

Ascitic non-HDL-C/ HDL-C Ratio: A Novel Prognostic Marker in Liver Cirrhosis

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Background and study aim: Laboratory tests were developed on ascitic fluid for assessing causes, mechanisms, and prognosis of the diseases. This study aimed to evaluate the prognostic value of ascitic lipids in cirrhosis with special consideration to the non-high-density lipoprotein cholesterol (non-HDL-C) and its related ratios.

Methods: This cross-sectional study included 120 cirrhotic patients with ascites. Ascitic fluid analysis for lipid contents and calculations of ratios were done. Ascitic lipid contents and ratios are used to assess liver disease severity and predict the mortality in patients groups with different Child-Pugh classes.

Results: Regarding Child-Pugh score, ascitic cholesterol, and HDL-C showed significantly higher levels in group C

when compared to groups A and B. Ascitic non-HDL-C and ascitic non-HDL-C/ HDL-C ratio had a statistically significant increasing trend among Child-Pugh groups. Serum-ascites cholesterol gradient showed no significant difference among Child-Pugh groups ($p>0.05$). Ascitic non-HDL-C/ HDL-C ratio had the highest positive linear correlation with cirrhosis severity scores ($p<0.0001$). High ascitic non-HDL-C/ HDL-C ratio patients had a higher mortality rate when compared to those with a lower ratio (38.5% versus 9.5%).

Conclusion: Ascitic non-HDL-C/HDL-C ratio was more beneficial than non-HDL-C level in cirrhosis severity prediction. Also, it could predict the cirrhotic patients' survival. So, Ascitic non-HDL-C/HDL-C ratio seems to be an excellent prognostic marker for cirrhosis.

INTRODUCTION

Cirrhosis appears as a context of chronic disease affecting the liver and is described as architectural distortion that occurred due to liver fibrosis and scarring followed by the development of regenerative nodules [1, 2]. Until the development of de-compensation signs as ascites or encephalopathy, cirrhosis is considered compensated.

Ascites is a prevalent complication of cirrhosis and its presence indicates the development of portal hypertension [3]. In compensated cirrhosis patients, about 60% of them will be manifested by ascites in a 10 years period. It tends to be detected clinically at a volume exceeding 1.5 Liter limit [4]. The pathogenesis mechanisms of ascites include the vasodilatation of the splanchnic vessels which increases

portal blood flow and intrahepatic resistance, in addition to hypoalbuminemia and the presence of extracellular fluid and sodium retention due to renal vasoconstriction and activation of Renin Angiotensin Aldosterone System (RAAS) [5].

Many sub-classifications for ascites were developed. Regarding the ascitic total protein concentration at a cutoff value of 2.5 mg/dL, ascites can be distinguished into transudate or exudate [6]. Also, regarding serum-ascites albumin gradient (SAAG), it can be classified into portal hypertension-related ascites and non-portal hypertensive one (SAAG are ≥ 1.1 and < 1.1 gm/dL, respectively) [7].

Serum-ascites lipid gradient (SALG) was utilized to clarify ascites causes (cirrhosis, malignancy, and tuberculosis) [8]. Ascitic cholesterol level and SALG were elevated in malignant ascites [9]. Studies reported that SALG was able to distinguish cirrhotic ascites from both tuberculous and malignant ascites [10-13]. However, SALG levels were not associated with cirrhosis progress [11, 12].

As previous studies detected that the low-density lipoproteins were the most represented portion of the ascitic fluid lipid in cirrhotic patients [14, 15]. The aim of this study is to evaluate the prognostic value of ascitic lipids in cirrhosis with special consideration to the non-high-density lipoprotein cholesterol (non-HDL-C). This study objective was comparing the role of non-HDL-C and its related ratios in predicting the severity of cirrhosis and survival in a 1 year period. The inter-compartment lipid transfer was dependent on the particles size, this was the study hypothesis.

