Effect of severity of steatosis as assessed ultrasonographically on hepatic vascular indices in non-alcoholic fatty liver disease

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Abstract

Early monitoring of non-alcoholic fatty liver disease (NAFLD) progression in obese patients is important to avoid the development of complications associated with fatty infiltration. The aim of this study was to investigate the relationship between the degrees of fatty infiltration and reduced vascular compliance in NAFLD patients in the three main hepatic vessels. Material and methods: Two hundred and forty subjects were enrolled in the study. They were divided into 4 groups: 60 controls, 60 grade 1 NAFLD patients, 60 grade 2 NAFLD patients and 60 grade 3 NAFLD patients. After US confirmation of the presence and grade of NAFLD, the peak and mean portal vein velocity (PPVV and MPVV, respectively), the hepatic artery resistance index (HARI), and the phasicity of the hepatic vein were measured.

Results: The PPVV was 19.6 ± 2.4 cm/sec in patients with grade 1 fatty liver, 17.6 ± 1.2 cm/sec in grade 2 and 15.4 ± 1.1 cm/sec in grade 3. The MPVV was 16.6 ± 2.4 cm/sec in patients with grade 1 fatty liver, 16.6 ± 2.9 cm/sec in grade 2 and 12.7 ± 0.7 cm/sec in grade 3. The HARI was 0.75 in patients with grade 1 fatty liver, 0.68 in grade 2 and 0.64 in grade 3. There was an inverse relationship between PPVV, MPVV and HARI and different grades of fatty liver in patients (p = 0.001 for PPVV (Figure 7) and HARI, p = 0.006 for MPVV). Conclusion: The values of the investigated liver blood flow parameters were inversely correlated with the fatty infiltration grading. Fatty infiltration can severely influence hepatic blood flow, pointing attention to the importance of early diagnosis and the need for hepatic vessel flow abnormalities characterization in the NAFLD population.

Keywords: non-alcoholic fatty liver, blood flow, ultrasonography
rhosis. Fat deposition in the liver is also present in a range of pathological conditions, such as infectious hepatitis, drug toxicity, malignancies, or inherited disorders [4].

On ultrasonography (US) fatty liver can be visualized as an increase in parenchymal echogenicity on gray-scale images and can be graded as mild, moderate, and severe [5-7]. The prevalence of nonalcoholic fatty liver by US examination has been reported to be at least 14% in developed countries [8,9]. Abdominal US examination was found to have a sensitivity of 82-89% and a specificity of 93% for identifying fatty infiltration of the liver [10].

The relative contribution of intrahepatic fat deposition to the hepatic vessels flow pattern alterations is unknown [11]. However, new Doppler US studies suggest that diffuse fatty infiltration of the liver might alter the flow properties in the hepatic veins and artery [12-14]. Recent findings by Balci et al [15], Erdogmus et al [16] and Mohammadina et al [17] suggest that portal venous velocity and pulsatility decrease in patients with fatty liver diseases. The alteration in Doppler waveform pattern of portal vein with fatty liver population indicates reduced vascular compliance in the liver [16].

Early monitoring of NAFLD progression in obese patients is important to avoid the development of the complications associated with fatty infiltration. US examinations are a convenient, non-invasive, and widely accessible method for monitoring the condition of liver parenchyma. Furthermore, it has other advantages over invasive biopsy sampling: it also enables the examiner to collect data on functional changes of the liver caused by fatty infiltration, such as those of the hepatic vessel flow.

Since fatty infiltration of the liver is a common condition not only in the obese population but also accompanying other forms of hepatic irregularities, it is of great significance to clarify the individual effect of NAFLD on hepatic blood flow patterns. Therefore, the aim of this study was to investigate the relationship between the degrees of fatty infiltration and reduced vascular compliance in NAFLD patients in the three main hepatic vessels (i.e. the portal vein, the hepatic artery and the hepatic vein).

