45 (41.7%)
Oesophageal varices	
Absent	33 (30.6%)
Present	75 (69.4%)
Grade I	22 (29.3%)
Grade II	29 (38.7%)
Grade III	24 (32%)
Class by child-pugh	
A	43 (39.8%)
B	49 (45.4%)
C	16 (14.8%)

[Table/Fig-1]: Clinical characteristics of patients.

Hypersplenism developed in 78 (72.2%) of patients, ascites was noticed in 63 (58.3%) of patients. Oesophageal varices were detected in 75 (69.4%) of patients: grade I in 22 (29.3%) of cases, grade II in 29 (38.7%) of patients, grade III in 24 (32%) of patients. A 43 (39.8%) of patients had LC of Child-Pugh class A, 49 (45.4%) and 16 (14.8%) of cases had Band C classes, respectively [Table/Fig-1]. With LC, a decrease in the serum content of all the studied parameters of the lipid profile was observed [Table/Fig-2,3], which was not interrelated with the gender and age of the patients [Table/Fig-4,5].



[Table/Fig-2]: Blood lipid profile in patients with LC.
Data presented in the form of median, interquartile range, minimal and maximal values.
*p<0.05 compared with control group (two sample Student's t-test)

Studied indicators (mmol/L)	Examined groups			
	Control, (n=45)	LC, (n=108)	t	p-value
Total cholesterol	4.62±0.08	3.44±0.09*	7.93	0.001
LDL cholesterol	2.75±0.08	2.05±0.07*	5.82	0.001
HDL cholesterol	1.50±0.04	1.07±0.04*	6.39	0.001
Triglycerides	1.12±0.04	0.95±0.05*	2.08	0.039

[Table/Fig-3]: Lipid profile in patients with liver cirrhosis.
*p<0.05 compared with control (two sample Student's t-test); Values presented as Mean±Standard Error (SE)

Studied indicators (mmol/L)	Examined groups			
	Control		LC	
	Male (n=21)	Female (n=24)	Male (n=52)	Female (n=56)
Total cholesterol	4.65±0.11	4.60±0.13	3.39±0.13*	3.49±0.14**
LDL cholesterol	2.78±0.11	2.73±012	1.99 ±0.09*	2.10±0.12**
HDL cholesterol	1.51±0.08	1.50±0.04	1.08±0.05*	1.05±0.06**
Triglycerides	1.12±0.06	1.12±0.05	0.92±0.07	0.98±0.08

[Table/Fig-4]: Association of parameters of lipidogram with sex of the patients with LC.
*p<0.05 compared with control male; **p<0.05 compared with control female (two sample Student's t-test); Values presented as Mean±SE

Studied indicators (mmol/L)	Examined groups			
	Control: age		LC: age	
	Middle-age (40-59 year), n=25	Old (≥60 year), n=20	Middle-age (40-59 year), n=58	Old (≥60 year), n=50
Total cholesterol	4.66±0.09	4.58±0.16	3.54±0.14*	3.33±0.12**
LDL cholesterol	2.81±0.10	2.68±0.13	2.04±0.10*	2.06±0.11**
HDL cholesterol	1.44±0.05	1.58±0.07	1.08±0.05*	1.05±0.06**
Triglycerides	1.13±0.05	1.11±0.06	0.89±0.05*	1.03±0.09

[Table/Fig-5]: Effect of age of patients with LC on blood lipid values.
*p<0,05 compared with control Middle-age; **p<0.05 compared with control Old (two sample Student's t-test); Values presented as Mean±SE

Regardless of the aetiology of LC, a reduced concentration of total cholesterol, LDL, HDL in the blood was determined [Table/Fig-6]. In alcoholic LC, HDL values were significantly lower than in cases of viral LC. In both HBV associated and HCV cirrhosis, decreased serum levels of total cholesterol, LDL and HDL were recorded; triglyceride levels were reduced only in cases of LC caused by HBV infection [Table/Fig-7].

Studied indicators (mmol/L)	Examined groups		
	Control (n=45)	LC, (n=53)	
		Alcoholic LC (n=55)	Viral LC (n=53)
Total cholesterol	4.62±0.08	3.33±0.13*	3.56±0.14*
LDL cholesterol	2.75±0.08	2.11±0.10*	1.99±0.11*
HDL cholesterol	1.50±0.04	0.91±0.04*	1.23±0.06*/**
Triglycerides	1.12±0.04	0.98±0.07	0.93±0.08

[Table/Fig-6]: Lipid spectrum in relation with aetiology of LC.
*p<0.05 compared with control; **p<0.05 in between groups of alcoholic LC and viral LC (Newman-Keuls test); Values presented as Mean±SE

Studied indicators (mmol/L)	Examined groups		
	Control (n=45)	Viral LC, n=53	
		HBV, (n=14)	HCV, (n=39)
Total cholesterol	4.62±0.08	3.85±0.29*	3.45±0.15*
LDL cholesterol	2.75±0.08	2.02±0.16*	1.98±0.14*
HDL cholesterol	1.50±0.04	1.26±0.13*	1.21±0.06*
Triglycerides	1.12±0.04	0.78±0.05*	0.98±0.10

[Table/Fig-7]: Association of type of virus with lipid disorders in LC.
*p<0.05 compared with control; **p<0.05 in between groups with HBV LC and HCV LC (Newman-Keuls test); Values presented as Mean±SE

The duration of the disease did not affect the reduced values of total cholesterol, LDL, HDL in the blood [Table/Fig-8]. The content of triglycerides in the blood in patients of LC with duration of more than 10 years was reduced, being lower than in the group of patients with a disease duration of less than 10 years.

