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FULL PAPER

A novel technique for the assessment of total liver blood flow in pregnancy: interrater and intrarater agreements

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Objective: To improve the technique for hepatic blood flow examination, with the objective of investigating the role for Doppler flowmetry of the liver in monitoring pregnant females with pre-eclampsia.

Methods: Two physicians independently examined a group of 50 healthy pregnant females. The main difference in the proposed technique is the measurement of the vessel cross-sectional area and the adjustment of the Doppler samples according to the diameter of each vessel. The portal vein was studied by using two approaches: in the epigastrium, to measure the diameter, and in the intercostal, for Doppler sample collection. The common hepatic artery was studied by using the epigastric approach. The average of three measurements of each vessel, in each subject, with intrarater and interrater agreements, was compared.

Results: The intraclass correlation coefficient for the intrarater flow measurements of the hepatic artery and portal vein ranged from 0.98 to 0.99 ($p < 0.0001$). The intraclass correlation coefficients for the interrater flow measurements was 0.93 for the flow of the portal vein ($p < 0.0001$), 0.94 for the flow of the hepatic artery ($p < 0.0001$) and 0.96 for the measurement of the portal vein diameter ($p < 0.0001$).

Conclusion: The new technique for evaluation of blood flow to the liver displayed excellent reproducibility, possibly because of the adjustment of the Doppler samples according to the diameter of each vessel.

Advances in knowledge: This approach will add reliability to the method. It opens a vast field of investigation given the importance of liver vascular lesions in pre-eclampsia.

INTRODUCTION

One of the limitations of liver Doppler ultrasonography in clinical practice is its low reproducibility,¹ i.e. the difference in the values obtained by the same observer or among observers. This may be related to insufficient standardization of the examination technique, with emphasis on the measurement of vessel caliber and Doppler sample volume. These factors may play a critical role in the reproducibility of the examination because 1 mm of uncertainty in the measurement of the diameter can lead to a 20% error in calculating the vessel area.²

Given the vascular origin of the liver lesion in pre-eclampsia, some authors have studied Doppler ultrasonography to evaluate the hepatic circulation in these cases. Kawabata et al³ compared the flow of the portal vein and hepatic artery at the time of hospitalization among 60 healthy pregnant females and 58 pregnant females diagnosed with

severe pre-eclampsia. In this last group, nine patients developed HELLP syndrome (haemolysis, elevated liver enzymes and low platelet count) between 2 and 4 days after admission. In the group of pregnant females with severe pre-eclampsia, the portal venous flow was reduced to 70% that of the control group, but the flow of the hepatic artery was increased by 30% and the total liver blood flow was maintained. In the subgroup of patients who developed HELLP syndrome, blood flows in both the portal vein and hepatic artery were reduced to 30% and 70% of those of the control group, respectively. The authors highlight the fact that the reduction in total liver blood flow preceded the onset of HELLP syndrome. Another study compared the liver Doppler ultrasonography results of 20 pregnant females with HELLP syndrome to those of 20 healthy pregnant females. The authors found similar results in that the blood flow in the hepatic artery in patients with HELLP syndrome was reduced to 30%, the blood flow in the portal vein was

reduced to 45% and the total blood flow was reduced to 40% that of the control group.⁴

Liver involvement in pre-eclampsia increases maternal morbidity and mortality. The pathophysiological mechanism of pre-eclampsia has as main events endothelial injury and exaggerated systemic inflammatory response, generated from placental hypoxia and release of pro-inflammatory and anti-angiogenic cytokines in circulation. In the liver, these events manifest through vasospasm and microangiopathic vascular lesion.⁵

We propose here an enhancement of the technique for study of total liver blood flow in order to increase its reproducibility and thus seek a role for Doppler ultrasonography of the liver in monitoring pregnant females with pre-eclampsia.

METHODS AND MATERIALS

The examinations were performed by two physicians (EPQA and VMM), both specialists in vascular ultrasonography with more than 20 years' experience, who developed the technique. The study was approved by the ethics committee of the University Hospital Gaffré e Guinle (Hospital Universitário Gaffré e Guinle). All the volunteers signed an informed consent form. From November 2015 to April 2016, 55 healthy pregnant females with a gestational age between 20 and 36 weeks, who attended the maternity ward of the Hospital Universitário Gaffré e Guinle, were selected randomly. Gestational age was confirmed based on the date of the last menstrual period and by ultrasonography performed before the 20th week of pregnancy. The females were grouped per full week of gestation. In addition to examination of hepatic blood flow, all pregnant females received obstetric examination on a routine basis at the time of the survey.

