Evaluation of Lipid Profile in Patients with Hepatitis C Virus Related Liver Cirrhosis

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Background: Liver cirrhosis is characterized by multiple metabolic changes. Hepatitis C virus (HCV) induced cirrhosis is one of the universal causes of morbidity and mortality. The peak prevalence of antibodies to hepatitis C virus (HCV) was reported in Egypt. The aim of this study was to evaluate lipid profile in patients with hepatitis C virus induced liver cirrhosis.

Study aim: To evaluate lipid profile in patients with hepatitis C virus induced liver cirrhosis.

Patients and Methods: This Case control study was conducted on 63 subjects divided into 21 subjects in each of 3 groups: Chronic hepatitis C group, cirrhotic groups and healthy control group. Full history taking and examination were done for all cases. Fasting serum lipid profile of serum total cholesterol , TG , HDL, LDL, VLDL were measured for all cases.

Results: There was statistically significant difference between the studied groups regarding total cholesterol and HDL cholesterol, TG and VLDL cholesterol. The best cutoff of HDL cholesterol in diagnosis of presence of liver cirrhosis was ≤37.71 mg/dl with area under curve 0.745, sensitivity 71.4%, specificity 71.4%, positive predictive value 55.6%, negative predictive value 83.3%m accuracy 71.4%. The best cutoff of VLDL cholesterol in diagnosis of presence of liver cirrhosis is ≤21.423 mg/dl with area under curve 0.78, sensitivity 84.5%, specificity 75.0%, positive predictive value 63.3%, negative predictive value 90.2%m accuracy 78.5%. Significant AUC was recorded with cutoff regard Serum cholesterol, Serum triglycerides, LDL and VLDL ≤157.5, ≤152.9, ≤83.2 and ≤30.59 respectively with perfect validity.

Conclusion: lipid parameters may be considered as a supporting method in appraising hepatic illness .

INTRODUCTION

Many chronic liver diseases including; fatty liver disease, alcoholism and viral hepatitis can induce liver cirrhosis (LC). Mostly cirrhotic patients remain without specific symptoms until reaching de compensation stage **[1]**.

Clinical evaluation of liver cirrhosis depends mainly on Child–Pugh score. However, the addition of subjective clinical criteria including ascites and hepatic encephalopathy restricted it. Model for end-stage liver disease (MELD) score, originally established to help predicting mortality in cirrhotic patients who undergo trans-jugular intrahepatic porto-systemic shunt procedures (TIPS). It was a good predictor of short-term mortality in cirrhotic patients. The main advantages of MELD score over the Child-Pugh score are being dependent on objective and accessible variables (serum creatinine, serum bilirubin, prothrombin time (PT) and international normalized ratio (INR) **[2].**

Ascites severity, degree of hepatic encephalopathy serum total bilirubin and serum albumin have been suggested to predict 90-day mortality independent of MELD score.

However, depending on subjective criteria such as severity of hepatic encephalopathy or ascites is considered unwanted **[3].**

Because liver is responsible for synthesis of lipoprotein and cholesterol, hepatocytes have important role in regulating the balance between lipid biosynthesis and transport **[4].**

Lipid metabolism is disturbed in cirrhosis and glycogen reserves are significantly decreased encouraging lipolysis and malnutrition [4]. Hepatitis C virus (HCV) infection can increase serum level of inflammatory cytokines and disturb endothelial function, therefore alter lipid metabolism **[5]**.

This study aimed to evaluate lipid profile in patients with hepatitis C virus induced liver cirrhosis.

SUBJECTS AND METHODS

Study design: Case control study.

Study setting: This study was completed in Tropical Department at Zagazig University hospitals and Al-Ahrar Teaching Hospital in the period from April 2021 to October 2021.

Sample size: This study included 63 patients. They were allocated into 2 groups.

Inclusion criteria:

1- Patients with HCV related liver cirrhosis diagnosed by clinical, laboratory and imaging modalities or histo-pathologically criteria in some cases.

2- Control group which was divided into two subgroups:

   a- Patients with chronic HCV infection and didn't fulfill the criteria of cirrhosis but they had elevated liver enzymes for more than 6 months with HCV markers (Antibodies and PCR).

   b- Healthy control.

Exclusion criteria:

Age <18 years old. Patients with non HCV related liver cirrhosis. Patients who had immune mediated inflammatory diseases. Patients who had malignancy including hepatocellular carcinoma. Patients who had primary affection of other organ. Patients with other diseases or drugs that could affect their the lipid metabolism.