Studied indicators (mmol/L)	Examined groups		
	Control (n=45	LC, n=108, duration of disease	
		Up to 10 years (n=86)	More than 10 years, (n=86)
Total cholesterol	4.62±0.08	3.43±0.10*	3.49±0.24*
LDL cholesterol	2.75±0.08	2.06±0.08*	1.99±0.17*
HDL cholesterol	1.50±0.04	1.04±0.04*	1.18±0.09*
Triglycerides	1.12±0.04	1.01±0.06	0.74±0.08*/**

[Table/Fig-8]: Effect of duration of LC on lipid indicators in blood.
*p<0.05 compared with control; ** p<0.05 in between groups with LC of duration up to 10 years and more than 10 years (Newman-Keuls test); Values presented as Mean±SE

Reduced levels of total cholesterol, LDL, HDL were not associated with the activity of LC and triglyceride levels decreased only with minimal activity of the process. The parameters of the lipid profile did not correlate with the activity of aminotransferases.

The appearance of ascites was characterised by comparatively lower serum levels of total cholesterol, LDL, HDL than in the compensated variant of the disease [Table/Fig-9]. The presence or absence of hypersplenism in patients with liver cirrhosis was not associated with a reduced concentration of total cholesterol and LDL in the blood, while HDL values were characterised by comparatively higher values in cases of hypersplenism [Table/Fig-10].

In liver cirrhosis, the content of total cholesterol, HDL and triglycerides in the blood was not associated with the presence of oesophageal varices. However, in patients with oesophageal varices, serum LDL values were statistically significantly lower than in those without port-caval anastomoses [Table/Fig-11]. Threshold LDL levels of more than 2.16 mmol/L were associated with a high

Studied indicators (mmol/L)	Examined groups		
		Presence of ascites, LC, n=108	
	Control, n=45	Absent, n=45	Present, n=63
Total cholesterol	4.62±0.08	3.66±0.13*	3.28±0.13*/**
LDL cholesterol	2.75±0.08	2.23±0.12*	1.92±0.09*/**
HDL cholesterol	1.50±0.04	1.17±0.06*	0.99±0.05*/**
Triglycerides	1.12±0.04	0.99±0.08	0.92±0.07

[Table/Fig-9]: Relation of ascites with lipid disorder in LC.
*p<0.05 compared with control; **p<0.05 in between groups with absence of ascites and presence of ascites (Newman-Keuls test); Values presented as Mean±SE

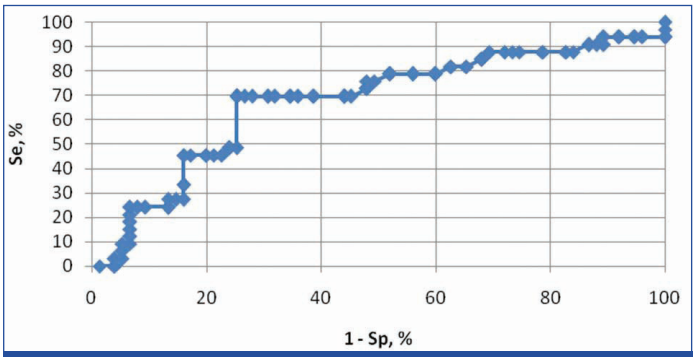
Studied indicators (mmol/L)	Examined groups		
		LC, n=108, Hypersplenism	
	Control, n=45	Absent, n=30	Present, n=78
Total cholesterol	4.62±0.08	3.45±0.15*	3.44±0.12*
LDL cholesterol	2.75±0.08	2.08±0.10*	2.04±0.10*
HDL cholesterol	1.50±0.04	0.89±0.06*	1.14±0.04*/**
Triglycerides	1.12±0.04	0.98±0.11	0.94±0.06

[Table/Fig-10]: Relation of hypersplenism with lipid markers in LC.
*p<0.05 compared with control; **p<0.05 in between groups with absence of hypersplenism and presence of hypersplenism (Newman-Keuls test); Values presented as Mean±SE

probability of absence of dilated oesophageal veins {OR 6.78, 95% CI (2.74-16.78)} and were characterised by moderate accuracy in this aspect (73.1%) [Table/Fig-12]. Indicators of Se, Sp, PPV, NPV for the above LDL values were 69.7, 74.7, 54.8 and 84.8%, respectively [Table/Fig-13].

