Hepatic vein Doppler flowmetry in patients with nonalcoholic steatosis*

Dopplerfluxometria da veia hepática em pacientes com esteatose não alcoólica

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Abstract **Objective:** To evaluate the correlation between right hepatic vein Doppler flowmetry and degree of steatosis, inflammation and fibrosis at biopsy in nonalcoholic fatty liver disease. **Materials and Methods:** Doppler ultrasonography was performed in 80 patients divided into two groups as follows: 40 patients diagnosed with nonalcoholic fatty liver disease and also submitted to biopsy, and a control group including 40 healthy adults with no risk factor for nonalcoholic fatty liver disease. The Doppler waveform patterns of right hepatic vein were classified into triphasic, biphasic and monophasic. Biopsy specimens were classified according to the degree of steatosis, inflammation and fibrosis. **Results:** Triphasic flow was observed in 38 (95%) patients of the control group and in nine (56%) patients with mild steatosis, whereas in patients with severe steatosis, the pattern was monophasic in 60%. A statistically significant difference was observed in the distribution of Doppler patterns ($p < 0.01$). A significant negative correlation between the Doppler waveform pattern of right hepatic vein and steatosis degree was observed ($r = 0.57$; $p < 0.01$). **Conclusion:** An abnormal Doppler waveform pattern of right hepatic vein in patients with nonalcoholic fatty liver disease may suggest the presence of decreased vascular compliance caused by fatty infiltration.

Keywords: Fatty liver; Hepatic steatosis; Ultrasonography; Doppler flowmetry; Hepatic vein; Liver biopsy.

Resumo **Objetivo:** Avaliar a correlação entre a dopplerfluxometria da veia hepática direita e o grau de esteatose, inflamação e fibrose à biópsia na doença hepática gordurosa não alcoólica. **Materiais e Métodos:** Foi realizada ultrassonografia com Doppler em 80 pacientes, sendo 40 portadores de doença hepática gordurosa não alcoólica, também submetidos à biópsia. Quarenta controles normais saudáveis, sem fatores risco para doença hepática gordurosa não alcoólica foram submetidos a ultrassonografia com Doppler. O padrão ao Doppler da veia hepática direita foi classificado em trifásico, bifásico e monofásico. Os espécimes de biópsia foram classificados conforme o grau de esteatose, inflamação e fibrose. **Resultados:** O fluxo foi trifásico em 38 (95%) dos controles e em 9 (56,3%) dos pacientes com esteatose discreta, enquanto nos com esteatose acentuada o padrão foi monofásico em 60%. Encontrou-se diferença significante na distribuição dos padrões ao Doppler ($p < 0,01$). Houve correlação negativa e significante entre o padrão ao Doppler da veia hepática direita e grau de esteatose ($r = –0,57$; $p < 0,01$). **Conclusão:** A alteração do padrão ao Doppler da veia hepática direita em pacientes com doença hepática gordurosa não alcoólica pode sugerir redução da complacência vascular consequente a infiltração gordurosa.

Unitermos: Fígado gorduroso; Esteatose hepática; Ultrassonografia; Dopplerfluxometria; Veia hepática; Biópsia hepática.

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INTRODUCTION

Steatosis is chemically defined as hepatic triglyceride content greater than 5% of the hepatic volume or of the liver weight[1] or, histologically, when 5% or more of the hepatocytes contain triglycerides[2].

Ultrasonography is the imaging method most frequently indicated in the diagnosis and evaluation of hepatic steatosis, considering its noninvasiveness, wide availability and low cost[3–5].

It has been described that the hepatic veins present a decrease in pulsatility in cases of fat infiltration, from the triphasic pulsatile pattern to a biphasic pattern and eventually to a monophasic flow where no oscillation in the flow velocity waveform is identified at pulsatile Doppler[6]. The objective of this study was to evaluate flow velocity waveform patterns in the right hepatic vein of patients with different steatosis degrees in nonalcoholic fatty liver disease confirmed by biopsy, and comparing them with each other and with a control group.

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MATERIALS AND METHODS

This is an observational study, in which the data were prospectively collected. It was developed in a single research center at Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil. The volunteers were consecutively included (Figure 1). The study was approved by the UFU Committee for Ethics in Research under number 065/08. Only those patients who signed a term of free and informed consent after verbal explanation were included in the present study.