SUBJECT AND METHODS

Study design:

A prospective, cross-sectional study carried out in Tropical Medicine, Internal Medicine and Clinical Pathology Departments, Faculty of Medicine, Zagazig University. The study was conducted amid the period from October 2017 to February 2019. "Informed consent was obtained from all individual participants included in the study." Follow-up was evaluated every month for a 1 year period; to assess the disease outcome (mortality).

Subjects:

The sample size was estimated with 80% power and 95% confidence interval. A total number of 120 cirrhotic patients with ascites were enrolled. Ascites was diagnosed by clinical and ultrasound examinations. All patients were classified into 3 groups according to Child-Pugh classification (A, B and C). Although ascites usually occurs in the setting of advanced liver disease (Child-Pugh class B and C), ascites was also encountered in early cirrhotic patients (Child-Pugh class A) despite normal serum albumin in some cases e.g. nonalcoholic steatohepatitis and markedly increased portal and hepatic venous pressures. Patients with spontaneous bacterial peritonitis,

primary dyslipidemia, chronic renal failure, hypothyroidism, and patients on chemotherapy were excluded from this study (Figure 1).

Samples:

After 12 hours of fasting, samples were collected. Whole blood samples were taken by venipuncture into BD Vacutainer® plastic Citrate Tubes and plain tubes. Citrate tubes were centrifuged promptly at 1500 x g for 15 minutes, while plain tubes were permitted to clot for 30 minutes then were centrifuged at 1200 x g for 10 minutes. The ascitic fluid was collected before any intervention. Under aseptic conditions, ultrasonography guided abdominal paracentesis was done according to the standard guidelines technique [16]. The ascitic fluid was centrifuged as serum samples.

Methods:

Plasma was tested on the Sysmex® CA-1500 System (Siemens, Kobe, Japan) for an international normalized ratio of prothrombin time (INR) estimation. Serum levels of total bilirubin, albumin, creatinine, and sodium were tested. The serum and the ascitic fluid lipid profile [Cholesterol, Triglycerides, and HDL-C] were measured. All tests performed on the Cobas 8000 Modular Analyzer series [C702 Module for chemistry tests and ISE Module for serum sodium] (Roche Diagnostics, Mannheim, Germany). These kits were designed for the serum but could be used in the analysis of body fluids according to the results of the validation study [17].

Calculations:

Body mass index (BMI) was estimated using the equation [weight (kg)/height (m²)]. Non-HDL-C was calculated as [cholesterol - HDL-C]. Serum ascitic gradient was calculated using the formula [Serum level - Ascitic fluid level]. The Child-Pugh score was based on serum albumin, serum bilirubin, INR, ascites and hepatic encephalopathy [18]. The Model for End-Stage Liver Disease (MELD) score was based on serum bilirubin, INR and serum creatinine. MELD-Na is a modified score corrected to serum sodium [19].

Statistical Analysis

This study data were parametrically distributed. The one-way analysis of variance (ANOVA) was

used to evaluate the significant difference among groups, and then the Least Significant Difference was utilized as a post hoc test. The Pearson correlation coefficient was used to ascertain the linear correlation. Receiver operating characteristic (ROC) was plotted to assess the performance. The binary logistic regression analysis was used to calculate the odds ratio. The Kaplan–Meier curves were used to survey survival. For statistical evaluation, data were analyzed using SPSS "version 16" (SPSS Inc., USA). The statistical significance was proved when p -value < 0.05 .

RESULTS

Cirrhotic patients' basic characteristics are presented in Table 1. The Child-Pugh score and MELD-Na score, as predictors of cirrhosis severity, were calculated.

Ascitic fluid lipids were assessed. Ascitic triglycerides had no statistically significant difference among Child-Pugh groups. Ascitic cholesterol and HDL-C had significant higher levels in group C when compared to groups A and B. Ascitic non-HDL-C, ascitic non-HDL-C/HDL-C ratio and ascitic non-HDL-C/cholesterol ratio, had statistically significant increasing trends among Child-Pugh groups (Table 2). SALG had no significant difference among Child-Pugh groups ($p > 0.05$) as presented in Table 2.