Process: The following data were reported for all patients after obtaining an informed consent:

1. **Medical history taking.**
2. **Clinical examination:**

General examination with attention to liver cell failure manifestations including jaundice, flabbing tremors and edema of the lower limb.

Local abdominal examination searching for signs of chronic liver disease, liver cell failure and portal hypertension eg (Organomegally and ascites). Ascities could be; mild which could be detected only by ultrasonography, moderate which could be detected by positive bilateral shifting dullness or tense which showed positive transmitted thrill).

3. **Abdominal ultrasonography** :
4. **Laboratory investigations in form of :**
   - LFT, HCV Antibody and PCR.
   - Fasting serum lipid profile assessment of Serum total cholesterol , TG , HDL, LDL, VLDL.
5. **Child-Pugh classification of the patients**
6. **MELD score calculation**
7. **Upper GI endoscopy for cirrhotic patients.**

PRINCIPLE :

Cholesterol esters resolve in fatty acids and cholesterol by Cholesterol esterase (CHE).Then cholesterol become oxidized by Cholesterol Oxidase (CHOD) and release hydrogen peroxide and Cholesterol-3-one. Red dye compound become formed by a reaction between the released hydrogen peroxide and a phenol substitute. The red color intensity is directly proportional to the whole cholesterol in the sample when read at500/520nm

For the enzymatic determination of Triglycerides according to the following reaction:

$$\text{Triglycerides} + H_2O \xrightarrow{LPL} \text{Fatty acids} + \text{Glycerol}$$

$$\text{Glycerol} + \text{ADP} + \text{ATP} \xrightarrow{GK} \text{Glycerol-3-phosphate}$$

$$\text{Glycerol-3-phosphate} + H_2O_2 + O_2 \xrightarrow{GPO} \text{Di hydroxyl acetone phosphate}$$

$$2\,H_2O_2 + \text{4-AAP} + \text{4-CHLOROPHENIL} \xrightarrow{POD} \text{colored compound} + H_2O$$

The intensity of the red color formed is directly proportional to level of triglycerides in the sample

HDL-Cholesterol is formed through precipitation of VLDL lipoproteins and LDL, thus HDL lipoproteins persist in solution. HDL-Cholesterol in supernatant is considered as a sample for assay of cholesterol conferring to the next reaction:

$$\text{Cholesterol ester} \xrightarrow{\text{CEH}} \text{fatty acids} + \text{Cholesterol}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-ona} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{p-HBA} \xrightarrow{\text{POD}} \text{Colored Comp.} + 4H_2O$$

Formed color is assesed at 546 nm and is proportional to concentration of HDL-Cholesterol in sample.

SPECIMEN COLLECTION:

Non hemolyzed fresh serum or plasma.

Statistical Analysis

Statistical analysis was done using SPSS version 24. Quantitative continuous variables were represented as the mean± SD & range, and qualitative variables were represented as absolute frequencies (number) & relative frequencies (percentage).

ANOVA (F) test was used when comparing more than two groups of normally distributed data.

Tukey Post-hoc Least Significant Difference (LSD) test was used to compare between each 2 group separately.

Compared categorical data was done using Chi-square test (χ2test) and fisher exact test.

ROC curve was used.

All tests were two sided. P-value< 0.05 was statistically significant (S), p-value < 0.001 was highly statistically significant (HS), and p-value ≥ 0.05 was statistically insignificant (NS).

RESULTS

Age was distributed as 54.95 ± 2.39, 55.0 ± 2.02 and 55.52 ± 2.71 respectively with no significant difference also There was no statistically significant difference between the studied groups regarding sex distribution **Table (1)**.

There is statistically significant difference between the studied patient groups regarding presence of jaundice, hepatomegaly, splenomegaly, banded OV, ascites, lower limb edema and encephalopathy **Table (2)**.

There is statistically significant difference between the studied groups regarding total cholesterol and HDL cholesterol, TG and VLDL cholesterol., the difference is significant among groups except cholesterol and LDL as the difference is only between HCV and other two groups without significant difference between them **Table (3).**

The best cutoff of HDL cholesterol in diagnosis of presence of liver cirrhosis is ≤37.71 mg/dL with area under curve 0.745, sensitivity 71.4%, specificity 71.4%, positive predictive value 55.6%, negative predictive value 83.3%m accuracy 71.4% **Table (4) & figure (1)**.