The inclusion criteria were as follows: singleton pregnancy; no history of smoking, alcoholism or use of illegal drugs; the absence of the use of drugs that could interfere with liver function or liver blood flow; the absence of chronic maternal diseases such as liver disease (with or without portal hypertension), kidney or heart disease, arterial hypertension, diabetes mellitus and anaemia; the absence of major foetal malformations assessed by using ultrasonography in the second trimester. The exclusion criteria were as follows: pregnant females with changes in the portal vein or common hepatic artery, such as partial or total thrombosis, aneurysm, arteriovenous fistula and anatomical variants; pregnant females who did not fulfil the fasting period of at least 6 h; and pregnant females who were unable to hold their breaths during the examination of hepatic blood flow.

Each volunteer was examined independently by one sonographer and soon thereafter by the other. For each subject, the measures of velocity and flow were performed three times in succession; and between a measure and another, the transducer was removed and repositioned for the acquisition of the following sampling. This procedure was followed by both examiners so that for each subject, six measurements of each vessel were performed. The values obtained by each examiner were organized by an assistant, so that the examiners could not compare each others' measurements during data collection.

The examinations were performed in all the volunteers by using the same apparatus (GE Healthcare S6), with capacity for a good image in B-mode, colour Doppler and spectral Doppler. A 2- to 5.2-MHz convex transducer was used. All the females were examined in the supine position, with a fasting period of at least 6 h and after at least 10 min of rest.

The entire length of the portal vein and common hepatic artery was analyzed. The analysis and the measurement of the diameter of the vessels were performed in B-mode, with just one-depth focus of each vessel. The dynamic range was adjusted to 45–50 dB, and the gain was reduced to eliminate artefacts of intraluminal reverberation.

For the study of the portal vein, two approaches were used, one for the measurement of the diameter of the vessel and analysis of its anatomical integrity, and another for the acquisition of Doppler sample volume. The diameter of the portal vein was measured in the axial scan, in the epigastrium, 1 cm before the origin of its left branch, with the patient breathing softly. At this location, the ultrasound beam can be easily positioned perpendicular to the vessel, allowing a sharp image of its parallel walls. Image magnification, gain reduction and dynamic range reduction were performed to increase the accuracy of the measurement. The cursors were placed with a tracker ball on the internal margins of the vessel wall (Figure 1).

For the measurement of portal flow, the transducer was transferred to the intercostal position, in the projection of the anterior axillary line at the height of the eighth or ninth intercostal spaces. At this location, the portal vein, neck of the gallbladder and inferior vena cava can be identified. Colour Doppler mapping of the portal flow was performed. The length of the sample volume was adjusted to an extent equal to or immediately below the internal diameter of the portal vein, previously established in B-mode. The pulsed Doppler cursor was placed before the bifurcation of the portal vein. In this location, an excellent beam

Figure 1. Measurement of the internal diameter of the portal vein 1 cm before the origin of its left branch. AHP, proper hepatic artery; pan, spleen; RD, right portal vein; RE, left portal vein; VP, portal vein.

angle, which must be $<60^\circ$, could be obtained. Adjustments were made for velocity scale, wall filter, increase and baseline. Thereafter, a precise correction of the angle of insonation was performed, and the Doppler sample of the average flow velocities [time-averaged mean velocity (TAMEAN)] was captured for 3–6 s, with the pregnant female holding her breath. Soon after, the cursors were positioned inside the colour map, with the measurement of the diameter of the vessel, to enable the software to automatically calculate the flow volume (Figure 2).

The common hepatic artery was studied by using an axial scan of the epigastrium, soon after its origin in the coeliac trunk. At this location, a straight segment of the artery can be observed and the ultrasound beam can easily be positioned perpendicular to the vessel, allowing a sharp image of its parallel walls. Image magnification, gain reduction and dynamic range reduction were performed to increase the accuracy of the measurement. The cursors were placed with a tracker ball on the internal margins of the vessel wall, as was performed in the measurement of the portal vein (Figure 3). For the measurement of liver arterial flow, the transducer had to be adjusted, at the same location, in order to obtain an angle of insonation of $\leq 60^\circ$. Colour Doppler mapping of arterial flow and adjustment of the length of the sample volume in one dimension, equal to or immediately below the one previously measured in B-mode, was performed. As with the measure of portal flow, adjustments were made to scale, in the wall filter, increase and baseline. Afterwards, precise correction of the beam angle was performed, and Doppler samples of the average flow velocities were obtained (TAMEAN) for at least 2–3 similar successive cycles, with the pregnant female holding her breath. Soon after, the cursors were positioned inside the colour map with the measurement of the diameter of the vessel, therefore the product software automatically calculated the volume of flow (Figure 4). The flow measurement of the common hepatic