Studied indicators (mmol/L)	Examined groups		
	Control, n=45	LC: Oesophageal varices, n=108	
		Absent, n=33	Present, n=75
Total cholesterol	4.62±0.08	3.48±0.14* (q=8.09, p<0.05)	3.42±0.12* (q=10.36, p<0.05)
LDL cholesterol	2.75±0.08	2.30±0.14* (q=3.84, p<0.05)	1.94±0.09* (q=8.40, p<0.05)/** (q=3.37, p<0.05)
HDL cholesterol	1.50±0.04	1.01±0.07* (q=8.92, p<0.05)	1.09±0.04* (q=9.08, p<0.05)
Triglycerides	1.12±0.04	0.99±0.11	0.94±0.06

[Table/Fig-11]: Indicators of lipidogram in relation with presence of oesophageal phleb-ectasii in LC.
*p<0.05 compared with control; **p<0.05 in between groups with absence of oesophageal varices and presence of oesophageal varices (Newman-Keuls test); Values presented as Mean±SE



[Table/Fig-12]: ROC curve of LDL ≥2.16 mmol/L and absence of oesophageal varices {OR 6.78, 95% CI (2.74-16.78)}.

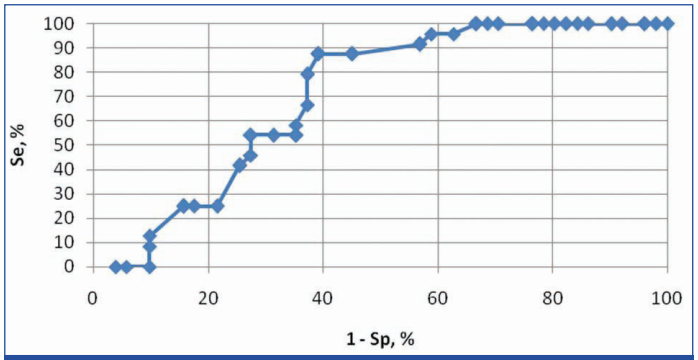
Indicator (mmol/L)	OR (95% CI)	p-value	Se (%)	Sp (%)	PPV (%)	NPV (%)	Ac (%)
LDL≥2.16	6.78 (2.74-16.78)	<0.05	69.7	74.7	54.8	84.8	73.1

[Table/Fig-13]: Diagnostic significance of lipids in predicting the absence of oesophageal varices in LC.

The authors did not investigate the relationship between reduced amounts of total cholesterol, LDL, HDL in the blood with the severity of oesophageal veins dilatation in LC. On the contrary, the decreased serum triglyceride content, noticed in patients with grade III oesophageal phlebectasias, was lower than the corresponding values in patients with lower gradations of oesophageal vein dilatation [Table/Fig-14]. Triglyceride values with a cut-off point less than 0.83 mmol/L indicated an increased chance of severe oesophageal veins dilatation {OR 10.85 (95% CI (2.86-41.19)} [Table/Fig-15], although they had little accuracy (69.3%) in its prediction indicators Se, Sp, PPV, NPV were 87.5, 60.8, 51.2 and 91.2%, respectively [Table/Fig-16].

Studied indicators (mmol/L)	Examined groups			
	Control (n=45)	LC: oesophageal varices, n=75		
		Grade I (n=22)	Grade II (n=29)	Grade III (n=24)
Total cholesterol	4.62±0.08	3.44±0.25*	3.45±0.17*	3.38±0.23*
LDL cholesterol	2.75±0.08	1.86±0.18*	2.07±0.16*	1.84±0.11*
HDL cholesterol	1.50±0.04	1.15±0.11*	1.04±0.07*	1.11±0.07*
Triglycerides	1.12±0.04	1.00±0.15**	1.08±0.10**	0.71±0.02*

[Table/Fig-14]: Correlation of stage of oesophageal varices in LC with lipid spectrum in blood.
*p<0.05 compared with control; **p<0.05 compared with oesophageal varices grade III (Newman-Keuls test); Values presented as Mean±SE



[Table/Fig-15]: ROC curve for triglycerides ≤0.83 mmol/L and oesophageal varices grade III {OR 10.85, 95% CI (2.86-41.19)}.

Indicator (mmol/L)	OR (95% CI)	p	Se (%)	Sp (%)	PPV (%)	NPV (%)	Ac (%)
Triglycerides ≤0.83	10.85 (2.86-41.19)	<0.05	87.5	60.8	51.2	91.2	69.3

[Table/Fig-16]: Diagnostic significance of triglycerides in the prediction of grade III oesophageal varices in LC.

The content of total cholesterol, LDL, HDL in the blood decreased with the severity of the disease according to the Child-Pugh classification, reaching minimum values in cases of class C, triglyceride levels were reduced only in decompensated LC. In patients with class C according to Child-Pugh, all the studied parameters were significantly lower than in the compensated variant of the disease, and LDL levels additionally differed from the corresponding values in those examined with class B [Table/Fig-17].