The best cutoff of VLDL cholesterol in diagnosis of presence of liver cirrhosis is ≤21.423 mg/dL with area under curve 0.78, sensitivity 84.5%, specificity 75.0%, positive predictive value 63.3%, negative predictive value 90.2%m accuracy 78.5% **Table (5) & figure (2).**

This table shows Performance of all of lipid profile in diagnosis of presence of cirrhosis; ≤157.5, ≤152.9, ≤83.2 and ≤30.59 respectively with perfect validity **Table (6).**

Table (1): Comparison among studied groups regarding demographic data.

Parameter	Groups			Test	
	Chronic hepatitis C group	Cirrhotic group	Control group	F/ χ^2	P
	N=21 (%)	N=21 (%)	N=21 (%)		
Age (years): Mean ± SD	54.95 ± 2.39	55.0 ± 2.02	55.52 ± 2.71	0.36	0.69
Gender:					
Female	7 (33.3%)	7 (33.3%)	8 (38.1%)	0.14	0.933
Male	14 (66.7%)	14 (66.7%)	13 (61.9%)		

F: One way ANOVA test. χ^2: chi square test. *: P< 0.05 is statistically significant.
**: P≤ 0.001 is statistically highly significant.

Table (2): Comparison between the studied groups between presenting symptoms and signs.

	Group		Test	
	Chronic hepatitis C group N=21 (%)	Cirrhotic group N=21 (%)	χ^2/ Fisher	P
Jaundice	0 (0%)	6 (28.6)	4.598	<0.001**
Hepatomegaly	11 (52.4%)	4 (19.0%)	5.965	<0.001**
Splenomegaly	0 (0%)	19 (90.0%)	37.36	<0.001**
Upper GI, banded OV	0 (0%)	14 (66.7%)	31.69	<0.001**
LL edema	0 (0%)	14 (66.7%)	31.69	<0.001**
Ascites:				
No	21 (100%)	7 (33.3%)		
Mild	0 (0%)	0 (0.0%)	26.745	<0.001**
Moderate	0 (0%)	9 (42.9%)		
Severe	0 (0%)	5 (23.8%)		
Encephalopathy:				
No	21 (100%)	7 (33.3%)		
Grade 1	0 (0%)	1 (4.8%)		
Grade 2	0 (0%)	7 (33.3%)	29.63	<0.001**
Grade 3	0 (0%)	4 (19.0%)		
Grade 4	0 (0%)	2 (9.5%)		

χ^2: chi square test. *: p<0.05 is statistically significant. **: P≤ 0.001 is statistically highly significant.

Table (3): Comparison between the studied groups regarding lipid profile.

	Groups			Test	
	Chronic hepatitis c group Median (range)	Cirrhotic group Median(range)	Control group Median(range)	F	P
Cholesterol	290.67 ± 30.74	113.24 ± 47.18	107.69 ± 12.41	205.22	<0.001**
LSD	P_1 <0.001**	P_2 0.866	P_3 <0.001**		
Triglycerides	183.4 ± 10.47	100.41 ±± 50.26	139.57 ± 12.89	38.38	<0.001**
LSD	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		
HDL.C	39.63 ± 7.05	31.23 ± 8.85	46.14 ± 5.35	46.36	<0.001**
LSD	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		
LDL.C	156.86 ± 14.03	57.1 ± 33.65	57.18 ± 19.73	121.53	<0.001**
LSD	P_1 <0.001**	P_2 >0.999	P_3 <0.001**		
VLDL.C	36.6 ± 2.09	20.09 ± 10.03	27.21 ± 1.79	39.83	<0.001**
LSD	P_1 <0.001**	P_2 <0.001**	P_3 <0.001**		

F: One way ANOVA test. ***:** p<0.05 is statistically significant **: p≤0.001 is statistically highly significant.
P1: the difference between HCV and Cirrhosis groups P2: the difference between Cirrhosis and control groups.
P3: the difference between HCV and control groups.

Table (4): Performance of HDL in diagnosis of presence of cirrhosis,

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≤37.71	0.745	71.4%	71.4%	55.6%	83.3%	71.4%	0.002*

AUC: Area under curve. PPV: Positive predictive value. NPV: Negative predictive value.
*: p≤0.05 is statistically significant.

