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High-density lipoprotein cholesterol and systemic arterial hypertension are associated with hepatic necroinflammatory activity in patients with chronic hepatitis C

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HIGLIGHTS

- HDL cholesterol levels <60 mg/dL were independently associated with necroinflammatory activity in chronic hepatitis C (CHC).
- CHC patients with hypertension are at an increased risk of developing necroinflammatory activity.
- In patients with CHC, liver fibrosis was independently associated with old age, steatosis, and HDL-C <60 mg/dL.
- Triglycerides levels ≥150 mg/dL were associated with lobular inflammatory activity in patients with CHC.

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ABSTRACT – Background – Approximately 71 million people are chronically infected with hepatitis C virus (HCV) worldwide. A significant number of these individuals will develop liver cirrhosis and/or hepatocellular carcinoma. Beyond the liver, there is a sizeable body of scientific evidence linking cardiovascular disease and chronic hepatitis C (CHC); however, the biological mechanisms behind the concurrence of these conditions have not been completely clarified yet. **Objective –** To evaluate associations between hepatic histology, clinical comorbidities and lipid profile in patients with CHC. To investigate associations between liver histology and demographic, nutritional, biochemical and virological parameters. **Methods –** Eight-five patients with CHC prospectively underwent hepatic biopsy. Liver fragments were obtained from each patient by percutaneous route using a Menghini needle. Fibrosis was evaluated according to the METAVIR scoring system, as follows: F0, no fibrosis; F1, fibrous portal expansion; F2, fibrous portal widening with few septa; F3, bridging fibrosis with architectural distortion; and F4, liver cirrhosis. The activity was classified based on the degree of lymphocyte infiltration and hepatocyte necrosis, from A0 to A3. The diagnosis of liver disease was based on clinical, biochemical, histological, and radiological methods. The data were analyzed by logistic regression models. **Results –** This cross-sectional study included 85 outpatients followed at the tertiary care ambulatory centre with a mean age of 57.2±10.7 years and 45 (52.9%) were females. There were 10 patients with cirrhosis. Patients with a

METAVIR F3-F4 were significantly older (*P*=0.02) and had higher levels of ALT (*P*=0.0006), AST (*P*<0.0001), γ-GT (*P*=0.03) and bilirubin (*P*=0.001) and higher prothrombin time than patients with F0-F2 score. Albumin levels (*P*=0.01) were significantly lower in METAVIR F3-F4. Age (OR=1.09; 95%CI=1.02–1.16; *P*=0.02), steatosis (OR=4.03; 95%CI=1.05–15.45; *P*=0.04) and high-density lipoprotein cholesterol (HDL-C) <60 mg/dL (OR=7.67; 95%CI=1.71–34.49; *P*=0.008) were independently associated with fibrosis. Hypertension (OR=6.36; 95%CI=1.31–30.85; *P*=0.02) and HDL-C <60 mg/dL (OR=9.85; 95%CI=2.35–41.39; *P*=0.002) were independently associated with necroinflammatory activity. Hypertension (OR=6.94; 95%CI=1.92–25.05; *P*=0.003) and HDL-C <60 mg/dL (OR=3.94; 95%CI=1.27–12.3; *P*=0.02) were associated with interface inflammatory activity. Triglycerides (TG ≥150 mg/dL) remained associated with lobular inflammatory activity. **Conclusion –** HDL cholesterol levels <60 mg/dL were independently associated with necroinflammatory activity in chronic hepatitis C. Patients with hypertension are at an increased risk of developing necroinflammatory activity.

Keywords – Hepatitis C virus; chronic hepatitis C; diabetes mellitus; hypertension; HDL cholesterol; liver histology.

INTRODUCTION

Blood screening for hepatitis C virus (HCV) and the revolutionary development of direct-acting antivirals (DAAs) have successfully saved millions of lives[1-3]. Although sustained virological response rates (SVR) linked to DAAs prescription have surpassed 95.0%[4,5], it is important to bear in mind that there are still various barriers to attain the World Health Organization (WHO) HCV elimination targets by 2030[6,7]. Among these obstacles, a truly universal and inclusive healthcare system, an outstanding screening to detect a submerged portion of patients chronically infected with HCV[8], the expensive DAAs therapy[9] and coronavirus disease 2019 (COVID-19) should be highlighted[10].

The Global hepatitis report 2017 estimated that 71 million people are chronically infected with HCV around the world[11]. A significant number of these individuals will develop liver cirrhosis and/or hepatocellular carcinoma after approximately two to three decades of viral infection[12]. Additionally, several comorbidities have been associated with HCV infection, including mixed cryoglobulinemia vasculitis, lymphoproliferative disorders, renal disease, rheumatoid arthritis-like polyarthritis, sicca syndrome, type 2 diabetes mellitus, insulin resistance and atherosclerosis[13,14].

In the metabolic disorder scenario, several studies demonstrated that the replication cycle of HCV is strongly linked to the lipid metabolism pathway in hepatocytes[15-19]. The development of enveloped viral particles relies on hepatic very-low-density lipoprotein (VLDL) assembly, and this process originates numerous circulating lipoviral particles, which are generated by a fusion of virions and lipoproteins[15-20]. Therefore, the lipid haemostasis is completely modified in patients with chronic hepatitis C (CHC) and this metabolic dysregulation might increase the risk of cardiovascular disease among HCV-infected persons[21-28].