In the period between October/2008 and November/2009, 182 patients with nonalcoholic fatty liver disease were evaluated at the fatty liver unit of Hospital das Clínicas of UFU. Among those patients, 40 were included in the present study after clinical evaluation, laboratory tests, ultrasonography and histopathological evaluation of liver fragments obtained by biopsy, thus constituting the group of steatotic patients.

Also, a group with 40 healthy volunteers without risk factors for nonalcoholic fatty liver disease, with minimal or no alcohol ingestion, neither diabetes nor obesity were included as a (nonsteatotic) control group. Such control group was submitted to the same evaluations as the patients with the disease, except for hepatic biopsy, for ethical reasons.

All the volunteers in the steatotic group met the specific inclusion and exclusion criteria described below. The volunteers included in the control group met the same exclusion and inclusion criteria, except for the presence of hepatic steatosis at ultrasonography. Additionally, for the control group, any evidence of insulin resistance and risk factor for nonalcoholic fatty liver disease were exclusion criteria.

Inclusion criteria were the following: presence of steatosis at US; age between 18 and 70 years, both sexes; written consent after verbal explanation.

Exclusion criteria were: alcohol ingestion > 140 g/week for men and > 70 g/week for women[7]; use of drugs known to be hepatotoxic, such as tetracycline, amiodarone, tamoxifen and alpha-methyldopa; presence of chronic viral hepatitis B or C; hemochromatosis; Wilson's disease; deficiency of alpha-1-antitrypsin; autoimmune hepatitis; other chronic liver diseases, portal hypertension, cirrhosis, ascites and liver failure; any form of coagulation disorder, no matter how slight it might be; presence of heart disease, acute coronary, cerebral or peripheral ischemia; presence of respiratory failure, kidney disease, ectopic or absent right kidney; focal hepatic lesions; focal renal lesions, which might interfere with the comparison between liver and kidney;pregnancy and lactation; refusal to participate in the study.

Clinical evaluation

Demographic data and the anthropometric measurements such as weight and height were obtained during outpatient consultations. The body mass index (BMI) was calculated by means of the Quetelet's formula: BMI = weight in kg/(height in m^2). Waist circumference was measured in centimeters, by utilizing an inelastic tape, at the midpoint located between the last rib and the iliac crest, at the end of normal expiration. The circumference of the hip was measured at the level of the greatest gluteal protuberance. The waist/hip ratio was obtained by the division of waist circumference by the hip circumference.

Laboratory tests

The following tests were performed: complete blood count, plasma lipid profile, coagulation, total protein and fractions, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, total bilirubin and fractions, glucose, insulin, markers for hepatitis B and C, autoantibodies, serum copper and ceruloplasmin and iron profile.