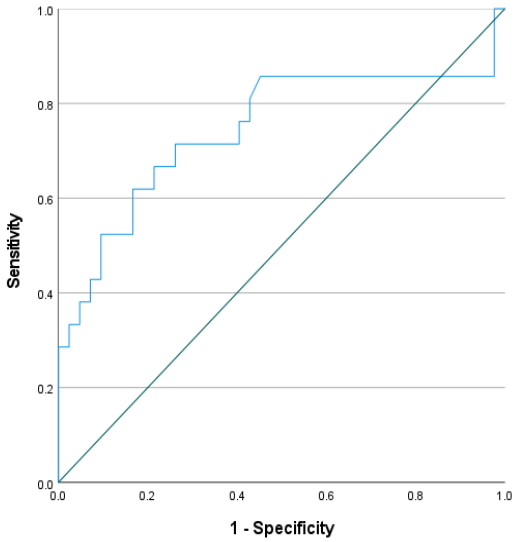

Figure (1): ROC curve showing performance of HDL cholesterol in diagnosis of presence of cirrhosis.

Table (5): Performance of VLDL in diagnosis of presence of cirrhosis.

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≤21.423	0.78	84.5%	75.0%	63.3%	90.2%	78.5%	<0.001**

AUC: Area under curve. PPV: Positive predictive value. NPV: Negative predictive value.
**: p≤0.001 is statistically highly significant.

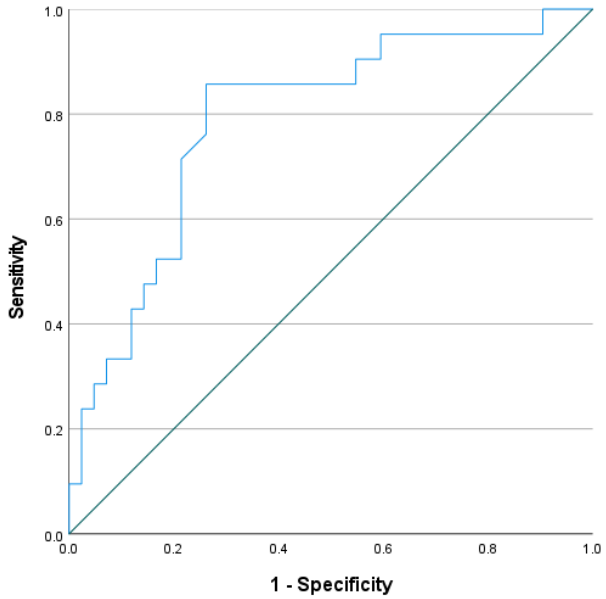

Figure (2) ROC curve showing performance of VLDL cholesterol in diagnosis of presence of cirrhosis.

Table (6): Performance of all of lipid profile in diagnosis of presence of cirrhosis.

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≤157.5	1.0	100.0%	100.0%	100.0%	100.0%	100.0%	<0.001**
≤152.9	1.0	100.0%	100.0%	100.0%	100.0%	100.0%	<0.001**
≤83.2	1.0	100.0%	100.0%	100.0%	100.0%	100.0%	<0.001**
≤30.59	1.0	100.0%	100.0%	100.0%	100.0%	100.0%	<0.001**

AUC: Area under curve. PPV: Positive predictive value. NPV: Negative predictive value.
**: p≤0.001 is statistically highly significant.
Significant AUC with cutoff regard Serum cholesterol, Serum triglycerides, LDL and VLDL ≤157.5, ≤152.9, ≤83.2 and ≤30.59 respectively with perfect validity.

DISCUSSION

Regarding the demographic data in the current study, age was distributed as 54.95 ± 2.39, 55.0 ± 2.02 and 55.52 ± 2.71 in chronic hepatitis C, cirrhotic, and control groups respectively with no significant difference. There was no statistically significant difference between the studied groups regarding gender distribution.

Yoo et al [1] conducted a study to verify the possible biomarkers for future development of liver cirrhosis. They used ultra-performance liquid chromatography (UPLC)-linear-trap quadrupole (LTQ)- Orbitrap mass spectrometry (MS) to measure the baseline serum metabolic profiles. The mean age of liver cirrhosis group was 43.8 years, 60.6% were males, and 39.4% were females. Regarding control group, the mean age was 45.2 years, 61.1% were males, and 38.9% were females. There was no significant difference between groups regarding age and gender. The cases in our study were older than these results.

Boemeke et al [6] reported a study on 150 patients to determine the association between reduced lipid profile and presented clinical outcome (pre transplantation death or liver transplantation). Regarding HCV cases, 62% of patients were males and the mean age was 63.1years.