Impaired glucose tolerance and type 2 diabetes mellitus have been frequently diagnosed in patients with chronic HCV infection independently of the hepatic disease severity[29-31]. A recent meta-analysis showed that individuals chronically infected with HCV were at increased risk of cardiovascular disease-related morbidity and mortality, especially patients with type 2 diabetes mellitus and hypertension[32]. On other hand, in patients with CHC, cirrhosis was associated with atherosclerosis independently of cardiovascular risk factors[33]. Furthermore, abnormalities in lipid metabolism and subsequent hepatic steatosis are reported to be risk factors for the progression of liver fibrosis in these patients[34,35].

Although there is a sizeable body of scientific evidence linking hepatitis C with an increased risk of cardiovascular disease, the biological mechanisms behind the concurrence of these conditions have not been completely clarified yet. Hence, we hypothesized that host factors, including clinical comorbidities and lifestyle data, have an influence on liver histopathological features. The present study aims to evaluate associations between hepatic histological findings

and type 2 diabetes mellitus, hypertension, and lipid profile in patients with CHC. We also aim to investigate associations between liver histology and demographic, nutritional, biochemical, and virological variables in patients with CHC.

METHODS

Study population and design

This prospective cross-sectional study included 112 consecutive outpatients (aged >18 years) with confirmed CHC diagnosis attending the Viral Hepatitis Outpatient Clinic, University Hospital, Belo Horizonte, Brazil, from 2017 to 2020. Each patient met the study's inclusion criteria for CHC, as confirmed by the presence of anti-HCV antibodies and HCV-RNA.

The Viral Hepatitis Outpatient Clinic is an outpatient care ambulatory of a metropolitan tertiary teaching hospital that admits patients for the treatment of chronic viral hepatitis. All patients signed the informed consent form. The study was designed and conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of the Federal University of Minas Gerais/UFMG (ETIC 0404.0.203.000-10; CAAE: 61481116.0.0000.5149).

All patients were examined for other hepatic diseases. The exclusion criteria were: pregnant or breastfeeding patients; presence of hepatic encephalopathy; hepatitis B virus (HBV)/HCV or HCV/human immunodeficiency virus (HIV) coinfection; current use of antiviral drugs, corticosteroids and statins or fibrates. Patients, who had causes of liver disease other than HCV infection and advanced diseases such as chronic kidney disease, heart failure, chronic pulmonary disease, and neoplasia, including hepatocellular carcinoma (HCC), were also excluded. All patients had undergone percutaneous needle liver biopsy. Patient was excluded from analysis if the biopsy were fragmented or had <6 portal tracts.

Twenty-seven patients were not included because five refused to participate, 16 were using hypolipemiant drugs as statins or fibrates and six had inadequate liver biopsy samples for histological assessment. Eighty-five outpatients with CHC remained in the study. The participants were from a similar socioeconomic level, as assessed by a previously validated questionnaire[36] based on income and educational level. All subjects were natives of Minas Gerais, a state with the following ethnic background, 33.0% of Portuguese ancestry, 33.0% of Amerindian ancestry, and 33.0% of African ancestry homogeneously present in each patient, irrespective of their phenotype[37].

Following the latest global guidelines: the European Association for the Study of the Liver (EASL)[38] and the American Association for the Study of Liver Diseases (AASLD)[39], the diagnosis of cirrhosis was based on clinical, biochemical, radiological and histological parameters[40]. The Child-Turcotte-Pugh[41] and AST to platelet ratio index (APRI)[42] scores were calculated for each participant based on medical data.

Histology

Liver fragments were obtained from each patient by percutaneous route using a Menghini needle. The specimens were 10.0% formalin-fixed and paraffin-embedded. A 3 µm-thick liver section was routinely stained with hematoxylin and eosin, Gomori's trichromic and sirius red method and the slides were blindly examined by one pathologist (GHDPS).

Fibrosis was evaluated according to the METAVIR scoring system[43], as follows: F0, no fibrosis; F1, fibrous portal expansion; F2, fibrous portal widening with few septa; F3, bridging fibrosis with architectural distortion; and F4, liver cirrhosis. Activity was classified based on the degree of lymphocyte infiltration and hepatocyte necrosis, from A0 to A3[43]. The degree of mononuclear and polymorphonuclear cell infiltration in the portal, periportal, lobular and perivenular regions was separately graded from 0 to 3 (0, absent; 1, mild; 2, moderate; 3, marked). The grading and staging of fatty liver were defined using criteria proposed by Brunt et al. for histological lesions[44]. To control biopsy size, each biopsy length was measured with a hand ruler, and the number of portal areas on one cross-section was counted[45,46].

Laboratory parameters

Following a 12-hour fasting, venous blood samples were obtained from all participants. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (γ-GT), alkaline phosphatase (ALP), albumin, total bilirubin, prothrombin activity (PA), creatinine, glycaemia, tri-

glycerides (TG), total cholesterol and high-density lipoprotein cholesterol (HDL-C) were evaluated by routine laboratory methods. Very low-density lipoprotein (VLDL-C) and low-density lipoprotein (LDL-C) were calculated by using Friedwald's equation.