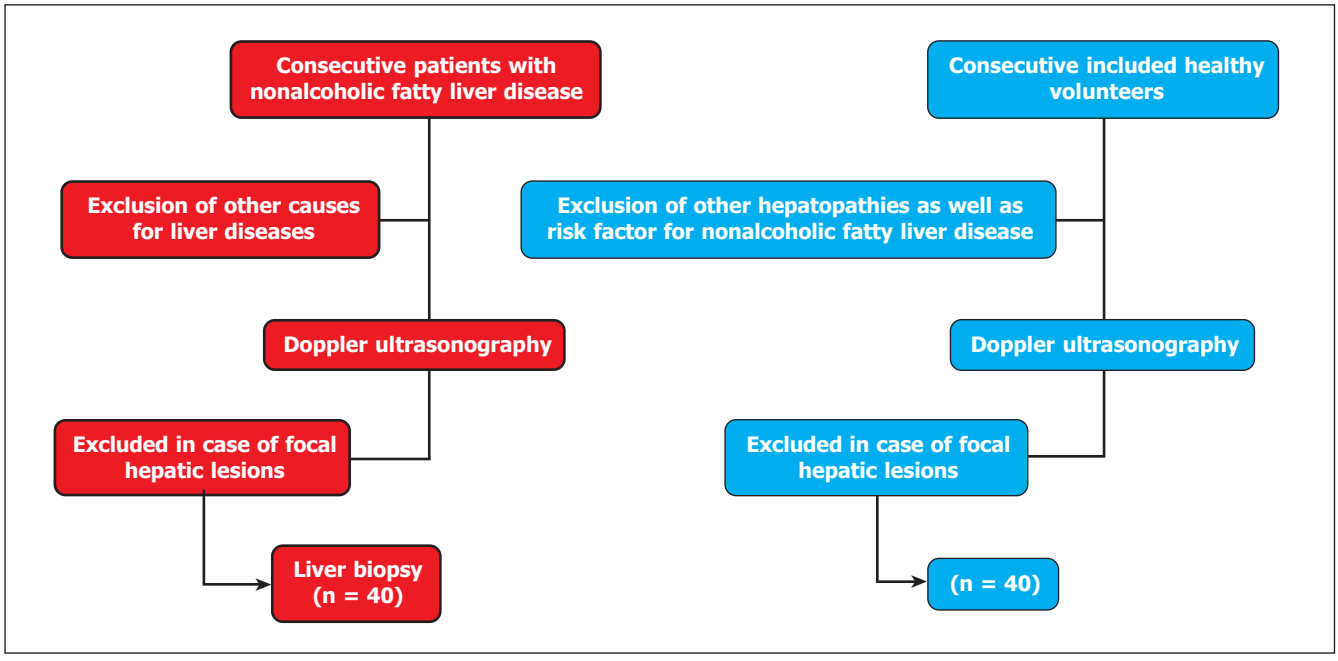

Figure 1. Flowchart representing the inclusion of individuals in the study.

Evaluation of insulin resistance

For the cases that did not met DM2 diagnostic criteria, and did not present glycemic risk for diabetes, the HOMA-IR (homeostasis model assessment for insulin resistance) index was calculated. For such purpose, material for insulin and glycemic tests was collected after fasting and the calculations were made according to the following formula: glycemia (mg/dl) × insulin (UI/ml) ÷ 405[8].

Sonographic evaluation

All the 80 patients were submitted to Doppler ultrasonography, with 2–5 MHz multifrequency transducer (Voluson 730 Pro V; General Electric, Milwaukee, WI, USA).

The patients were examined under fasting conditions, between 8:00 and 10:00 a. m., in dorsal decubitus, with arms extended besides their head.

All liver segments were scanned, and the presence of vascular malformations, cysts, focal parenchymal lesions was ruled out.

The color Doppler mode was utilized for identification of the hepatic veins, and to evaluate their pulsatility pattern and flow direction. The evaluation of the right hepatic vein was standardized at the level of the 10th or 11th right intercostal space during a short breath-hold period, at a distance of 2.0 cm distal to the hepatic veins confluence, to avoid artifacts in the waveform pattern. The analysis was recorded for at least four cardiac cycles (approximately 6 seconds). The transducer angle was 30°. The waveform patterns were classified into three groups: normal triphasic waveform with a short reversed flow, biphasic waveform without reversed flow but fluttering of more than 10% of the mean phasic amplitude, and monophasic flat waveform with fluttering of < 10% of the mean phasic amplitude (Figure 2). Because of alterations in the vessel diameter, of up to 2 mm per cardiac cycle at systole and diastole, besides different flow directions, velocimetry in the hepatic veins was not calculated.