Studied indicators (mmol/L)	Examined groups			
	Control (n=45)	LC: Child-pugh class, n=108		
		Class A (n=43)	Class B (n=49)	Class C (n=16)
Total cholesterol	4.62±0.08	3.71±0.14*	3.35±0.13*	3.02±0.27*/**
LDL cholesterol	2.75±0.08	2.32±0.13*	1.94±0.10*/**	1.66±0.15*/**
HDL cholesterol	1.50±0.04	1.15±0.05*	1.07±0.06*	0.85±0.10*/**
Triglycerides	1.12±0.04	1.06±0.09	0.95±0.08	0.69±0.05*/**

[Table/Fig-17]: Effect of severity of LC on lipid profile in blood.
*p<0.05 compared with control; **p<0.05 compared with class A (Newman-Keuls test); Values presented as Mean±SE

There was a negative correlation found between the Child-Pugh scale values with total cholesterol and HDL cholesterol (r=-0.28; p<0.05; r=-0.27; p<0.05, respectively), and its relationship with triglycerides tended to be reliable (r=-0.18; p=0.059). There was

a positive correlation between HDL and triglycerides with serum albumin levels (r=+0.20; p<0.05; r=+0.23; p<0.05, respectively), as well as triglycerides with values of prothrombin index (r=+0.23; p<0.05) [Table/Fig-18].

Indicator (mmol/L)	Child-pugh class	Albumin	Prothrombin index	Bilirubin
Total cholesterol	r=-0.28; p=0.003	r=+0.16; p=0.11	r=+0.01; p=0.92	r=+0.01; p=0.89
LDL cholesterol	r=-0.12; p=0.23	r=+0.02; p=0.83	r=-0.14; p=0.14	r=+0.02; p=0.81
HDL cholesterol	r=-0.27; p=0.005	r=+0.20; p=0.03	r=+0.12; p=0.22	r=-0.07; p=0.42
Triglycerides	r=-0.18; p=0.059	r=+0.23; p=0.04	r=+0.23; p=0.02	r=+0.03; p=0.77

[Table/Fig-18]: Correlation of lipid spectrum with parameters of severity of LC.

Hyperlipidaemia was observed in 19 (17.6%) of patients with LC. In cases of alcoholic LC, hyperlipidaemia was more common than absence of hyperlipadaemia (73.7% and 46.1% respectively, χ^2=3.7; p=0.053), while demographic, clinical and laboratory markers of the disease were not associated with its occurrence [Table/Fig-19].

Parameters	Hyperlipidaemia, 19 (17.6%)	No hyperlipidaemia, 89 (82.4%)	Significance
Alcoholic LC, n (%)	14/19 (73.7%)	41/89 (46.1%)	χ^2=3.7; p=0.053
Male sex, n (%)	7/19 (36.8%)	45/89 (50.6%)	χ^2=0.69; p=0.40
Age (years)	56.42±1.64	56.93±1.01	t=0.22, p=0.83
Duration of LC, (years)	5.74±0.84	7.33±0.65	t=1.08, p=0.28
AST, U/l	67.23±8.82	79.84±9.22	t=0.62, p=0.54
ALT, U/l	41.59±5.06	51.72±4.85	t=0.93, p=0.35
Bilirubin μmol/L	67.69±14.64	42.43±8.62	t=1.27, p=0.21
Albumin, g/L	33.68±1.53	35.14±0.66	t=0.91, p=0.36
Prothrombin index, %	76.26±3.83	82.33±1.24	t=1.89, p=0.06
Child-Pugh scale, points	7.68±0.51	7.12±0.19	t=1.18, p=0.24
Child-Pugh class, A/B/C	5/10/4 (26.3%/52.6%/21.1%)	38/39/12 (42.7%/43.8%/13.5%)	χ^2=1.14; p=0.29
Ascites present, n (%)	11/19 (57.9%)	52/89 (58.4%)	χ^2=0.05; p=0.17
Oesophageal varices present, n (%)	12/19 (63.2%)	63/89 (70.8%)	χ^2=0.15; p=0.29
Hypersplenism present, n (%)	13/19 (68.4%)	65/89 (73.0%)	χ^2=0.02; p=0.99

[Table/Fig-19]: Association of lipid spectrum with parameters of severity of LC.

Hypocholesterolaemia observed in 88 (81.5%) of patients with LC, hypotriglyceridaemia seen in 52 (48.1%) of cases, hypocholesterolaemia and/or hypotriglyceridaemia seen in 93 (86.1%) of cases. Patients with or without hypocholesterinaemia and/or hypotriglyceridaemia did not differ in sex, age, aetiology and duration of the disease, levels of AST, ALT, bilirubin, albumin, prothrombin index, Child-Pugh scale, presence of oesophageal varices and hypersplenism. In cases of hypocholesterolaemia and/or hypotriglyceridaemia, ascites was relatively more common (64.5% and 20.0%, respectively, χ^2=8.8; p=0.003), Child-Pugh scale class A was less common (35.5% and 66.7%, respectively, χ^2=4.0; p=0.045), and the values of the Child-Pugh scale were higher (7.39±0.19 and 6.20±0.35, respectively, p=0.023).

DISCUSSION

According to the presented data, in LC, there was a reduced content of total cholesterol, HDL, LDL and triglycerides in the blood, which coincides with the results of other authors [15,16,25]. Nevertheless, the possibility of normal or elevated serum levels of triglycerides, LDL, VLDL in patients with liver cirrhosis cannot be ruled out against the background of a decrease in other indicators of the blood lipid spectrum [17,18].