Antibody to HCV was investigated by a commercial ELISA (AxSYM HCV, version 3.0; Abbott GmbH & Co., Wiesbaden, Germany), and a qualitative PCR confirmed HCV status for HCV RNA (AMPLICOR 2.0 assay Roche Diagnostics, Branchburg, NJ) according to the manufacturer's instructions. HCV genotyping and viral load were determined by using a commercial test (Cobas Taq Man HCV test V.2.0; Roche Molecular Systems, Pleasanton, CA) and a line probe assay (VERSANT HCV genotyping assays; Bayer's Diagnostic Corporation, Tarrytown, NY), respectively. The assays were performed according to the manufacturer's recommendations. Viral load and HCV genotyping were accessible in 75 (88.2%) of the included patients.

Alcohol consumption assessment

The current and previous history of alcohol use was assessed. Particularly related to alcohol use, the cut-off scores were based upon a low-risk drinking level of no more than 20 grams of alcohol per day, 5 days per week, or a weekly level of no more than 100 grams[47].

Clinical comorbidities

Hypertension was diagnosed according to the 2016 European Guidelines on cardiovascular disease prevention in clinical practice[48]. Diabetes mellitus (DM) was defined using the 2014 American Diabetes Association Guidelines diagnosis and classification of DM[49]. Dyslipidaemia was diagnosed according to the 2013 American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA) guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the ACC/AHA[50].

Anthropometry assessment

The weight and height were measured with a mechanical platform scale (FILIZOLA®, São Paulo, Brazil). The body mass index (BMI) was calculated using the formula BMI = weight/height^2.

Statistical analysis

Data were analysed with IBM SPSS (Armonk, NY: IBM Corp.) statistical software package version 26.0. Descriptive statistics provided demographic, clinical, alcohol consumption, nutritional, and biochemical data. The Shapiro-Wilk test was used to evaluate whether the data were normally distributed. The asymptotic Pearson's chi-square test and Fisher's exact test were used to compare the percentages. The Mann-Whitney U and, for means, the Student's *t*-test or ANOVA were used to compare medians. For comparison between serum concentration of HDL-C and grade and staging of histological findings, the Kruskal-Wallis test was used.

Pearson's or Spearman's correlation was used to analyse the strength of the association between HDL-C serum levels, triglycerides/HDL-C ratio (TGL/HDL-C) and hepatic necroinflammatory activity.

Associations between histological features [Model 1, Fibrosis (F0–F2 vs F3–F4); Model 2, hepatic necroinflammatory activity; Model 3, interface hepatitis; Model 4, lobular necrosis] and the following variables: demographic (sex, age), clinical comorbidities (type 2 diabetes mellitus and hypertension), alcohol consumption data [alcohol use (<20 g/day vs ≥20 g/day)] and biochemical data [HDL-C (<60 mg/dL vs ≥60 mg/dL) and triglycerides (<150 mg/dL vs ≥150 mg/dL)] were evaluated by univariate analysis. All variables with *P* values <0.25 were included in the entire models of logistic regression. Odds ratio (OR) and 95%CI were used to estimate the risk. The Hosmer-Lemeshow test was used to assess the adequacy of the models. Variables with more than missing data >10% were not selected for the multivariate analysis models. The level of significance was set at *P* values ≤0.05.

RESULTS

Characteristics of the patients

Patients with advanced liver fibrosis (Metavir score, F3 or F4) were signifcantly older and had significantly higher levels of ALT, AST, γ-GT and bilirubin, as well as higher prothrombin time than patients with score F0–F2. Otherwise, albumin levels were significantly lower in the former. (TABLE 1).

TABLE 1. Demographic, clinical, alcohol consumption, biochemical and virological data of patients with chronic hepatitis C according to the stage of liver fibrosis (n=85)[1].

Variables	Fibrosis stage (F0–F2) n=62	Fibrosis stage (F3–F4) n=23	*P* value
Demographics			
Sex			
Male n (%)	29 (46.8)	11 (47.8)	0.93
Female n (%)	33 (53.2)	12 (52.2)	
Age (years)[a]	55.5±11.0	61.8±8.9	0.02
Body mass index (kg/m^2)[a]	26.4±4.2	26.0±2.9	0.68
Comorbidities n (%)			
Type 2 diabetes mellitus	13 (21.0)	6 (26.1)	0.62
Hypertension	23 (37.1)	12 (52.2)	0.21
Steatosis	38 (61.3)	19 (82.6)	0.07
Alcohol consumption data n (%)			
Current alcohol use (>20 g ethanol/day)	12/60 (20.0)	2/22 (9.1)	0.33
Past alcohol use	52/60 (86.7)	20/22 (90.9)	0.72
Biochemical and haematological data			
Albumin (g/dL)[a]	4.±0.4	3.9±0.6	0.002
ALT(U/L)[b]	46.0 (38.0–78.0)	79.5 (53.8–132.0)	0.006
AST (U/L)[b]	43.0 (32.0–66.0)	78.5 (64.8–118.5)	<0.0001
ALP (U/L)[b]	88.0 (70.8–126.5)	114.0 (81.8–176.0)	0.10
γ-GT (U/L)[b]	57.0 (30.0–106.0)	84.5 (50.8–200.8)	0.03
Total bilirubin (mg/dL)[b]	0.70 (0.50–0.85)	1.10 (0.60–1.79)	0.001
Prothrombin time (seconds)[b]	14.1 (13.3–14.7)	15.8 (13.0–17.4)	0.01
Glycaemia (mg/dL)[b]	91.0 (84.0–110.5)	91.5 (84.3–104.3)	0.90
Triglycerides (mg/dL)[b,c]	85.0 (69.0–142.5)	87.0 (70.0–115.5)	0.29
Total cholesterol (mg/dL)[b,c]	175.0 (146.0–200.5)	156.0 (139.0–188.5)	0.14
Non-HDL cholesterol (mg/dL)[b,c]	117.0 (87.0–143.5)	111.0 (93.5–130.5)	0.51
HDL cholesterol (mg/dL)[b,c]	55.0 (44.0–69.0)	48.0 (41.5–57.3)	0.23
Virological parameters[d]			
HCV-RNA log10 (IU)/mL)[b]	5.9 (5.4–6.3)	5.7 (5.5–6.2)	0.67
Genotype 1 n (%)	42/53 (79.2)	20/22 (90.9)	0.32