Our findings regarding serum total cholesterol revealed that there was statistically significant increase in chronic HCV group in comparison with control group but no significant difference in LC group with mean value 113.24 in LC group. Regarding serum Triglycerides there is statistically significant increase in HCV group in comparison with control group and statistically significant decrease in LC group with mean value 100.41 in LC group. Regarding HDL there is statistically significant decrease in HCV group and statistically significant decrease in LC group in comparison with control group with mean value 31.23 in LC. Regarding LDL there is significant increase in HCV group and non-significant decrease in LC group with mean value 57.1 in LC. Regarding VLDL there is significant increase in HCV group and significant decrease in LC group with mean value in LC 20.09 .

Yoo et al [1] reported that after correcting for certain variables (sex, age, BMI, drinking status and smoking), waist circumference, body weight, blood pressure, blood glucose, triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol, and hs-CRP did not have any statistical significant difference between the LC group and control group. These results were in contradiction of the present results, this could be because their data were collected from National Health Insurance Scheme (NHIS), and they did not analyze the charts of the patients and performed uniform test for diagnosis of LC.

In accordance with the present study; except for triglycerides, **Chrostek et al [7]** reported that in LC group, the mean cholesterol level was 153.9 mg/dl, the triglycerides level was 75 mg/dl, the HDL level was 49.7 mg/dl.

Regarding HDL levels our results were agreed with **Chida et al [8]** as they found that the mean LDL-cholesterol and HDL-cholesterol levels were 83±27 mg/dl and 50±16 mg/dl, respectively.

Our results were in agreement with **Deb *et al* [9]** as they found that the mean cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol levels were 170.7 mg/dl, 99.9 mg/dl, 83±27 mg/dl, and 50±16 mg/dl, respectively.

Marzouk et al and Harrison et al [10,11] showed that the increased serum level of triglycerides ,LDL, HDL and total cholesterol are associated with improved rates of sustained virological response (SVR). However in accordance of our study **Vespasiani-Gentilucci et al [12]** showed that insulin resistance in HCV cases may be attributed to increased level of lipid profile and alterations of glucose metabolism.

Our study reported that the best cutoff of HDL cholesterol in diagnosis of presence of liver cirrhosis was ≤37.71 mg/dl with area under curve 0.745, sensitivity 71.4%, specificity 71.4%, positive predictive value 55.6%, negative predictive value 83.3% and accuracy 71.4%. The best cutoff of VLDL cholesterol in diagnosis of presence of liver cirrhosis is ≤21.423 mg/dl with area under curve 0.78, sensitivity 84.5%, specificity 75.0%, positive predictive value 63.3%, negative predictive value 90.2% and accuracy 78.5%. Significant AUC was recorded with cutoff regard Serum cholesterol, Serum triglycerides, LDL and VLDL ≤157.5, ≤152.9, ≤83.2 and ≤30.59 respectively with perfect validity.

A study done by **Jiang et al [13]** reported that TG, TC, HDL and LDL levels were reduced as

the MELD score was increased in decompensated cirrhotic patients. A TC level ≤108 mg/dl and a MELD score ≥ 21 were described to be independent prognostic factors of less than 1 year survival in cirrhotic patients.

Habib et al [14] studied 413 patients with liver cirrhosis due to different causes and described that within 1 year cirrhotic patients with HDL serum levels <30 mg/dl, had a higher need for liver transplantation. They also reported a significant association between biochemical markers (bilirubin, albumin and INR), serum level of HDL, and MELD score.

CONCLUSION

Lipid parameters may be considered as a supporting method in appraising hepatic illness. The amount of decrease in total cholesterol, HDL, LDL in cirrhotic patients is associated with progression of HCV related cirrhosis and incidence of cirrhosis related complications.

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Conflict of interest: None

Ethical considerations: The study was done with agreement from the Ethical Committee of Faculty of Medicine , Zagazig University , and an Institutional Review Board (IRB) was obtained . Written consent was obtained from all the patients or their parents. Privacy and confidentiality of the obtained data was insured for all participants**.**

.HIGHLIGHTS

- Because liver is responsible for synthesis of lipoprotein and cholesterol, hepatocytes have important role in regulating the balance between lipid biosynthesis and transport.
- This study assessed the changes in lipid profile in chronic hepatitis c virus patients and hepatitis c virus related cirrhosis .
- We correlated lipid profile changes including total cholesterol, HDL, LDL and triglycerides to the severity of hepatitis c virus related cirrhosis and the possible development of complications.

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