The decrease in the parameters of the blood lipid profile in patients with LC did not depend on gender and age. In an earlier study, it was found that there was no relationship between serum lipid levels and the age of patients with HCV associated liver pathology [20].

This study did not establish the relationship between the indicators of total cholesterol, LDL, triglycerides with the aetiology of the disease, however, in alcoholic LC, HDL values were comparatively lower than in LC of viral aetiology. It is assumed that the nature of deviations in lipid metabolism in LC depends on the etiological factor. Nevertheless, changes in the blood lipid spectrum in alcoholic LC, as a rule, did not differ from those in the viral aetiology of the process, with the exception of lower HDL values and higher triglycerides in alcoholic LC [15,16,26].

According to present data, the content of lipids and lipoproteins in the blood was not associated with the activity of LC, which generally corresponds to previously obtained data on the absence of a relationship between blood lipids and the degree of viremia, biochemical and histological activity and the stage of viral liver pathology [20,27]. However, it is possible for lower triglyceride or total cholesterol values to be present in cases of elevated aminotransferase values in patients with HBV infection [27,28].

The parameters of the blood lipid spectrum in patients were negatively associated with manifestations of portal hypertension in the form of comparatively lower values of total cholesterol, LDL, HDL with the appearance of ascites, LDL in cases with the formation of oesophageal varices, as well as triglycerides against the background of the third-grade dilatation of the oesophageal veins. Threshold values of LDL of more than 2.16 mmol/L have been established, which make it possible with a high probability to exclude the presence of oesophageal varices in LC, as well as the values of triglycerides (less than 0.83 mmol/L), which characterise patients with a high risk of having oesophageal varices of the 3rd grade have been determined.

Oesophageal varices are one of the most severe manifestations of portal hypertension. The priority of early detection and assessment of the state of oesophageal phlebectasias in LC is quite obvious, allowing timely preventive measures for the development of bleeding. The diagnostic value of non invasive predictors of dilated oesophageal veins is widely discussed, taking into account the possibility of their damage during endoscopic examination. Previously, the prognostic significance of a number of parameters of haemostatic homeostasis and endothelial dysfunction in the detection of oesophageal phlebectasias was established [29,30]. It was noted that in patients with non alcoholic fatty liver disease with severe fibrosis or compensated cirrhosis, a decrease in total blood cholesterol acted as a predictor of future complications (including dilated oesophageal veins) [21]. Apparently, the revealed relationship is due to the fact that the formation and/or aggravation of portal hypertension is responsible for the violation of the flow of cholesterol and fatty acids with the portal blood flow to the liver and the subsequent decrease in the content of lipids and lipoproteins in the blood.

According to present data, blood lipid profile parameters were characterised by minimal values in patients with LC with class C according to Child-Pugh, negatively correlating with the values of the Child-Pugh scale and positively with the levels of albumin and prothrombin index. The conjugation of lipid metabolism markers

with the Child-Pugh and Model for End-stage Liver Disease (MELD) scales in LC was found earlier [12,16], although not in all studies [17,26]. In patients with LC, the content of total cholesterol and LDL in the blood had a predictive ability in relation to class C, and in total cholesterol, LDL and triglycerides in relation to MELD values>24 [16]. In our opinion, the found association may be based on a violation of the synthetic function of the liver, which plays an important role in metabolism, synthesis, transport, and clearance of lipids and lipoproteins [2].

Hyperlipidaemia, registered in 17.6% of patients, was associated with alcoholic aetiology of the process, which confirms the increased risk of coronary heart disease in cases of alcoholic LC [11,19]. It is known that the alcoholic nature of hepatic pathology was a risk factor for obstructive form of IHD and calcification of coronary arteries [11,31]. In our opinion, patients with alcoholic LC with elevated levels of blood lipids constitute a risk group for the development of IHD, which requires further targeted diagnostic research in this direction.

On the contrary, the majority of patients with LC had hypocholesterolaemia and/or hypotriglyceridaemia, which coincides with the previously obtained data [12,13]. Hypolipidaemia was more often observed in decompensated forms of the disease, which raises the question of optimising the elements of nutritional support for this category of patients in order to improve the blood lipid profile.

Thus, patients with LC develop blood lipid spectrum disorders associated with an unfavourable course of the disease. Lipid metabolism markers associated with the appearance and weighting of oesophageal varices in LC can be a useful tool in the non invasive diagnosis of oesophageal varices.

The effect of virological features of the disease (presence or absence of HBeAg in HBV cases, HCV genotype, degree of viremia of HCV, HBV) on lipid profile disorders in patients with viral LC were not evaluated. It is known that HCV infection is closely associated with lipid disorders, since the virus uses the host's lipid metabolism to maintain its life cycle [32]. The HCV enters the hepatocyte through ApoE receptors, which normally binds to LDL, then the viral genome replicates, binding to VLDL, which masks HCV and makes it inaccessible to the human immune system [33]. The mechanisms by which HCV virus leads to hypocholesterolaemia have not been fully elucidated. It is suggested that HCV reduces the activity and amount of microsomal triglyceride transporter protein, which impairs the production and secretion of VLDL and LDL [13].