1: the french METAVIR; Cooperative Study Group[31]. F: fibrosis stages; ALT: alanine aminotransferase; ALP: alkaline Phosphatase; AST: aspartate aminotransferase; γ-GT: gama-glutamyltransferase; HCV: hepatitis C virus; RNA: Ribonucleic Acid. ; a: mean ± standard deviation; b: Median and interquartile range (IQR), 25th–75th percentile; c: data from 78 subjects (91.8%); d: data from 75 subjects (88.2%). *P* values ≤0.05 were considered significant. Pearson's chi-square test and Fisher's exact test were used to compare categorical variables. The t test and Mann-Whitney U test were used for comparison of normally and not normally distributed continuous variables, respectively.

Overweight, type 2 diabetes mellitus and hypertension were associated with hepatic necroinflammatory activity (TABLE 2), Additionally, increased levels of ALT, AST, bilirubin, triglycerides and decreased levels of HDL-C were associated with liver inflammation and necrosis (TABLE 2).

Histological findings and HDL-C cholesterol

It is well defined that HDL-C levels ≥60 mg/dL and <40 mg/dL are associated with decreased and increased risk of cardiovascular diseases (CVD), respectively[51-54]. Thus, taking into account that the me-

TABLE 2. Demographic, clinical, alcohol consumption, biochemical and virological data of the included patients according to the liver necroinflammatory activity (n=85)[1].

Variables	Without hepatic necroinflammatory activity (A0) n=18	With hepatic necroinflammatory activity (A1–A3) n=67	*P* value
Demographics			
Sex			
Male n (%)	9 (50.0)	31 (46.3)	0.78
Female n (%)	9 (50.0)	36 (53.7)	
Age (years)[a]	56.1±8.6	57.5±11.3	0.61
Body mass index (kg/m^2)[a]	24.5±4.5	26.8±3.6	0.02
Comorbidities n (%)			
Type 2 diabetes mellitus	1 (5.6)	18 (26.9)	0.05
Hypertension	3 (16.7)	32 (47.8)	0.02
Steatosis	9 (50.0)	48 (71.6)	0.08
Alcohol consumption data n (%)			
Current alcohol use (>20 g ethanol/day)	6 (33.3)	8 (12.5)	0.04
Past alcohol use	17 (94.4)	55 (85.9)	0.33
Biochemical and haematological data			
Albumin (g/dL)[b]	4.4 (4.1–4.7)	4.3 (4.1–4.5)	0.15
ALT(U/L)[b]	44.0 (28.0–49.0)	69.0 (44.0–95.0)	<0.001
AST (U/L)[b]	36.0 (28.5–46.5)	65.0 (40.0–86.0)	<0.001
ALP (U/L)[b]	88.0 (65.5–172.0)	105.0 (73.0–142.0)	0.61
γ-GT (U/L)[b]	43 (23.1–129.5)	71.0 (38.0–125.0)	0.10
Total bilirubin (mg/dL)[b]	0.60 (0.40–0.70)	0.79 (0.51–1.21)	0.009
Prothrombin time (seconds)[b]	14.1 (13.2–14.6)	14.2 (13.0–15.4)	0.51
Glycaemia (mg/dL)[b]	89.0 (78.0–97.0)	91.0 (85.0–114.0)	0.07
Triglycerides (mg/dL)[b,c]	80.0 (61.0–112.5)	87.0 (69.0–142.0)	0.02
Total cholesterol (mg/dL)[b,c]	175.0 (146.5–187.5)	163.0 (140.0–201.0)	0.99
Non-HDL cholesterol (mg/dL)[b,c]	104.0 (83.8–132.3)	116.0 (91.5–142.0)	0.20
HDL cholesterol (mg/dL)[b,c]	61.0 (45.0–86.5)	51.0 (41.0–58.0)	0.02
Virological parameters[d]			
HCV-RNA log10 (IU)/mL)[b]	5.69 (5.33–5.99)	5.97 (5.63–6.32)	0.06
Genotype 1 n (%)	14 (82.4)	48 (82.8)	0.97

1: the french METAVIR Cooperative Study Group[31]. A: METAVIR necroinflammatory grading; ALT: alanine aminotransferase; ALP: alkaline Phosphatase; AST: aspartate aminotransferase; γ-GT: gama-glutamyltransferase; HCV: hepatitis C virus; RNA>: Ribonucleic Acid. a: mean ± standard deviation; b: median and interquartile range (IQR), 25th–75th percentile; c: data from 78 subjects (91.8%); d: data from 75 subjects (88.2%). *P* values ≤0.05 were considered significant. Pearson's chi-square test and Fisher's exact test were used to compare categorical variables. The *t* test and Mann-Whitney U test were used for comparison of normally and not normally distributed continuous variables, respectively.

dian of HDL-C in the study population without necroinflammatory activity (A0; n=18) was 61.0 mg/dL, we divided the patients into two groups (HDL-C <60 mg/dL and ≥60 mg/dL) to evaluate associations of the variable with histological findings (TABLE 3).