artery is indeed the trickiest moment of the examination due to the difficulty to find the proper angle to obtain Doppler samples, a procedure that requires a longer training time. In difficult patients, we lower the Doppler frequency to approximately 1.8–2.0 MHz, keeping a 4.0-MHz B-mode frequency or lowering to 3.0 MHz. Besides that, we can turn off the colour mapping, using only duplex scanning, in order to increase the frame rate. Finally, if difficulties persist, we replace the convex probe for a sector (Figures 5 and 6) or a microconvex probe (Figures 7 and 8). Note that the vessels are measured in B-mode with low gain, dynamic range <50 dB and image amplification. Thus, we measure the intraluminal diameter of the vessel and place the measurements with the cursors in the colour map when measuring the flow.

For each subject, the averages of the three measurements of the flow of each vessel obtained by each examiner (A and B) were considered. The hepatic blood flow was calculated by using the sum of the flow of the portal vein with the flow of the common hepatic artery.

Statistical analysis was performed by using the following parameters, measured by using Doppler ultrasonography, for the portal vein and hepatic artery as the dependent variables:

- caliber: vessel diameter measured in millimetres
- TAMEAN: the average of the curve of average velocities obtained in a specific time interval and expressed in cm s^{-1}
- flow volume: evaluated in ml min^{-1} .

All the parameters studied were expressed in averages and standard deviations. The pattern of distribution of the values of the variables was analyzed by using the Kolmogorov–Smirnov normality test. In order to test the hypothesis that the proposed technique is more reproducible than the traditional technique, we assessed possible intrarater and interrater agreement differences. We evaluated differences in averages between the observers and for the same observer by using the Stuart *t* test for the following variables: caliber of the portal vein, flow volume in the portal vein, caliber of the

Figure 2. Measurement of the portal flow right above the inferior vena cava and below the neck of the gallbladder in an intercostal approach. Note the length of the sample volume adjusted to the caliber of the portal vein. Also note the measurement of the vessel diameter placed inside the colour map for automatic calculation. vb, gallbladder; vci, inferior vena cava; VP, portal vein.

Figure 3. Measurement of the internal diameter of the common hepatic artery in axial resolution. Ao, aorta; AHC, common hepatic artery; vci, inferior vena cava; VP, portal vein.

Figure 4. Measurement of the common hepatic artery flow. Note the measurement of the vessel diameter placed inside the colour map for automatic calculation. AHC, common hepatic artery.

common hepatic artery and flow volume in the common hepatic artery. In addition, we applied Pearson's correlation test for the values found for the same variables. Then, the Bland–Altman graphs were used for a better visual interpretation of the differences.

RESULTS

55 healthy pregnant females were recruited for the study. Five examinations were not performed due to technical difficulties. The difficulties encountered were due to obesity, distention due to abdominal gas and intolerance to the supine position. The pregnant females had an average age of 28 years (range, 18–40 years), and the average gestational age was 28 weeks (range, 20–36 years). The intrarater and interrater variabilities were not significant as demonstrated by using the Stuart *t*-test and correlation coefficients (Table 1).

The intraclass correlation coefficient was used for evaluation of the intrarater variability in assessment of flow in the portal vein and common hepatic artery (Table 2).

Figure 5. Use of the sector probe on the common hepatic artery (CHA) in B-mode. A, Aorta; CHA, common hepatic artery; SA, splenic artery.

Figure 6. Use of the sector probe on the common hepatic artery (CHA) in colour Doppler. CHA, common hepatic artery.

We also used the Bland–Altman graphs for better visualization of possible intrarater and interrater differences (Figure 9).

DISCUSSION

The liver receives a double blood supply, 75% from the portal vein and 25% from the hepatic artery. Colour Doppler ultrasonography is a useful method for vascular evaluation of the liver in patients who received organ transplantation, with liver diseases and suspicion of portal hypertension among other indications.⁶ However, the study of hepatic blood flow has been relatively limited.