Characteristic disorders of the lipid profile in HCV infection are due to the use of host lipoproteins by the virus (especially genotype 3) in the life cycle, which changes the final pathways of cholesterol synthesis [34,35]. It was previously noticed that lipid profile disorders in HCV infection were more pronounced in cases of genotype 3a, [36], did not depend on the level of viremia and virus genotype [20], positively correlated with the severity of hepatic steatosis and negatively correlated with the response to antiviral therapy [19,35].

Secondly, in patients with alcoholic LC, the relationship of lipid disorders with the presence or absence of alcoholic hepatitis, the duration of alcohol abuse and the dose of ethanol taken, has not been studied. It is known that alcohol consumption is a common cause of secondary hyperlipidaemia, manifested by elevated levels of total cholesterol, triglycerides, VLDL, phospholipids in the blood and is more common in alcoholic steatosis and steatohepatitis, than in cirrhosis [37-40].

Thirdly, the association of lipid spectrum abnormalities with morphological manifestations of LC has not been studied, although in general, the relationship of reduced blood lipid levels with biochemical or histological activity, stage of HCV or HBV-associated liver pathology was not found [20,41,42].

Finally, the study of lipid metabolism markers in the course of observation of patients with LC would make it possible to establish the ability of lipid profile deviations in terms of predicting lethal outcomes and severe complications of the disease. In patients who died from acute liver failure, relatively lower levels of HDL were determined [43], and in patients with liver cirrhosis with an unfavourable 3-month and 12-month prognosis, lower values of cholesterol, triglycerides, HDL, and LDL were observed [42]. The content of total cholesterol in the blood of less than 2 mmol/L had an accuracy of 75% in predicting mortality in LC [44], and values of ≤2.8 mmol/L were predictors of 3-month and 12-month mortality, which allows them to be used in predicting the mortality of patients with LC, taking into account the imperfection of the MELD scale [43]. At the same time, there were no differences in the incidence of hypocholesterolaemia and/or hypotriglyceridaemia in surviving and deceased patients over a 4-year follow-up period in patients with LC [13].

Limitation(s)

The present study was limited by small sample size. There is a need of a large multicentred clinical analysis of patients for generalising the results. A comparative analysis of virological features of the disease, presence or absence of HBeAg in HBV cases, HCV genotype, degree of viremia of HCV, HBV on lipid profile disorders, which were not studied in our current research must be analysed for clarifying the extent of lipid disorder with aetiology of LC.

CONCLUSION(S)

With LC, there was a decrease in the serum content of total cholesterol, triglycerides, HDL, LDL, regardless of gender and age of patients, associated with the severity of portal hypertension and the severity of the disease. Low density lipoprotein values of more than 2.16 mmol/L were associated with an increased chance of absence of oesophageal varices in patients with LC, triglyceride levels less than 0.83 mmol/L predict the presence of grade 3 oesophageal varices.

REFERENCES

[1] Feingold KR, Grunfeld C. Lipids: A key player in the battle between the host and microorganisms. J Lipid Res. 2012;53(12):2487-89. https://doi.org/10.1194/jlr.E033407.
[2] Aizawa Y, Seki N, Nagano T, Abe H. Chronic hepatitis C virus infection and lipoprotein metabolism. World J Gastroenterol. 2015;21:10299-313. https://doi.org/10.3748/wjg.v21.i36.10299.
[3] Koroy PV, Vitkovskaya MA, Raevskaya AI, Dudov TR, Yagoda AV. Ischemic heart disease in liver cirrhosis: Modern realities. Attending Physician. 2020;2:06-09. (In Russ.). https://dx.doi.org/10.26295/OS.2020.77.35.001.
[4] Tsai MC, Yang TW, Wang CC, Wang YT, Sung WW, Tseng MH, et al. Favorable clinical outcome of nonalcoholic liver cirrhosis patients with coronary artery disease: A population-based study. World J Gastroenterol. 2018;24(31):3547-55. https://dx.doi.org/10.3748/wjg.v24.i31.3547.
[5] Moon YJ, Kwon HM, Jung KW, Jeong HW, Park YS, Jun IG, et al. Risk stratification of myocardial injury after liver transplantation in patients with computed tomographic coronary angiography-diagnosed coronary artery disease. Am J Transplant. 2019;19(7):2053-66. https://doi.org/10.1111/ajt.15263.
[6] Patel SS, Nabi E, Guzman L, Abbate A, Bhati C, Stravitz RT, et al. Coronary artery disease in decompensated patients undergoing liver transplantation evaluation. Liver Transpl. 2018;24(3):333-42. https://doi.org/10.1002/lt.25069.
[7] Kazankov K, Munk K, Øvrehus KA, Jensen JM, Siggaard CB, Grønbaek H, et al. High burden of coronary atherosclerosis in patients with cirrhosis. Eur J Clin Invest. 2017;47(8):565-57. https://doi.org/10.1111/eci.12777.
[8] Patel SS, Lin FP, Rodriguez VA, Bhati C, John BV, Pence T, et al. The relationship between coronary artery disease and cardiovascular events early after liver transplantation. Liver Int. 2019;39(7):1363-71. https://doi.org/10.1111/liv.14092.
[9] VanWagner LB, Harinstein ME, Runo JR, Darling C, Serper M, Hall S, et al. Multidisciplinary approach to cardiac and pulmonary vascular disease risk assessment in liver transplantation: An evaluation of the evidence and consensus recommendations. Am J Transplant. 2018;18(1):30-42. https://doi.org/10.1111/ajt.14531.
[10] McCaughan GW, Crawford M, Sandroussi C, Koorey DJ, Bowen DG, Shackel NA, et al. Assessment of adult patients with chronic liver failure for liver transplantation in 2015: Who and when? Intern Med J. 2016;46:404-12. https://doi.org/10.1111/imj.13025.
[11] An J, Shim JH, Kim SO, Lee D, Kim KM, Lim YS, et al. Prevalence and prediction of coronary artery disease in patients with liver cirrhosis: A registry-based matched case-control study. Circulation. 2014;130(16):1353-62. https://doi.org/10.1161/CIRCULATIONAHA.114.009278.