Because recently, non-HDL-C was shown to be a better predictor of cardiovascular disease (CVD) death than LDL-C, we included non-HDL-C values instead of LDL-C in the statistical analyses. Liver necroinflammatory activity score, portal inflammatory infiltrate portal neutrophils and lymphocytes, periportal inflammatory infiltrates, periportal lymphocytes and lobular neutrophils were significantly associated with HDL-C <60 mg/dL (TABLE 3).

TABLE 3. Histological findings in the liver of patients with chronic hepatitis C with and without HDL-C cholesterol <60 mg/dL (n=78).

Histological findings[1,2]	HDL-C cholesterol ≥60 mg/dL n=25	HDL-C cholesterol <60 mg/dL n=53	*P* value
Liver fibrosis grade [F0 to F4, n (%)][1]	2 (8.0)/ 13 (52.0)/ 7 (28.0)/ 0 (0.0)/ 3 (12.0)	1 (1.9)/ 22 (41.5)/ 12 (22.6)/ 13 (24.5)/ 5 (9.4)	0.07
Necroinflammatory activity grade [A0 to A3, n (%)][2]	11 (44.0)/ 13 (52.0)/ 1 (4.0)/0 (0)	7 (13.20)/ 38 (71.7)/ 8 (15.1)/0 (0)	0.008
Steatosis grade [1, ≤33%; 2, >33% to <66%; 3, ≥6%][3]	6 (24.0)/ 19 (76.0)/ 0 (0.0)	20 (37.7)/ 31 (58.5)/ 2 (2.6)	0.26
Inflammatory infiltrate grade [absent n (%)/mild n (%)/moderate n (%)/marked n (%)][4]			
Portal inflammatory infiltrate n (%)	0 (0.0)/ 14 (56.0)/ 11 (44.0)/ 0 (0.0)	0 (0.0)/ 18 (34.0)/ 28 (52.8)/ 7 (13.2)	0.04
Cells' type [absent n (%)/mild n (%)/moderate n (%)/marked n (%)][4]			
Neutrophils	17(68.0)/ 8 (32.0)/ 0 (0.0)/ 0 (0.0)	23(43.4)/ 30 (56.6)/ 0 (0.0)/ 0 (0.0)	0.04
Lymphocytes	0 (0.0)/ 14 (56.0)/ 11 (44.0)/ 0 (0.0)	0 (0.0)/ 18 (34.0)/ 27.0 (50.9)/ 8 (15.1)	0.05
Histiocytes	23 (92.0)/ 2 (8.0)/ 0 (0.0)/ 0 (0.0)	44 (83.0)/ 9 (17.0)/ 0 (0.0)/ 0 (0.0)	0.29
Periportal inflammatory infiltrate n (%)	12 (48.0)/ 12 (48.0)/ 1 (4.0)/ 0 (0.0)	8 (15.1)/ 41 (77.4)/ 4 (7.5)/ 0 (0.0)	0.008
Neutrophils	23 (92.0)/ 2 (8.0)/ 0 (0.0)/ 0 (0.0)	41 (77.4)/ 12 (22.6)/ 0 (0.0)/ 0 (0.0)	0.12
Lymphocytes	12 (48.0)/ 12 (48.0)/ 1 (4.0)/ 0 (0.0)	8 (15.1)/ 41 (77.4)/ 4 (7.5)/ 0 (0.0)	0.008
Histiocytes	25 (100.0)/ 0 (0.0)/ 0 (0.0)/ 0 (0.0)	52 (98.1)/ 1 (1.9)/ 0 (0.0)/ 0 (0.0)	0.49
Inflammatory infiltrate [absent n (%)/mild n (%)/moderate n (%)/marked n (%)][4]			
Lobular inflammatory infiltrate n (%)	11 (44.0)/ 14 (56.0)/ 0 (0.0)/ 0 (0.0)	12 (22.6)/ 41 (77.4)/ 0 (0.0)/ 0 (0.0)	0.06
Cells' type [absent n (%)/mild n (%)/moderate n (%)/marked n (%)][4]			
Neutrophils	24 (96.0)/ 1 (4.0)/ 0 (0.0)/ 0 (0.0)	48 (90.6)/ 5 (9.4)/ 0 (0.0)/ 0 (0.0)	0.04
Lymphocytes	11 (44.0)/ 14 (56.0)/ 0 (0.0)/ 0 (0.0)	12 (27.6)/ 40 (75.5)/ 1 (2.0)/ 0 (0.0)	0.13
Histiocytes	25 (100.0)/ 0 (0.0)/ 0 (0.0)/ 0 (0.0)	52 (98.1)/ 1 (1.9)/ 0 (0.0)/ 0 (0.0)	0.49
Centrilobular inflammatory infiltrate n (%)	18 (72.0)/ 7 (28.0)/ 0 (0.0)/ 0 (0.0)	43 (81.1)/ 10 (18.9)/ 0 (0.0)/ 0 (0.0)	0.37
Neutrophils	25 (100.0)/ 0 (0.0)/ 0 (0.0)/ 0 (0.0)	52 (98.1)/ 1 (1.9)/ 0 (0.0)/ 0 (0.0)	0.49
Lymphocytes	18 (43.0)/ 72 (81.1)/ 0 (0.0)/ 0 (0.0)	7 (28.0)/ 10 (18.9)/ 0 (0.0)/ 0 (0.0)	0.36
Histiocytes	25 (100.0)/ 0 (0.0)/ 0 (0.0)/ 0 (0.0)	53 (100.0)/ 0 (0.0)/ 0 (0.0)/ 0 (0.0)	–