**Material and methods**

Gray scale US examination of the liver parenchyma and color Doppler ultrasonographic (DUS) examination of the portal vein (PV), hepatic artery (HA) and hepatic vein (HV) was performed in 180 patients who were consecutively admitted to the Gastroenterology outpatient clinic and referred to the Radiology department for abdominal US, and in 60 healthy subjects. The patients were divided into three groups (each group consisted of 60 patients) based on the degree of fatty infiltration: mild fatty liver (grade 1), moderate fatty liver (grade 2) and severe fatty liver (grade 3) according to US appearance of hepatosteatosis. The control subjects were classified as grade 0. They were selected from the hospital staff who have to undergo routine laboratory examination every 6 months. The study was approved by the University Institutional Review Board and Ethics Committee. A written informed consent was obtained from all participants in the presence of a witness. Only hepatitis B surface antigen (HBS Ag) and hepatitis C antibody (HCV Ab) negative patients were enrolled. The body mass index (BMI) was calculated for every patient included in the study.

Subjects with heart disease, acute or chronic liver disease, acute or chronic kidney disease, any malignancy, alcohol consumption, pregnancy, liver masses, taking any medication with adverse effect on liver or cardiovascular drugs and those who had underwent abdominal or thoracic surgery were excluded.

B-mode examination for grading of fatty liver was done by a single radiologist with two years’ experience in abdominal US and color Doppler examination of controls and patients by a radiologist with 6 years’ experience in this field using the same system- a Toshiba, Nemio 30 machine with a 3.5 MHz convex transducer. The radiologist performing color Doppler examination was blinded with regard to B-mode US and laboratory findings of patients and control subjects.

All patients also had laboratory examination for both transaminases (ALT: alanine aminotransferase, AST: aspartate aminotransferase) which was ordered by their gastroenterologist. Only 18 patients in normal group had laboratory results for AST and ALT so their ALT and AST was not included in final analysis.

US examinations were performed after 8 to 12 hours fasting. Each subject was examined in the supine and 60° left lateral positions during quiet inspiration and asked to stop breathing during shallow inspiration for recording the correct spectral Doppler for at least 6 seconds. The presence or absence and grading of fatty infiltration of the liver were recorded. Grade 0 of fatty infiltration was considered to be the normal liver echogenicity. In grade 1 (mild) fatty infiltration, echogenicity was slightly increased, with normal visualization of the diaphragm and the intrahepatic vessel borders. The grade 2 (moderate) of fatty infiltration was established when echogenicity was moderately increased, with slightly impaired visualization of the diaphragm or intrahepatic vessels. In grade 3 (severe) of fatty infiltration, echogenicity was markedly increased with poor or visualization of the diaphragm, the intrahepatic vessels, and the posterior portion of the right lobe.

The Doppler parameters were measured three times in the same place and the mean value was included in the
The peak portal vein velocity (PPVV) and mean portal vein velocity (MPVV) in spectral analysis were calculated in all subjects. The point of measurement was the proximal part of the portal vein before bifurcation and Doppler angle was set at 45° (fig 1). The spectral Doppler waveform was determinate in the middle part of the hepatic vein-3-4 cm away from the inferior vena cava (IVC) to avoid any influence of waveform alterations in the IVC via the right lateral intercostals during quiet inspiration. We classified all patients and controls into three groups according to spectral waveform patterns: group 1- regular triphasic waveform (fig 2), group 2- biphasic waveform without a reverse flow (fig 3), and group 3- monophasic waveform (fig 4). The hepatic artery resistive index (HARI) was measured in porta hep-
In the area where the hepatic artery passes anterior to the portal vein (fig 5).

Statistical analysis was performed using SPSS (version 16, Chicago, IL, USA). Data are expressed as mean ± standard deviation for numerical variables. We used independent chi-square test, T-test and one-way analysis of variance (ANOVA) to analyze data. P-values ≤0.05 was considered to be statistically significant.

Results

Two hundred and forty subjects participated, 120 male and 120 female. Mean age was 48.23 ± 9.6 years (range: 20-73 years). They were divided into 4 groups: 60 controls (30 male, 30 female), 180 patient with fatty liver including 60 grade 1 fatty liver patients (27 male, 33 female), 60 grade 2 fatty liver patients (32 male, 28 female) and 60 grade 3 fatty liver patients (31 male, 29 female).