The correlations of ascitic lipids with the factors that can predict the cirrhosis severity (Child-Pugh score and MELD-Na score) were presented in Table 3. Ascitic non-HDL-C/HDL-C ratio

had the highest positive linear correlation with cirrhosis severity scores ($r=0.89$ with Child-Pugh score, and $r=0.82$ with MELD-Na score) ($p < 0.0001$).

ROC curve analysis was used to test the ability of the ratio in predicting the cirrhosis severity as regards the Child-Pugh score, the area under the ROC of ascitic non-HDL-C/HDL-C ratio was 0.917 (Figure 2). The optimal cutoff value was set at 2.9, the ascitic non-HDL-C/HDL-C ratio had a sensitivity of 78.6%, a specificity of 100%, predictability for late grades of 100%, and predictability for the early grade of 66.7%.

In the Univariate analysis, ascitic non-HDL-C/HDL-C ratio can predict significantly the cirrhosis severity scores. It had an odds ratio of 6.66 [95% confidence interval (CI): 2.7-16.3] with Child-Pugh score ($p < 0.0001$). Also, it showed a significant odds ratio with MELD-Na score [1.5 (95% CI: 1.12 – 1.91)] ($p=0.001$). In the Multivariate Logistic Regression Test, the ratio was adjusted for age, sex, BMI, etiology, and duration of the disease. It had an adjusted odds ratio of 7.34 [95% CI: 2.64-20.4] with Child-Pugh score ($p < 0.0001$). Also, it showed a significant adjusted odds ratio with MELD-Na score [3.71 (95% CI: 1.2 – 11.3)] ($p=0.021$). So, ascitic non-HDL-C/HDL-C ratio was suggested as an independent predictor of cirrhosis severity.

The mortality rate was assessed in a one-year period; patients with high non-HDL C/HDL C ratio had 38.5% mortality rate, whereas it was 9.5% in patients with low ratio values. Cirrhotic patients with low non-HDL-C/HDL ratio had significantly longer survival than those with a high ratio ($p < 0.0001$) (Figure 3).

Table 1: Demographic, clinical and biochemical characteristics of the studied groups.

Parameter	Cirrhotic patients (No. = 120)
Age (years)	58 ± 8.5
Sex (Male/ Female)	64/56 (53.3/46.7)
Duration of disease	4.9 ± 3.2
Etiology	
• Hepatitis C	80 (66.6)
• Hepatitis B	12 (10)
• Hepatitis B & C	8 (6.7)
• Autoimmune	8 (6.7)
• Cryptogenic	12 (10)
BMI (Kg/m ²)	28.95 ± 3.13
INR	1.67 ± 0.55
Total bilirubin (mg/dL)	3.7 ± 2.79
Albumin (g/dL)	3 ± 0.64
Creatinine (mg/dL)	1.98 ± 1.25
Serum sodium (mmol/L)	138.65 ± 3.26
Serum cholesterol (mg/dL)	135.6 ± 27.9
Serum triglyceride (mg/dL)	103.2 ± 23.3
Serum HDL-C (mg/dL)	39.5 ± 8.43
Serum Non-HDL-C (mg/dL)	96.08 ± 23.2
Child-Pugh Grade	
• A	36 (30)
• B	30 (25)
• C	54 (45)
Child-Pugh score	9.2 ± 2.75
MELD-Na score	21.65 ± 7.6
1 year Mortality	34 (28.3)

No.: number of subjects; BMI: Body mass index; INR: International normalized ratio of prothrombin time; HDL-C: high-density lipoprotein cholesterol; MELD-Na: Model for End-Stage Liver Disease corrected to sodium

Data are presented as No. (%) or mean ± SD

Conversion for total bilirubin, albumin, and creatinine from mg/dL to SI (in $\mu\text{mol/L}$): multiply by 17.1, 15.2, and 88.4 respectively.

Conversion for cholesterol, and HDL-C from mg/dL to SI (in mmol/L): multiply by 0.0259.