Histological evaluation

Hepatic biopsy specimens were collected under US guidance by means of

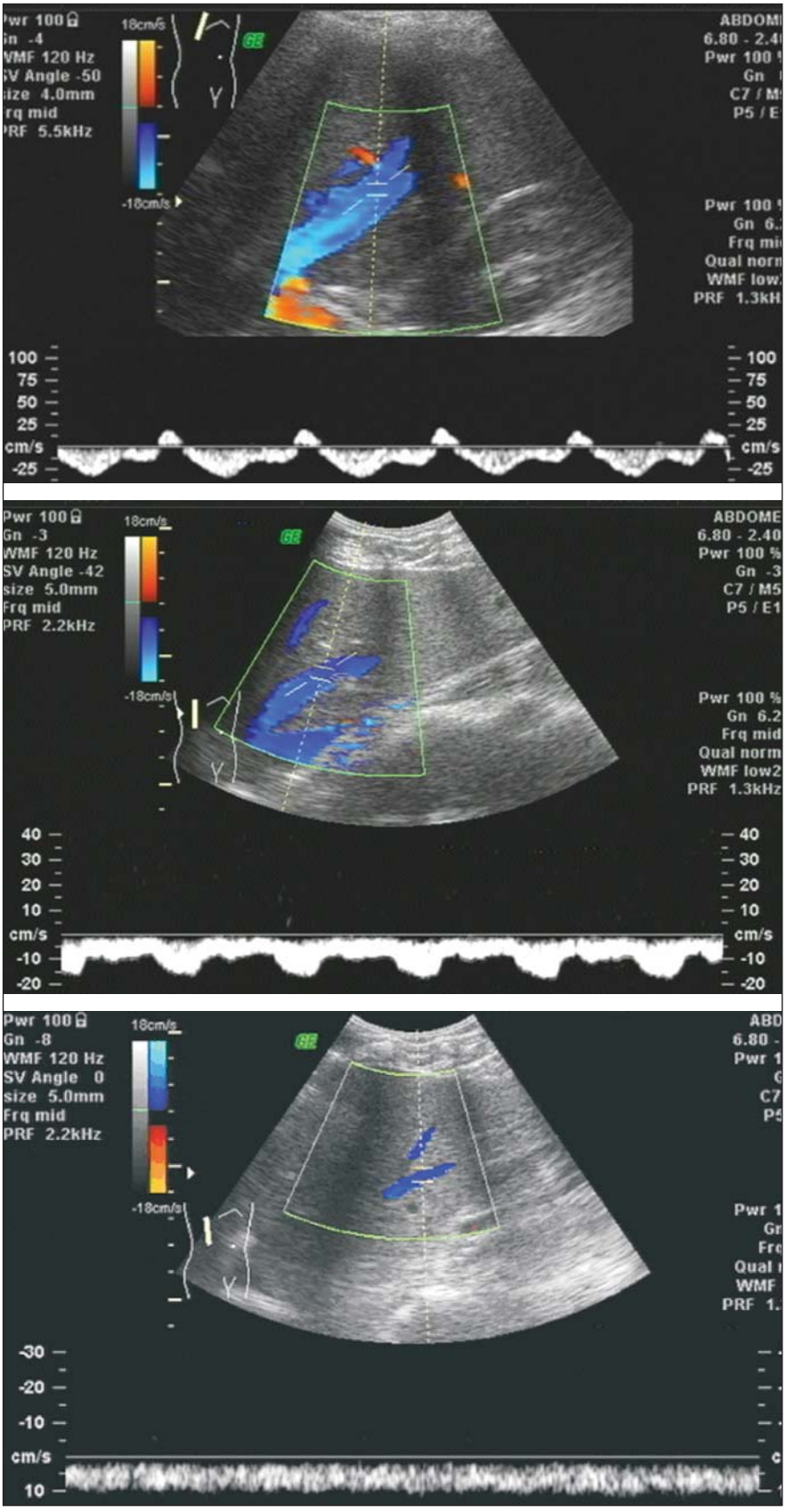

Figure 2. Hepatic vein flow pattern at Doppler. On the upper image, triphasic pattern; on the central image, biphasic pattern; on the lower image, monophasic pattern.

Trucut needle, at the right hepatic lobe. The hepatic tissue fragment with approximately 10 mm was fixed in 4% formol-saline and stained with hematoxylin-eosin and Masson's trichrome techniques according to the method adopted at the division of Pathological Anatomy of Hospital das Clínicas of UFU. A single observer, an experienced pathologist, performed a blind evaluation of the material. The biopsy was performed one week after the ultrasonography.

The histopathological evaluation was based on the reviewed standardized classification of the Pathology Committee of the NASH Clinical Research Network[2], which designated and validated the histologic characteristics and a score system for nonalcoholic fatty liver disease activity, for the purposes of clinical studies.

The histopathological variables were described and subdivided into hepatocellular changes, fibrosis and inflammatory infiltrate. The hepatocellular changes that were systematically searched were the following: steatosis, hepatocellular ballooning and Mallory bodies. Steatosis was classified as mild (up to 33% of hepatocytes infiltrated by fat droplets), moderate (from 33% to 66%) and severe (more than 66% of fatty infiltration in the hepatocytes). The inflammatory infiltrate intensity was classified into mild, moderate and accentuated. Fibrosis was described according to location in the hepatic acinus, appearance and intensity.

Statistical analysis

The statistical analysis was performed with the aid of the software Statistical Package for the Social Sciences (SPSS for Windows, version 16.0) (SPSS Inc.; Chicago, IL, USA).

The Lilliefors normality test was utilized in the analysis of the data distribution. The continuous variables were expressed as mean ± standard deviation, median and quartiles, as appropriate, and compared by means of the analysis of variance between means (ANOVA) and Tukey's range test for *post-hoc* analysis. The categorical variables were expressed by absolute (*n*) and relative frequencies, and were analyzed by means of the Fisher's and Mann-Whitney's tests. The correlation analyses were performed by utilizing the Spearman coefficient.

All significance tests were bilateral, adopting a significance level of 0.05 (α = 5%), with descriptive levels (p) lower than this value being considered as statistically significant.