[1]The grade of fibrosis was evaluated according to the METAVIR fibrosis score: F0, no fibrosis; F1, portal fibrous expansion; F2, portal fibrous widening with few septa; F3, bridging fibrosis with architectural distortion; and F4, liver cirrhosis; [2]the grade of necroinflammatory activity was evaluated considering METAVIR necroinflammatory grading: [3]the degree of inflammation was classified from A0 to A3 based on the evaluatio n of lymphocyte infiltration and hepatocyte necrosis; the grade of steatosis was evaluated according to Brunt et al.: grade 1, ≤33%; grade 2, >33% to <66%; grade 3, ≥66%; [4]the degree of inflammatory cells (mononuclear and polymorphonuclear), was scored from 0 to 3 (0, absent; 1, mild; 2, moderate and 3 marked) separately in all hepatic sites. *P* values ≤0.05 were considered significant. The Kruskal-Wallis test was used for the comparison of two or more groups of sample data.

Correlation between the degree of hepatic necroinflammatory activity and HDL-C and triglycerides to HDL-C ratio (TG/HDL-C)

Serum levels of HDL-C were inversely correlated with the degree of hepatic necroinflammatory activity (r=-0.33; *P*=0.003). TG/HDL-C ratio (also considered a risk factor of CVD) was positively correlated with hepatic necroinflammatory activity (r=0.32, *P*=0.004), perilobular inflammatory activity (r=0.30, *P*=0.008), periportal lymphocytes (r=0.32, *P*=0.004) and lobular lymphocytes (r=0.33, *P*=0.003).

Variables associated with the main hepatic histological changes

Next, to evaluate the variables associated with the main hepatic histological changes, we created four models (model 1, variables associated with fibrosis; model 2, variables associated with necroinflammatory activity; model 3, variables associated with interface inflammatory activity; model 4, variables associated with lobular inflammatory activity). The data were analysed by logistic regression models, and the results are summarised in TABLE 4.

TABLE 4. Variables associated with liver fibrosis and inflammatory activity in patients with chronic hepatitis C (n=85).

Independent variable	Univariate analysis	Multivariate analysis		
	P	OR	95%CI	*P*
Model 1 - dependent variable – fibrosis (F0–F2 vs F3–F4)				
Sex	0.93	–	–	–
Age	0.02	1.09	1.02–1.16	0.02
Body mass index	0.68	–	–	–
Hypertension	0.21	–	–	–
Type 2 diabetes mellitus	0.62	–	–	–
Steatosis	0.07	4.03	1.05–15.45	0.04
Current alcohol use (≥20 g/day)	0.33	–	–	–
Triglycerides (≥150 mg/dL)	0.29	–	–	–
HDL cholesterol <60 mg/dL	0.06	7.67	1.71–34.49	0.008
Model 2 - dependent variable – hepatic necroinflammatory activity				
Sex	0.78	–	–	–
Age	0.61	–	–	–
Body mass index	0.02	1.06	0.89–1.27	0.51
Hypertension	0.02	6.36	1.31–30.85	0.02
Type 2 diabetes mellitus	0.05	2.03	0.18–22.42	0.56
Steatosis	0.08	5.35	1.31–21.74	0.02
Current alcohol use (≥20 g/day)	0.04	0.36	0.08–1.61	0.18
Triglycerides (≥150 mg/dL)	0.02	1.02	0.99–1.04	0.12
HDL cholesterol <60 mg/dL	0.06	9.85	2.35–41.39	0.002
Model 3 - dependent variable – interface inflammatory activity				
Sex	0.91	–	–	–
Age	0.08	1.05	0.99–1.11	0.14
Body mass index	0.06	1.12	0.96–1.31	0.16
Hypertension	0.002	6.94	1.92–25.05	0.003
Type 2 diabetes mellitus	0.05	1.50	0.26–8.70	0.65
Steatosis	0.47	–	–	–
Current alcohol (≥20 g/day)	0.03	0.50	0.13–1.96	0.32
Triglycerides (≥150 mg/dL)	0.56	–	–	–
HDL cholesterol <60 mg/dL	0.02	3.94	1.27–12.23	0.02
Model 4 - dependent variable – lobular inflammatory activity				
Sex	0.69	–	–	–
Age	0.92	–	–	–
Body mass index	0.28	–	–	–
Hypertension	0.82	–	–	–
Type 2 diabetes mellitus	0.08	2.90	0.57–14.72	0.20
Steatosis	0.21	–	–	–
Current alcohol use (≥20 g/day)	0.48	–	–	–
Triglycerides (≥150 mg/dL)	0.001	1.02	1.01–1.04	0.01
HDL cholesterol <60 mg/dL	0.05	2.32	0.79–6.83	0.13

Variables associated with fibrosis

In the univariate analysis, age, steatosis, and HDL-C <60 mg/dL were selected and remained associated with fibrosis (F0–F2 vs F3–F4) in the multivariate analysis (Model 1) (TABLE 4).