Conversion for triglyceride from mg/dL to SI units (in mmol/L): multiply by 0.0113.

Table 2: Ascitic fluid lipid analysis results.

Parameter	Cirrhotic patients (No. = 120)	Child-Pugh A (No.=36)	Child-Pugh B (No.=30)	Child-Pugh C (No.=54)
Ascitic cholesterol (mg/dL)	35.77±10.97	28.53 ± 5.99	34.2 ± 10.18	41.5 ± 11.1 ^{a,b}
Ascitic triglyceride (mg/dL)	25.52±10.11	27.28 ± 8.74	23.12± 11.41	25.67±10.32
Ascitic HDL-C (mg/dL)	8.54±2.89	9.92 ± 3.2	9.3 ± 3.03	7.2±1.96 ^{a,b}
Ascitic non-HDL-C (mg/dL)	27.23±10.25	18.62±5.18	24.92±7.49 ^a	34.26±9.23 ^{a,b}
Ascitic non-HDL-C/ HDL-C ratio	3.46±1.38	2.06±0.75	2.77±0.62 ^a	4.79±0.55 ^{a,b}
Ascitic non-HDL-C/ cholesterol ratio	0.65 ± 0.48	0.65 ± 0.94	0.73 ± 0.41 ^a	0.83 ± 0.16 ^{a,b}
Serum ascitic cholesterol gradient (mg/dL)	99.9 ± 28.66	102.5±10.2	100.98±37.91	97.57±31.8
Serum ascitic triglyceride gradient (mg/dL)	75.5 ± 26.78	78.1±24.6	71.96±28.3	75.78±28.09
Serum ascitic HDL-C gradient (mg/dL)	31.29 ± 9.03	31.52±6.06	2.3±5.97	32.8±11.58
Serum ascitic Non-HDL-C gradient (mg/dL)	68.6 ± 23.51	70.97±8.38	72.68±32.55	64.77±24.69

No.: number of subjects; HDL-C: high-density lipoprotein cholesterol

Data are presented as mean ± SD

a: Significant difference compared with group A

b: Significant difference compared with group B

Conversion for cholesterol, and HDL-C from mg/dL to SI (in mmol/L): multiply by 0.0259.

Conversion for triglyceride from mg/dL to SI units (in mmol/L): multiply by 0.0113.

Table 3: Correlation between cirrhosis scores and ascitic fluid lipids.

Parameter	Child-Pugh Correlation		MELD-Na Correlation	
	R	p	R	p
Ascitic cholesterol	0.47	<0.0001*	0.40	0.001*
Ascitic HDL-C	-0.42	0.001*	-0.43	0.001*
Ascitic non-HDL-C	0.62	<0.0001*	0.55	<0.0001*
Ascitic non-HDL-C/ HDL-C ratio	0.89	<0.0001*	0.82	<0.0001*
Ascitic non-HDL-C/ cholesterol ratio	0.78	<0.0001*	0.73	<0.0001*

r: Correlation coefficient; MELD-Na: Model for End-Stage Liver Disease corrected to sodium
HDL-C: high-density lipoprotein cholesterol

* Significant

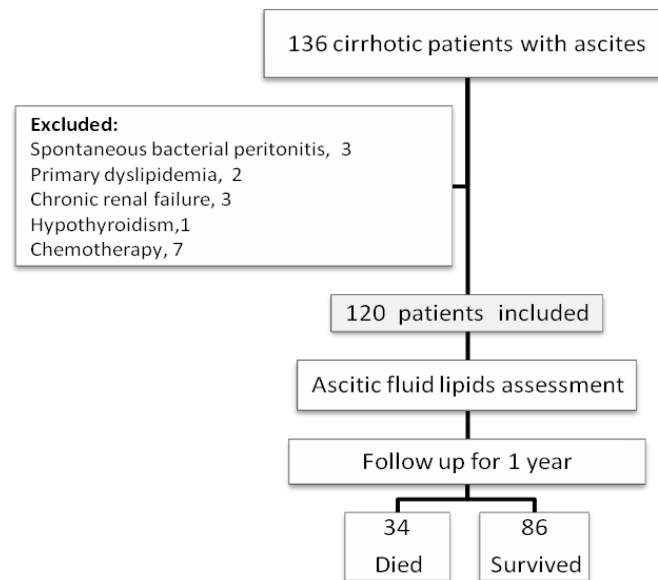


Figure 1: This study flowchart.