RESULTS

Clinical characterization of patients and controls

The clinical characteristics of steatotic patients as compared with controls are shown on Table 1.

The groups were paired with respect to age and sex. The ages of volunteers in the control group ranged from 27 to 70 years, with a mean age of 42.48 ± 11.52 years. The mean age of the steatotic patients was 47.10 ± 10.18 years. By utilizing the variance analysis (ANOVA for one variable) no significant difference was found between mean ages (p = 0.061). The control group comprised 11 men and 29 women, while the steatotic group comprised seven men and 33 women. By utilizing the Fisher's test, no statistically significant difference was observed between these ratios (p = 0.284). No statistically significant difference was found between the groups with respect to the albumin serum level, bilirubin, platelet count and transferrin saturation index.

The patients with nonalcoholic fatty liver disease presented altered obesity, dyslipidemia, insulin resistance, hepatic enzymes indices and ferritin levels as compared with the control group.

Histological evaluation

The histological evaluation was only performed in the group of diseased patients, confirming the presence of steatosis in all of them. Among those patients, 16 presented mild steatosis, i.e., with up to 33% of affected hepatocytes; 19 presented moderate steatosis (33% to 66% of affected hepatocytes); and five presented severe steatosis, with more than 66% of the hepatocytes infiltrated by fat droplets.

At biopsy, none of the patients presented advanced fibrosis producing nodulations (cirrhosis). Thirty of the patients presented some degree of fibrosis, and in nine of them (22.5%) bridging fibrosis was described.

Table 1 Clinical and laboratory characteristics of the group with steatosis and the control group, expressed in mean ± standard deviation, except for the categorical variables, expressed, whenever indicated, in absolute and percentage frequencies.

	Steatosis (n = 40)	Control (n = 40)	p
Age (years)	47.10 ± 10.18	42.48 ± 11.52	0.090
Male patients (%)	7 (17.50%)	11 (27.20%)	0.284
Albumin (g/dl)	4.68 ± 0.53	4.68 ± 0.41	0.977
Platelets/1000	274.03 ± 76.43	255.59 ± 48.71	0.206
TSI	30.03 ± 13.42	32.95 ± 11.44	0.341
Total bilirubin (mg/dl)	0.65 ± 0.58	0.79 ± 0.42	0.269
ANF (% reagents)	7 (17.50%)	6 (15.00%)	0.931
BMI (kg/m^2)	32.57 ± 6.04	22.40 ± 2.43	0.000
Glycemia (mg/dl)	105.25 ± 23.53	85.13 ± 7.46	0.000
HOMA-IR	2.94 ± 2.78	0.88 ± 0.49	0.000
Total cholesterol (mg/dl)	198.63 ± 37.16	180.44 ± 40.39	0.045
Triglicerydes (mg/dl)	162.03 ± 83.51	88.38 ± 40.57	0.000
HDL (mg/dl)	45.33 ± 10.42	59.95 ± 10.95	0.000
Aspartate aminotransferase*	0.73 ± 0.53	0.38 ± 0.11	0.000
Alanine aminotransferase*	1.03 ± 0.73	0.41 ± 0.18	0.000
Gamma-glutamyltransferase*	1.49 ± 1.32	0.46 ± 0.23	0.000
Alkaline phosphatase*	0.75 ± 0.28	0.52 ± 0.16	0.000
Ferritin (ng/ml)	193.23 ± 116.86	122.55 ± 135.49	0.019

TSI, transferrin saturation index in %; ANF, anti-nuclear factor; BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance; HDL, high density lipoprotein. * The hepatic enzymes were described in relation to the respective upper normality limit.

Sonographic evaluation

Differences were observed in the hepatic vein flow velocity waveform pattern at Doppler between the steatotic and the control groups. In the control group, the predominant waveform pattern was triphasic, while in the group with the disease the pattern was either biphasic or monophasic. At Doppler, 22 of 40 steatotic patients (55%) presented abnormal hepatic vein pattern, with 16 (40%) with monophasic pattern and six (15%) with biphasic pattern.

At Mann-Whitney test, the difference in the frequency of the abnormal hepatic vein flow velocity waveform pattern between the two groups was statistically significant ($p < 0.0001$).

A reversed and statistically significant correlation between the fatty infiltration degree and the hepatic vein flow velocity waveform pattern was observed ($r = –0.57 \pm 0.08$; $p < 0.01$).