Variables associated with necroinflammatory activity

In the univariate analysis, BMI, hypertension, type 2 diabetes mellitus, steatosis, current alcohol use (≥20 g/day), TG and HDL-C (<60 mg/dL) were selected. In the multivariate analysis, hypertension, steatosis and HDL-C <60 mg/dL remained associated with the presence of hepatic necroinflammatory activity (Model 2) (TABLE 4).

Variables associated with inflammatory interface activity

In the univariate analysis, age, BMI, hypertension, type 2 diabetes mellitus, current alcohol use (≥20 g/day) and HDL-C (<60 mg/dL) were selected. In the multivariate analysis, hypertension and HDL-C <60 mg/dL remained associated with inflammatory interface activity (Model 3) (TABLE 4).

Variables associated with lobular inflammatory activity

In the univariate analysis, age, BMI, type 2 diabetes mellitus, TG and HDL-C were selected. In the multivariate analysis, TG (TG ≥150 mg/dL) remained associated with lobular inflammatory activity (Model 4) (TABLE 4).

Clinical comorbidities, high density lipoprotein cholesterol, HCV viral load and HCV genotype

Neither viral load nor HCV genotype was associated with type 2 diabetes mellitus, hypertension, TG ≥150 mg/dL and HDL-C <60 mg/dL.

DISCUSSION

HCV is recognised as the virus that is most frequently associated with nonhepatic manifestations[55]. Thus, in addition to chronic liver disease and its complications, HCV infection causes several systemic conditions, which may markedly affect morbidity, mortality and quality of life[12,13,55]. To the best of our knowledge, this is the first study to demonstrate that low HDL-C levels are associated with hepatic necroinflammatory activity in patients with CHC. Furthermore, TGL/HDL-C ratio positively correlated with hepatic necroinflammatory and lobular inflammatory activity.

Several lines of evidence have demonstrated that in patients chronically infected with HCV, the virus's replication cycle is tightly associated with the host metabolism of lipids and lipoproteins[15-18]. In this way, HCV interferes with the crosstalk between liver, lipoprotein metabolism and cholesterol balance[15-18]. During the HCV life cycle, the virus makes use of the host lipoprotein machinery, occasioning the secretion of altered lipoproteins carrying viral constituents from the liver, which are termed lipo-viral-particles (LVPs)[22]. In particular, low serum levels of total cholesterol, LDL-C and apolipoprotein B (apoB) have been found in patients with CHC[56,57].

However, the mechanisms involved in the modulation of lipoprotein by HCV infection are not completely understood. Earlier evidences suggest that LVPs could be involved in the regulation of the immune response and affect the anti- and proinflammatory mediator balance[58,59]. In the current study, serum levels of HDL-C were inversely correlated with the degree of hepatic necroinflammatory activity. Moreover, high TG/HDL-C ratio was significantly correlated with hepatic necroinflammatory activity, perilobular inflammatory activity, periportal lymphocytes and lobular lymphocytes. Therefore, these altered lipid profiles may aggravate the intrahepatic inflammatory milieu in patients chronically infected with HCV.

By contrast, it has been shown that HDL-C suppresses cytokine and chemokine production by monocytes, macrophages, and monocyte-derived dendritic cells, downregulates costimulatory molecule production and decreases antigen presentation[60]. In the HCV scenario, a previous study from Italy has shown that HDL-C levels were inversely correlated with serum concentrations of interleukin-6 (IL-6)[61]. We should keep in mind that HCV does not associate with a direct cytopathic effect on host cells, and most of the liver-related abnormalities are linked to virus-mediated modification of host biological processes such as immune response[61] and various metabolic pathways[15-18,22].

In the current study, low HDL-C was associated with liver fibrosis. It is well known that the liver

plays a crucial role in lipid metabolism; thus, alterations of hepatic function are linked to modifications of circulating lipids[62]. Cirrhosis is associated with a decline in serum concentrations of HDL-C[63,64], LDL-C[65] and apo A-I, the principal protein constituent of HDL-C units[66]. With respect to HDL-C levels, an inverse association between this lipid molecule and several adverse outcomes has been shown in patients with advanced liver disease[62-64]. Habib et al. (2005) showed that low HDL-C in noncholestatic cirrhotic subjects might be an indicator of liver function and poor prognosis[62]. In our study, HDL-C was not correlated with albumin, total bilirubin, PA and AST/ALT ratio. These results should be attributed to our small data set with the inclusion of only 10 patients with advanced liver disease.

In the present study, in addition to HDL-C <60 mg/dL, arterial hypertension was associated with the hepatic necroinflammatory activity. To date, the influence of arterial hypertension on liver histology abnormalities of patients with CHC has barely been described. In a previous study conducted in USA, Corey and cols. evaluating the histological effect of angiotensin II blocking agents in CHC-patients, observed that HCV-infected individuals with hypertension had higher fibrosis degree than those without hypertension[67]. The authors also observed that hypertensive patients who received angiotensin II blocking agents had less fibrosis than hypertensive individuals who did not receive angiotensin-converting enzyme (ACE) inhibitors[67]. Based on findings of an earlier investigation, including patients infected with HCV, hypertensive individuals receiving angiotensin-blocking therapy had a substantial decrease in the extent of hepatic inflammatory activity[68]. Although longitudinal studies are required to advance this understanding, the use of liver biopsies in clinical practice, at least in the hepatitis B and C settings, is significantly declining worldwide[69].