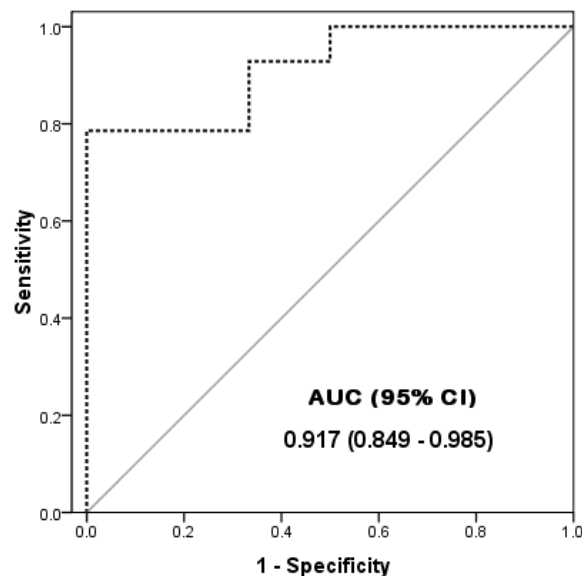


Figure 2: Receiver operating characteristic (ROC) curve of ascitic non-HDL-C/ HDL-C ratio. AUC: Area under curve; CI: Confidence interval.

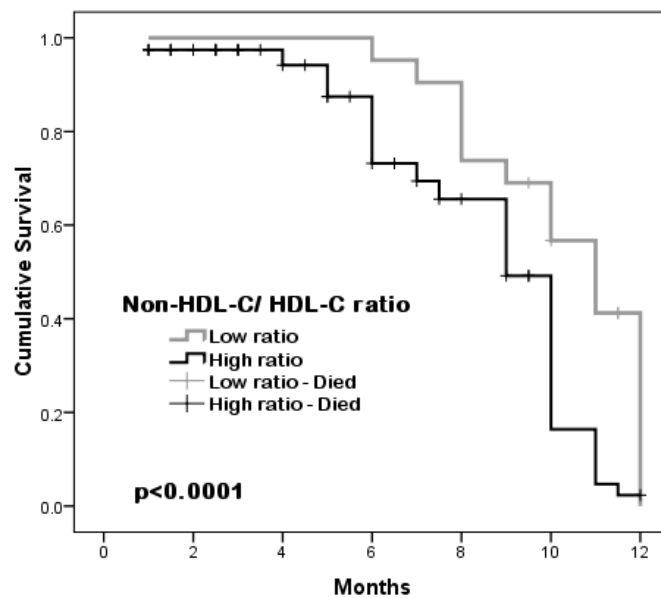


Figure 3: Kaplan–Meier survival curves according to the different levels of non-HDL-C/ HDL ratio in cirrhotic patients.

DISCUSSION

Cirrhosis is the terminal sequence of chronic liver diseases. The highest incidence of mortality from cirrhosis was reported in Egypt which makes it an essential field of investigation [20]. The ascites is a prevalent event in cirrhotic patients [21]. Laboratory tests were developed on ascitic fluid for assessing causes, mechanisms, and prognosis of the disease as well as the response to treatment [22].

Previously, the serum lipid profile was reported to be decreased in cirrhotic patients with ascites. This finding was explained by the reduction of their biosynthesis by cirrhotic liver [23, 24]. This reduction in serum lipid profile was reported to be a characteristic criterion for cirrhotic ascites, as it was able to distinguish it from other causes of ascites [8]. Ascitic lipid profile was also evaluated in ascites of different causes [25–29]. This study aimed to evaluate the ascitic lipid profile in liver cirrhosis as an attempt to find a reliable prognostic biomarker. To our knowledge, this is the first study investigating the role of ascitic non-HDL-C and its related ratios in the assessment of liver cirrhosis prognosis. In agreement with previous studies [13, 14], this study confirms that the inter-compartment lipid transfer was dependent on the particles size.