The difference in the frequency of the abnormal hepatic vein flow velocity waveform pattern among the subgroups of steatotic patients was not statistically significant ($p = 0.33$). Table 2 demonstrates the distribution of flow velocity waveform patterns according to the degree of steatosis detected at histology.

Table 2 Distribution of the right hepatic vein flow waveform patterns at Doppler, in the control group and in the group with steatosis at biopsy, expressed in absolute and percentage frequencies.

Group	Monophasic pattern	Biphasic pattern	Triphasic pattern	Total
Control	1 (2.5%)	1 (2.5%)	38 (95.0%)	40.0
Mild steatosis	5 (31.3%)	2 (12.5%)	9 (56.3%)	16.0
Moderate steatosis	8 (42.1%)	3 (15.8%)	8 (42.1%)	19.0
Severe steatosis	3 (60.0%)	1 (20.0%)	1 (20.0%)	5.0

DISCUSSION

Nonalcoholic fatty liver disease is a common cause of chronic hepatopathy in insulin-resistant individuals[9]. The disease presentations range from steatosis, with possible progression to steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma[10]. At ultrasonography, the most frequent presentation is the finding with an appearance suggestive of steatosis[11].

In the present study, a control group of individuals with no risk factor risk for nonalcoholic fatty liver disease was utilized for comparison. Such group was confirmed to be comprised of healthy individuals, without any sign characterizing insulin resistance, which clearly differentiated it from the group of patients with the disease. The mean HOMA-IR index for the healthy volunteers was quite low (0.88) and the cutoff point between the control group and the group with the disease (> 1.35) was lower than the ones reported in other Brazilian studies[10,11].

In the present sample, the mean values for hepatic enzymes in relation to the upper normal limit demonstrated to be compatible with very subtle increases, with normal aminotranferases levels in most of the cases, as already described in the literature. On the other hand, at histology, all the patients submitted to biopsy met some criteria for nonalcoholic steatohepatitis, confirming, as previously known, that hepatic enzymes are falsely normal in the majority of such patients[12,13].

In the setting of portal hypertension, the clinical value of Doppler in the hepatic and portal veins hemodynamics is well established. On the other hand, the contribution of steatosis, inflammation and fibrosis in the setting of nonalcoholic fatty liver disease, to change the hepatic and portal veins flow velocity waveform is the focus of more recent interest.

In the present study, Doppler ultrasonography and hepatic biopsy were prospectively performed in a same week, in a group of volunteers with diffuse and homogeneous steatosis caused by nonalcoholic fatty liver disease, with the objective of reducing eventual biases. Other conditions that might interfere with Doppler, such as pregnancy, cardiac failure, valvulopathies and chronic pulmonary disease, were ruled out.

Dietrich et al.[14], in a study published in 1998, demonstrated that the relationship between the left hepatic lobe and the heart, often leads to artifacts in the Doppler signal, particularly because of heart motion. For this reason, the evaluation of the left hepatic vein, particularly, is not always reliable. In 35 of 135 patients the evaluation of the middle hepatic vein was only possible during deep inspiration, with respiration artifacts initially producing a monophasic flow velocity waveform, then changing to biphasic and triphasic during the examination. The most reproducible evaluation of the flow velocity waveform was obtained on the right hepatic vein through the intercostal pathway, at a 30º angle, as performed in the present study.

Other studies approaching hepatic vein flow velocity waveform also have not found its capacity to differentiate groups with the disease (Table 3), finding frequencies similar to those observed in the present study regarding the pathological hepatic vein pattern in patients with the disease and in the controls[6,14,15].

CONCLUSION

The triphasic hepatic vein flow velocity waveform pattern was more frequent in the controls as compared with the diseased patients. However, no statistically significant difference was observed between the

Table 3 Characteristics and partial results of the main published studies utilizing the hepatic vein flow pattern, as compared with the present study.

Authors	Sample	*n*	Pattern	Pathological right hepatic vein pattern	
				With disease	Control
Dietrich et al., 1998[14]	HCV	135	Biopsy	53%	25%
Karabulut et al., 2004[15]	Obese	186	Biopsy	45%	1%
Oguzkurt et al., 2005[6]	Steatosis	90	Biopsy	43%	2%
The present study	NAFLD	80	Biopsy	52%	5%

HCV, chronic hepatitis C virus; NAFLD, nonalcoholic fatty liver disease.

right hepatic vein flow velocity waveform patterns in the three steatotic sub-groups.

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