The limitations of our study should be considered. The subjects included were recruited from a referral centre and may not represent all patients with CHC. In addition, the cross-sectional nature of this investigation precluded the possibility of recognizing any cause-effect relationship between serum HDL-C levels, and hepatic histological findings in patients with CHC.

Concerning the liver biopsy, 10 to 12 portal tracts within the specimen are generally considered relevant to achieve an accurate diagnosis. Although we have included liver fragments containing 6 to 9 portal tracts, in all these cases, the pathologist was able to make a detailed interpretation. In a previous study, Coral et al. suggested that less than 11 portal tracts may still be suitable for the assessment of adequate staging portal tracts in chronic hepatitis, and more than five portal tracts did not adversely influence the staging[70].

In summary, the results of our study may influence clinical decision-making and contribute to the development of effective strategies to control potential and modifiable host factors implicated in the hepatic disease progression in individuals chronically infected with HCV.

CONCLUSION

High levels of serum HDL cholesterol may have a protective role against fibrosis and necroinflammatory activity in chronic hepatitis C. Patients with hypertension are at an increased risk of developing necroinflammatory activity.

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Authors' contribution

Souza GHP, Vidigal PVT and Silva LD: designed research (project conception, development of overall research plan, and study oversight); Souza GHP and Vieira DA conducted research (hands-on conduct of data collection); Vidigal PVT, Rocha GA and Lima AS provided essential reagents or provided necessary materials; Souza GHP, Vidigal PVT and Silva LD: analysed data or performed statistical analysis; Souza GHP, Vidigal PVT, Rocha GA and Silva LD wrote the paper; Souza GHP, Vidigal PVT, Rocha GA and Silva LD had primary responsibility for final content. All authors critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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Souza GHP, Silva LD, Vieira DA, Rocha GA, Lima AS, Vidigal PVT. Lipoproteína de alta densidade e hipertensão arterial sistêmica estão associados à atividade necroinflamatória hepática em pacientes com hepatite C crônica. Arq Gastroenterol. 2023;60(3):287-99.

RESUMO – Contexto – Aproximadamente 71 milhões de pessoas estão infectadas pelo vírus da hepatite C em todo o mundo. Um número significativo desses indivíduos desenvolverá cirrose hepática e/ou carcinoma hepatocelular. Além do fígado, há evidências científicas que associam doenças cardiovasculares e hepatite C crônica; no entanto, os mecanismos biológicos implicados na ocorrência dessas condições ainda não foram completamente esclarecidos. **Objetivo –** Avaliar a associação entre histologia hepática, comorbidades clínicas e perfil lipídico em pacientes com hepatite C crônica. Investigar associações entre histologia hepática e parâmetros demográficos, nutricionais, bioquímicos e virológicos. **Métodos –** Oitenta e cinco pacientes com hepatite C crônica foram prospectivamente submetidos à biópsia hepática. Biópsias hepáticas foram obtidas de cada paciente por via percutânea com agulha de Menghini. A fibrose foi avaliada de acordo com o sistema de pontuação METAVIR, como segue: F0, sem fibrose; F1, expansão portal fibrosa; F2, alargamento portal fibroso com poucos septos; F3, fibrose em ponte com distorção arquitetônica; e F4, cirrose hepática. A atividade foi classificada com base no grau de infiltração de linfócitos e necrose de hepatócitos, de A0 a A3. O diagnóstico da doença hepática foi baseado em métodos clínicos, bioquímicos, histológicos e radiológicos. Os dados foram analisados por modelos de regressão logística. **Resultados –** Neste estudo transversal, realizado em um ambulatório do hospital universitário, foram incluídos 85 pacientes que tinham média de idade de 57,2±10,7 anos, sendo 45 (52,9%) do sexo feminino. Havia 10 pacientes com cirrose. Os pacientes com METAVIR F3–F4 eram significativamente mais velhos (*P*=0,02) e tinham níveis mais elevados de ALT (*P*=0,0006), AST (*P*<0,0001), γ-GT (*P*=0,03) e bilirrubina (*P*=0,001) e, maior tempo de protrombina do que pacientes com escore F0-F2. Os níveis de albumina (*P*=0,01) foram significativamente mais baixos naqueles classificados como METAVIR F3–F4. Idade (OR=1,09; IC95%=1,02–1,16; *P*=0,02), esteatose (OR=4,03; IC95%=1,05–15,45; *P*=0,04) e HDL-C <60 mg/dL (OR=7,67; 95%IC=1,71–34,49; *P*=0,008) foram independentemente associados à fibrose. Hipertensão (OR=6,36; IC95%=1,31–30,85; *P*=0,02) e HDL-C <60 mg/dL (OR=9,85; IC95%=2,35–41,39; *P*=0,002) foram independentemente associados à atividade necroinflamatória. Hipertensão (OR=6,94; IC 95%=1,92–25,05; *P*=0,003) e HDL-C <60 mg/dL (OR=3,94; IC95%=1,27–12,3; *P*=0,02) foram associados à atividade inflamatória de interface. Os triglicerídeos (TG >150 mg/dL) permaneceram associados à atividade inflamatória lobular. **Conclusão –** Níveis de coleterol HDL <60 mg/dL foram independentemente associados à atividade necroinflamatória na hepatite C crônica. Pacientes com hipertensão têm risco aumentado de desenvolver atividade necroinflamatória.

Palavras-chaves – Vírus da hepatite C, hepatite C crônica, diabetes mellitus, hipertensão, colesterol HDL, histologia hepática.

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