[12] Bassani L, Fernandes SA, Raimundo FV, Harter DL, Gonzalez MC, Marroni CA. Lipid profile of cirrhotic patients and its association with prognostic scores: A cross-sectional study. Arq Gastroenterol. 2015;52(3):210-15. https://doi.org/10.1590/S0004-28032015000300011.
[13] Boemeke L, Bassani L, Marroni CA, Gottschall CBA. Lipid profile in cirrhotic patients and its relation to clinical outcome. Arq Bras Cir Dig. 2015;28(2):132-35. https://doi.org/10.1590/S0102-67202015000200012.
[14] Privitera G, Spadaro L, Marchisello S, Fede G, Purrello F. Abnormalities of lipoprotein levels in liver cirrhosis: Clinical relevance. Dig Dis Sci. 2018;63(1):16-26. https://doi.org/10.1007/s10620-017-4862-x.
[15] Som K, Swaika BC, Pramanik S, Chakraborty P, Gantait K. Lipid profile in alcoholic and non alcoholic patients of chronic liver disease- a comparative and analytical study in a rural-based tertiary care centre. J Assoc Physicians India. 2019;67(4):22-24.
[16] Suman C, Kumar BR, Prabhakar B. Lipid profile in assessing the severity of cirrhosis. IAIM. 2016;3(6):113-23.
[17] Mandal SK, Sil K, Chatterjee S, Ganguly J, Chatterjee K, Sarkar P, et al. A study on lipid profiles in chronic liver diseases. Natl J Med Res. 2013;3(1):70-72.
[18] Phukan JP, Sinha A, Deka JP. Serum lipid profile in alcoholic cirrhosis: A study in a teaching hospital of north-eastern India. Niger Med J. 2013;54(1):05-09. https://doi.org/10.4103/0300-1652.108886.
[19] Loria P, Marchesini G, Nascimbeni F, Ballestri S, Maurantonio M, Carubbi F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying aetiology. Atherosclerosis. 2014;232(1):99-109. https://doi.org/10.1016/j.atherosclerosis.2013.10.030.
[20] Alavian SM, Miri SM, Tabatabaei SV, Keshvari M, Behnava B, Elizee PK, et al. Lipid profiles and hepatitis C viral markers in HCV-infected thalassemic patients. Gut Liver. 2011;5(3):348-55. https://doi.org/10.5009/gnl.2011.5.3.348.
[21] Bhala N, Angulo P, Van der Poorten D, Lee E, Hui JM, Saracco G, et al. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: An international collaborative study. Hepatology. 2011;54(4):1208e16.
[22] Abby Philips C, Sahney A. Oesophageal and gastric varices: Historical aspects, classification and grading: Everything in one place. Gastroenterol Rep (Oxf). 2016;4(3):186-95. Doi: 10.1093/gastro/gow018.
[23] Peng Ying, Qi Xingshun, Guo, Xiaozhong. Child–Pugh Versus MELD Score for the Assessment of Prognosis in Liver Cirrhosis. Medicine. 2016;95(8):e2877. Doi: 10.1097/MD.0000000000002877.
[24] Lee Y, Siddiqui WJ. Cholesterol Levels. [Updated 2021 Jul 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. https://www.ncbi.nlm.nih.gov/books/NBK542294/.
[25] Arain SQ, Talpur FN, Channa NA, Ali MS, Afridi HI. Serum lipid profile as a marker of liver impairment in hepatitis B cirrhosis patients. Lipids Health Dis. 2017;16(1):51. https://doi.org/10.1186/s12944-017-0437-2.
[26] Chrostek L, Supronowicz L, Panasiuk A, Cylwik B, Gruszewska E, Flisiak R. The effect of the severity of liver cirrhosis on the level of lipids and lipoproteins. Clin Exp Med. 2014;14(4):417-21. https://doi.org/10.1007/s10238-013-0262-5.
[27] Kwarteng JK, Owusu L, Afihene M, Mica E, Opare-Sem O, Arthur FK. Lowered serum triglyceride levels among chronic hepatitis B-infected patients in Ghana. J Sci Technol (Ghana). 2012;32(3):01-10. https://doi.org/10.4314/just.v32i3.1.
[28] Agbecha A, Usoro CA, Etukudo MH. Serum lipids in chronic viral hepatitis B patients in Makurdi, Nigeria. J Health Res. 2017;4(2):81-86. https://doi.org/10.4103/2348-3334.201981.
[29] Koroy PV. Clinical, pathogenetic and prognostic importance of disorders of haemostatic homeostasis in chronic liver diseases: dissertation thesis of the doctor of medical sciences. Stavropol, 2010. (In Russian). https://medical-diss.com/medicina/kliniko-patogeneticheskoe-i-prognosticheskoe-znachenie-narusheniy-gemostaticheskogo-gomeostaza-pri-hronicheskih-zabolevan.