In a study of reproducibility of liver Doppler ultrasonography, an interrater coefficient of variation of 0.83 was obtained in measuring the cross-sectional area of the hepatic artery and 0.53 for the portal vein. In blood flow measurement, the coefficient of interrater variation was 0.85 for the hepatic artery and 0.39 for the portal vein.² A review on the haemodynamic evaluation of patients with portal hypertension through Doppler ultrasonography studies

Figure 7. Use of the microconvex probe on the common hepatic artery (CHA) in B-mode. Ao, Aorta; CHA, common hepatic artery; CT, celiac trunk; ICV, inferior vena cava; PV, portal vein; SA, splenic artery.

Figure 8. Use of the microconvex probe on the common hepatic artery (CHA) in duplex Doppler. CHA, common hepatoartery.

concluded that blood flow in the portal vein can usually be measured, although the reproducibility of this examination is low. On the other hand, measurement of the blood flow in the hepatic artery was considered impossible owing to the technical difficulties of the examination.¹ A study on the velocimetry of the hepatic artery, which compared non-pregnant females and healthy pregnant females, pointed out technical difficulties in obtaining samples for measurement of total hepatic blood flow.⁷ Guidelines for studies on reproducibility and reliability of measures underscore the importance of intraclass correlation coefficients of at least 0.90–0.95 in controlled research environments to support decisions in clinical practice.⁸

Table 2. Intraclass correlation coefficient in the evaluation of intrarater agreement in the evaluation of volume of flow in the portal vein and common hepatic artery

Observer	Portal vein	Common hepatic artery
A	0.982	0.997
B	0.984	0.998

Published research on liver flowmetry have in common aspects such as preparation of the subject, which must involve fasting and a few minutes of rest; the use of a 2- to 5-MHz convex transducer; adjustments in the apparatus; and analysis of wave profiles of two to four cardiac cycles, with breath-hold. However, measuring the vessel diameter is not a rule among researchers; even the choice of which vessel is to be studied can vary.

With regard to blood flow, some studies assessed the common hepatic artery,^{2,7,9} and others evaluated the proper hepatic artery.^{10,11} In subjects with a normal anatomy, the common hepatic artery becomes the proper hepatic artery after the departure of the right gastric branch. Unfortunately, this branch is rarely detected on ultrasonography; therefore, the flow in the hepatic artery proper is difficult to assess accurately. The examination in fasting ensures that the flow to the right gastric and duodenal arteries is minimized and enables the use of the common hepatic artery to compose the estimation of total hepatic blood flow.² In the present study, the liver arterial flow was studied through the common hepatic artery because, in addition

Table 1. Paired *t*-test results and correlation coefficients for intrarater and interrater variabilities of the new technique for evaluation of total liver blood flow in pregnancy

<i>n</i> = 50	Mean values	Mean difference	CI 95% of the difference	<i>p</i> -value	Correlation coefficient	<i>p</i> -value
Intrarater differences						
CHA FV ^a (A1–A3)	A1: 486.7 A3: 483.9	−2.88	−17.20 to 11.44	0.68	0.98	<0.0001
CHA FV (B1–B3)	B1: 468.1 B3: 468.3	0.26	−10.24 to 10.75	0.96	0.99	<0.0001
PV FV (A1–A3)	A1: 1027.5 A3: 1026.2	−0.62	−16.74 to 15.49	0.93	0.98	<0.0001
PV FV (B1–B3)	B1: 1044.3 B3: 1032.8	−11.5	−29.49 to 6.45	0.20	0.98	<0.0001
Interrater differences						
CHA FV (A–B)	A: 488.4 B: 468.4	−20.02	−44.97 to 4.93	0.11	0.94	<0.0001
CHA caliber ^b (A–B)	A: 5.09 B: 5.07	−0.03	−0.10 to 0.05	0.52	0.94	<0.0001
PV FV (A–B)	A: 1023.0 B: 1037.5	14.4	−18.52 to 47.43	0.38	0.93	<0.0001
PV caliber (A–B)	A: 11.31 B: 11.38	0.07	−0.03 to 0.17	0.15	0.96	<0.0001

CHA, common hepatic artery; CI, confidence interval; FV, flow volume; A, Rater A; B, Rater B; A1, Rater A first evaluation; A3, Rater A third evaluation; B1, Rater B first evaluation; B3, Rater B third evaluation; PV, portal vein.

^aFlow volume (ml min^{−1}).

^bCaliber (mm).