The ascitic lipid profile role in cirrhosis severity evaluation was assessed by evaluating each

parameter levels as regards the Child-Pugh stages. This study found that ascitic triglyceride had similar values in different Child-Pugh groups. Ascitic cholesterol and HDL-C had significantly different values in group C when compared to groups A and B. In group C patients, despite increased ascitic cholesterol, the HDL-C was decreased. Regarding values of ascitic non-HDL-C, ascitic non-HDL-C/ HDL-C ratio and ascitic non-HDL-C/ cholesterol ratio, each showed an increasing trend among different Child-Pugh groups.

SALG was previously introduced as a simple screening test for the differentiation of various causes of ascites [8, 10–13]. This study showed that SALG levels had no significant difference among Child-Pugh groups. Consistently, Morsy et al. and Dubey et al. reported that SALG was non-significantly correlated with cirrhosis severity [11, 12]. Although they found that SALG had different values among different Child-Pugh groups, this difference was of no statistical significance.

Hypocholesterolemia was significantly associated with Child-Pugh score and with MELD [30, 31]. This study revealed that high cholesterol levels in the ascitic fluid were correlated with (Child-Pugh and MELD-Na scores). Although total cholesterol and its fractions in the ascitic fluid may predict the cirrhosis severity, ascitic non-HDL-C/ HDL-C ratio had the highest direct correlation with both

severity scores. Ascitic non-HDL-C/ HDL-C ratio may be considered as an independent predictor of cirrhosis severity. It had significant adjusted odds ratios of 7.3 and 3.7 with Child-Pugh and MELD-Na scores respectively. The ascitic non-HDL-C/ HDL-C ratio had a perfect specificity (100%) with considerable sensitivity (78.6%) for the differentiation between early and late Child-Pugh grades.

This study found that cirrhotic patients with high ascitic non-HDL-C/ HDL-C ratio patients had a higher mortality rate when compared to those with a lower ratio (38.5% versus 9.5%). Ascitic non-HDL-C/ HDL ratio seems to be a predictor for cirrhotic patients' survival.

Although the Child-Pugh score is the most utilized prognostic score in cirrhosis, it has some limitations. Firstly, it depends on some chosen factors and other important factors have not been considered. Additionally, it includes subjective factors as ascites and encephalopathy [32]. Likewise, MELD score has a limitation as it incorporates some factors which are affected by other conditions as sepsis, hemolysis, and diuretics usage [33]. In this way, looking for a novel prognostic marker is an important issue. This study suggests that ascitic non-HDL-C/HDL-C ratio seems to be a prognostic parameter for cirrhosis.

Few limitations met this study. It was a cross-sectional study performed on a relatively small sample size in a single center. Also, the follow-up time was short for prognosis assessment. So, further studies are recommended on a larger sample size to validate this study results. Also, studies are required to evaluate this ratio applicability in ascites of other etiologies.

CONCLUSION

Ascitic non-HDL-C/HDL-C ratio was more beneficial than non-HDL-C in cirrhosis severity prediction. Also, it could predict the cirrhotic patients' survival. So, Ascitic non-HDL-C/HDL-C ratio seems to be an excellent prognostic marker for cirrhosis.

Abbreviations:

BMI: Body mass index

HDL-C: High-density lipoprotein cholesterol

INR: International normalized ratio of prothrombin time

MELD-Na: Model for End-Stage Liver Disease corrected to sodium

ROC: Receiver operating characteristic

SAAG: Serum-ascites albumin gradient

SALG: Serum-ascites lipid gradient

Ethical approval: This study was conducted according to the principles expressed in the Declaration of Helsinki and the protocol was inspected and approved by the Faculty of Medicine (Zagazig University) Institutional Review Board.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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