[30] Yagoda AV, Koroy PV. Text Book on Liver pathology and function of platelets (clinical and pathogenetic analysis). Stavropol: Stavropol State Medical University, 2008. (In Russian).
[31] Danielsen KV, Wiese S, Hove J, Bendtsen F, Møller S. Pronounced coronary arteriosclerosis in cirrhosis: Influence on cardiac function and survival? Dig Dis Sci. 2018;63(5):1355-62. https://doi.org/10.1007/s10620-018-5006-7.
[32] Popescu CI, Dubuisson J. Role of lipid metabolism in hepatitis C virus assembly and entry. Biol Cell. 2010;102:63-74.
[33] Felmlee DJ, Hafirassou ML, Lefevre M, Baumert TF, Schuster C. Hepatitis C virus, cholesterol and lipoproteins- impact for the viral life cycle and pathogenesis of liver disease. Viruses. 2013;5:1292-24.
[34] Bassendine MF, Sheridan DA, Bridge SH, Felmlee DJ, Neely RD. Li-pids and HCV Semin. Immunopathol. 2013;35:87-100.
[35] Clark PJ, Thompson AJ, Vock DM. Hepatitis C virus selectively per-turbs the distal cholesterol synthesis pathway in a genotype-specific manner. Hepatology. 2012;56:49-56.
[36] Siagris D, Christofidou M, Theocharis GJ, Pagoni N, Papadimitriou C, Lekkou A, et al. Serum lipid pattern in chronic hepatitis C: Histological and virological correlations. J Viral Hepat. 2006;13(1):56-61.
[37] Ristic-Medic D, Takic M, Vucic V, Kandic D, Kostic N, Glibetic M. Abnormalities in the serum phospholipids fatty acid profile in patients with alcoholic liver cirrhosis. J Clin Biochem Nutr. 2013;53:49-54.
[38] Van de Wiel A. The effect of alcohol on postprandial and fasting triglycerides. Int J Vasc Med. 2012;2012;4.
[39] Vodnala D, Rubenfire M, Brook RD. Secondary causes of dyslipidemia. Am J Cardiol. 2012;1110:823-25.
[40] Kwarteng JK, Owusu L, Afihene M, Mica E, Opare-Sem O, Arthur FK. Lowered serum triglyceride levels among chronic hepatitis B-infected patients in Ghana. J Sci Technol (Ghana). 2012;32(3):01-10.
[41] Miyazaki T, Honda A, Ikegami T, Saitoh Y, Hirayama T, Hara T, et al. Hepatitis C virus infection causes hypolipidemia regardless of hepatic damage or nutritional state: An epidemiological survey of a large Japanese cohort. Hepatol Res. 2011;41(6):530-41.
[42] Jiang M, Liu F, Xiong WJ, Zhong L, Xu W, Xu F, et al. Combined MELD and blood lipid level in evaluating the prognosis of decompensated cirrhosis. World J Gastroenterol. 2010;16(11):1397-401.
[43] Etogo-Asse FE, Vincent RP, Hughes SA, Auzinger G, Le Roux CW, Wendon J, et al. High density lipoprotein in patients with liver failure; relation to sepsis, adrenal function and outcome of illness. Liver Int. 2012;32(1):128-36.
[44] Janicko M, Veselíny E, Lesko D, Jarcuska P. Serum cholesterol is a significant and independent mortality predictor in liver cirrhosis patients. Ann Hepatol. 2013;12:581-87.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Hospital Therapy, Stavropol State Medical University, Stavropol, Russia.
2. Assistant Professor, Department of Hospital Therapy, Stavropol State Medical University, Stavropol, Russia.
3. Professor, Department of Hospital Therapy, Stavropol State Medical University, Stavropol, Russia.
4. Professor, Department of Hospital Therapy, Stavropol State Medical University, Stavropol, Russia.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Vijaya Jawahar Sarithala,
No. 12/432, First Floor, Ramachandra Lodge, Tadipatri-515411, Andhra Pradesh, India.
E-mail: jay_sv2006@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 01, 2021
- Manual Googling: Jan 26, 2022
- iThenticate Software: Apr 28, 2022 (10%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Nov 29, 2021**
Date of Peer Review: **Jan 27, 2022**
Date of Acceptance: **Mar 08, 2022**
Date of Publishing: **Jun 01, 2022**