Figure 9. Bland-Altman graphs of the intrarater and interrater differences in measuring blood flow in the portal vein and in the common hepatic artery. (a) Portal vein intrarater difference for Rater A; (b) portal vein intrarater difference for Rater B; (c) common hepatic artery intrarater difference for Rater A; (d) common hepatic artery intrarater difference for Rater B; (e) portal vein interrater difference; (f) common hepatic artery interrater difference. A, Rater A; A1, Rater A first evaluation; A3, Rater A third evaluation; B, Rater B; B1, Rater B first evaluation; B3, Rater B third evaluation; CHA, common hepatic artery; FV, flow volume; PV, portal vein; SD, standard deviation.

Table 3. Overview of Doppler flowmetry parameters

(I) Measurement of vessel diameter in B-mode
(1) image of the vessel at a perpendicular position to the ultrasound beam
(2) only one depth focus of each vessel
(3) reduction of dynamic range to 45–50 dB
(4) vessel image amplification
(5) gain reduction
(6) cursors placed on the internal margins of the vessel wall.
(II) Doppler flowmetry data acquisition
(1) colour Doppler mapping (optional)
(2) adjustment of the length of the sample volume in one dimension equal to or immediately below the one previously measured in B-mode
(3) Doppler angle $\leq 60^\circ$
(4) time-averaged mean velocity of 3–6 s in the portal vein and for at least 2–3 cycles in the hepatic artery
(5) positioning of the cursors with the measurement of the internal vessel diameter in the colour map or greyscale image.

to the anatomical issue described earlier, the common hepatic artery is straighter, which makes the measurement of its diameter more consistent. The single access to the common hepatic artery is the epigastric approach, since the intercostal approach is not able to reach this artery. On venous blood flow, the diameter of the portal vein is most often measured in the intercostal approach, when it crosses with the hepatic artery proper.^{11,12} This marker may be inadequate given the anatomical variations of the intersection of these vessels in the liver. Although the intercostal approach facilitates the achievement of a better angle for the Doppler, it does not allow a direct visualization of its entire path, and the measure of the portal vein section in an almost vertical position is hampered by the lack of lateral resolution, making it difficult to discriminate between the echogenic wall and the anechoic lumen of the vessel. The measurement of the vessel size is a critical point for the reproducibility of the tests, taking into account that an apparently minimal difference in vessel diameter will be maximized in the calculation of flow, which is given by the product of the cross-sectional area of the vessel and blood flow velocity. As a result, this new technique proposes a careful measure of the vessel diameter by using approaches to focus the ultrasound beam at 90° for both vessels, allowing a clear

vision of the limits of the vascular walls. The magnification and other device settings allow for a more accurate measure of the diameter of vessels.

Another particularly relevant issue is the volume of the Doppler sample. In small vessels, as in the case of the hepatic artery, blood flow velocities vary from the centre to the periphery of the vessel. For all velocities to be included in the calculation of the averages, the size of the Doppler sample volume should match the diameter of the vessel. The majority of flowmetry studies use fixed values for the sample volume.^{11,12} The new technique proposed takes into account the vessel size for the calculation of the sectional area and therefore of the flow in the hepatic artery and portal vein.

The new technique proposed, with emphasis on the improvement of the measurement of vessel size, and the adjustment of the sample volume allows expressive reproducibility. The intrarater and interrater correlation coefficients were ≥ 0.98 and 0.93, respectively.

Although data collection was performed by qualified examiners, which probably influenced the results obtained, the high levels of reproducibility, even in a research environment, indicate a high potential for use of the tests in clinical practice⁸ (Table 3). The limitations of the study pertain primarily to the limitations of the method, Doppler ultrasonography, in evaluating blood flow. Although in some situations its evaluation capacity in real time and sensitivity to instant changes have advantages, this feature hampers the clinical interpretation of its results. A large part of the variability found among individuals is not related to a specific condition but to differences, e.g. in cardiac output—which varies according to the temperature of the environment and the body—the heart rate, rest and fasting duration. Several authors have described the variability of Doppler results in the same individual and between individuals for non-pregnant population.^{13,14} Nevertheless, Doppler ultrasonography of the portal vein and hepatic artery is a good method in the evaluation of flow, e.g. in the liver with cirrhosis or post-transplantation.^{15,16}

The new technique opens up new prospects for the application of the evaluation of hepatic blood flow in liver diseases in general and during pregnancy. Doppler ultrasonography is a safe examination method at any stage of pregnancy and has relatively low cost and potential to contribute to the study of pregnancies complicated by hypertensive disease.

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