The mean BMI was 25.6 ± 2 kg/m² in the control group and 29.5 ± 4.1 kg/m² in the fatty liver group (p<0.001). The mean BMI was 28 ± 2.5 kg/m² in patients with grade 1 fatty liver, 29.5 ± 4.2 kg/m² in grade 2 and 31 ± 4.7 kg/m² in grade 3 (p<0.001) (fig 6).

The PPVV was 24.8 ± 2.6 cm/sec in control subjects and 17.5 ± 2.4 cm/sec in the fatty liver group. The MPVV was 20.4 ± 1.5 cm/sec in the control and 15.3 ± 7.7 cm/sec in the fatty liver group. The mean HARI was 0.77 in the normal and 0.69 in the fatty liver group. The difference between the control and the fatty liver group was significant in all the above parameters (p<0.001).

The PPVV was 19.6 ± 2.4 cm/sec in patients with grade 1 fatty liver, 17.6 ± 1.2 cm/sec in grade 2 and 15.4 ± 1.1 cm/sec in grade 3. The MPVV was 16.6 ± 2.4 cm/sec in patients with grade 1 fatty liver, 16.6 ± 2.9 cm/sec in grade 2 and 12.7 ± 0.7 cm/sec in grade 3. The HARI was 0.75 in patients with grade 1 fatty liver, 0.68 in grade 2 and 0.64 in grade 3. There was an inverse relationship between PPVV, MPVV and HARI and different grades of fatty liver in patients [p = 0.001 for PPVV (fig 7) and HARI, p = 0.006 for MPVV (fig 8)]. Table I summarizes the correlation between vascular indices (PPVV, MPVV, HARI) and grading of the fatty liver.

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<th>Table I. The relationship between BMI, peak portal vein velocity, mean portal vein velocity and grading of the fatty liver.</th>
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*Correlation is significant at the 0.01 level (2-tailed); BMI-body mass index; PPVV-peak portal vein velocity; MPVV-mean portal vein velocity

Fig 6. Positive association between grade and BMI (r=0.303, p<0.001)

Fig 7. Negative association between grade and PPVV (r=0.701, p<0.001)
In control subjects the hepatic vein flow was triphasic in 54 (90%), biphasic in 6 (10%) and monophasic in no (0%) cases. Hepatic vein was triphasic in 47 (78%), biphasic in 9 (15%) and monophasic in 4 (7%) patients with grade 1 fatty liver. Hepatic vein was triphasic in 34 (57%), biphasic in 21 (35%) and monophasic in 5 (8%) patients with grade 2 fatty livers. Hepatic vein was triphasic in 24 (40%), biphasic in 24 (40%) and monophasic in 12 (20%) patients with grade 3 fatty livers. There was a significant difference in HV phasicity between fatty liver patients and controls (p<0.001). Table II summarizes the correlation between PPVV, MPVV and grading of the fatty liver.

The mean AST was 21.33 ± 6.83 in grade 1, 23.08 ± 6.83 in grade 2 and 25.13 ± 8.07 in grade 3 fatty liver. The mean ALT was 21.82 ± 10.27 in grade 1, 25.02 ± 13.07 in grade 2 and 36.42 ± 16.99 in grade 3 fatty liver.

**Discussion**

In our study, we investigated characteristic flow parameters of the three main hepatic vessels in order to describe the effect of NAFLD on liver vascular compliance. The measured parameters were PPVV and MPVV for assessing the portal vein flow properties, HARI for the hepatic artery and phasicity of the blood flow for the hepatic vein.

In a healthy individual, the portal vein flow accounts for about 80% of the total liver circulation [18]. PPVV showed a constant tendency of decrease as the grade of fatty infiltration increased. However, the value of MPVV was the same for the first two grades of fatty liver, and a further decrease was observed in the most severe grade of fatty infiltration. Interestingly, the tendency of decrease for HARI among different grades of fatty liver was distinct from that observed for MPVV. The flow values characteristic for the hepatic artery were similar for grade 2 and grade 3 fatty infiltration; however, we observed a greater difference between grades 1 and 2. These observations indicate that HARI is probably a more useful marker for early detection of hepatic blood flow alterations caused by NAFLD than MPVV, since notable alterations of this parameter are already present at the early grades of the disease.

Our findings indicate that the phasicity of the hepatic vein measured at the middle part of the vessel is inversely proportional to the grade of fatty infiltration of the liver. While there was no monophasic flow detected in control subjects, and only 10% of them had biphasic flow, 22% of grade 1 fatty liver patients, 43% of grade 2 fatty liver patients and 60% of grade 3 fatty liver patients had abnormal flow phasicity, which shows a constant tendency of increase as the severity of NAFLD progresses.

We found that the values of the investigated liver blood flow parameters inversely correlate with the grade of fatty infiltration. An important component of this relationship might be the increased intra-abdominal pressure in patients with higher BMI values, which has a deteriorating effect on hepatic blood flow. The other important component could be the subjectivity of the fatty liver assessment by the investigator.

Previous investigations in pediatric populations suggest that, in accordance with our findings in adults, there are positive correlations of BMI and grade of NAFLD with HARI, and inverse correlations with phasicity of the HV in obese children [19,20]. These results indicate that the hepatic arterial perfusion is decreased with increasing levels of BMI in children with NAFLD.

Paralleling previous and current findings in NAFLD, patients with alcoholic cirrhosis have also been shown to have distinct alterations in portal vein flow properties [21,22]. In these studies, the velocity in the portal vein significantly correlated with the stage of liver disease. The average MPVV in study-investigating patients with...
alcoholic cirrhosis was 12.56 cm/sec [23]. Surprisingly, we measured similar values (an average MPVV of 12.7 cm/sec) in grade 3 NAFLD patients. This fact indicates that fatty infiltration can severely influence the hepatic blood flow (causing damage that is similar to the effects of alcohol in this respect), and points attention to the importance of early diagnosis and the urgent need of the characterization of hepatic vessel flow abnormalities in the NAFLD population.

Reduced parenchymal and vascular compliance has already been suggested as the cause of bi- and monophasic venous flow in NAFLD [12,13]. Alterations on the cellular level also contribute to these observations: the limited distensibility of the liver capsule leads to the compression of hepatic veins by the increased volume of hepatocytes due to intracellular fat deposition.

A major limitation of our study is that the US diagnosis of NAFLD was not confirmed by biopsy. However, we applied strict exclusion criteria to provide the homogeneity of the studied population and to ensure that no other pathologies of the liver that could have influenced our measurements were present. Furthermore, it has been demonstrated that grades of NAFLD assessed by ultrasonography significantly correlated with histopathologic evaluation of steatosis [24,25].

The presence of fibrosis in the liver parenchyma will cause an increase in echogenicity and hepatitis causes the decrease of the liver echogenicity. These two conditions in turn can influence the subjective evaluation of overall liver echogenicity and grading of steatosis [26].

Fibrosis of liver parenchyma can cause damage to the sinusoids, which will in turn increase the resistance to the portal vein flow, dilation of portal vein and consequently a decrease in the portal vein flow velocity. When portal venous inflow decreases, hepatic artery diastolic flow increases to compensate the diminished portal vein flow and can lead to a reduction in hepatic artery’s RI [26]. In this condition, the liver parenchyma should be carefully evaluated to rule out focal or diffuse liver fibrosis [26].

In liver fibrosis or diffuse liver disease, the parenchymal compliance of liver decrease and hepatic vein spectral periodicity shows decrease periodicity with progression of fibrosis.

Due to decrease in the parenchymal compliance in liver fibrosis and diffuse liver disease, hepatic vein spectral periodicity decreases [26]. So the more liver fibrosis progresses, the less the periodicity of hepatic vein becomes.

In conclusion, the cause of the alterations of hepatic blood flow patterns we have detected in NAFLD patients is probably reduced vascular compliance of the liver caused by fatty infiltration. Our results indicate that NAFLD alone in the lack of complicating factors (such as infections, drug toxicity, malignancy, or inherited metabolic disorders) notably alters flow properties of the hepatic vessels.

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Conflict of interest: none

Abreviations:


References