BMC Medical Imaging

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Detection of liver and spleen stiffness in rats with portal hypertension by two-dimensional shear wave elastography

YongJian Chen[1], JingYun Li[2], Qin Zhou[1], GuoRong Lyu[1,2*] and ShiLin Li[1]

Abstract

Background: The measurement of liver stiffness (LS) and spleen stiffness (SS) based on ultrasound elastography can be used for non-invasive assessment of portal hypertension (PH). However, there are few studies on the corresponding mechanism of increased spleen stiffness. Our aim was to use two-dimensional shear wave elastrography (2D-SWE) to evaluate the relationship between LS and SS and the severity of PH in rats. And explore the mechanism of the increase of LS and SS in PH.

Methods: Sixty male Sprague–Dawley rats were randomly divided into portal hypertension (PH group, n = 45) and normal control (NC group, n = 15). At 12 weeks, LS and SS was detected by 2D-SWE in vivo. Related hemodynamic parameters and portal vein pressure (PVP) was measured. Spleen and liver 2D-SWE detection was performed again after sacrifice. Pathological changes were observed.

Results: The SS and LS were increased in PH group ($P < 0.05$). The SS decreased after sacrifice, and what's more the magnitude of SS decline significantly higher in PH group than in NC group ($P < 0.05$). The correlation between SS and PVP is stronger than LS ($r = 0.624$, $P < 0.001$). SS has positive correlation with indexes of hyperdynamic circulation, but LS was weakly. The correlation between SS and the pathological grade ($r = 0.633$, $P < 0.001$) was lower than that in LS ($r = 0.905$, $P < 0.001$). Multiple linear regression analysis revealed that SS, portal vein inner diameter (PVD) and splenic vein blood flow velocity (SVV) were significantly associated with PH.

Conclusions: Spleen and liver measurement by 2D-SWE may be helpful in evaluating PVP. The correlation between SS and PVP is stronger than LS in rats measured by 2D-SWE. Hemodynamic circulation are important in the elevation of SS with portal hypertension. Pathological changes also have a degree of influence, but have more significance for the elevation of LS. SS may be a more effective noninvasive predictor of PH than LS.

Keywords: Portal hypertension, Two-dimensional shear wave elastography, Spleen stiffness, Liver stiffness, Hemodynamics

Background

Portal hypertension (PH) refers to the obstruction of blood flow and/or increased blood flow in the portal vein system under the action of various etiologies, resulting in a continuous increase in the pressure of the portal vein and its tributaries. It is a complex clinical syndrome. Ascites, splenomegaly, and collateral circulation formation and opening are the three common clinical

*Correspondence: lgr_feus@sina.com
[1] Department of Ultrasound, The Second Affiliated Hospital of Fujian Medical University, No. 34 North Zhongshan Road, Licheng District, , Quanzhou 362000, Fujian, China
Full list of author information is available at the end of the article

manifestations of PH. And at the present, the most commonly used clinical evaluation is hepatic venous press gradient (HVPG) [1]. It is a complicated, expensive, and invasive test available only in specialized centers, which subjects have a poor tolerance [2]. Therefore, non-invasive assessment of the degree of PH is a critical research topic. Recent studies have shown that ultrasound elastography measurement of liver (LS) and spleen stiffness (SS) has good clinical application value in predicting esophageal varices in patients with cirrhosis and portal hypertension [3–6]. Among them, two-dimensional shear wave elastrography (2D-SWE) is an effective noninvasive diagnostic tool for predicting the presence of esophageal varices [4, 7], and is highly valuable in diagnosing clinically significant portal hypertension (CSPH) [8–10]. Some studies believe that SS has better evaluation of the severity of PH than LS[11], and other scholars believe that SS combined with LS is an excellent predictor of CSPH[12, 13]. Moreover, clinical studies and metanalysis have already shown that there is a close correlation between LS and HVPG when the value $\leq$ 10–12 mmHg, but above this threshold the strength of the correlation decreases markedly, and that SS reflects portal pressure better than LS [11, 14]. However, it is still unclear why the spleen elastic modulus increases in portal hypertension. To address this, this study used an animal model and two-dimensional shear wave elastrography (2D-SWE) to detect liver and spleen stiffness, and explored the relationship between LS, SS, and portal pressure, and hemodynamics and pathological changes, to evaluate the relationship between LS and SS in assessing the severity of portal hypertension.

Methods

Test object

Sixty Male Sprague–Dawley (145–195 g) rats were purchased from Shanghai Slack Laboratory Animal Co. Ltd. They were reared adaptively with standard basic feed for 7 days, and maintained at an appropriate temperature and humidity.

Establishment of portal hypertension rat model

Using a completely random design, according to the experimental method of Königshofer [15], rats were divided into two groups, portal hypertension (PH group, n = 45) and normal control (NC group, n = 15). The PH group used the CCl_4 (Sinopharm, Beijing, China) induction method to create the model. After local skin disinfection, a CCl_4 corn oil solution (1 mL/kg body weight, twice a week) was injected subcutaneously, while the NC group was injected with corn oil only. During the experiment, the rats' general physical signs (coat color, mental state), movement flexibility, and body weight changes were observed and their body mass was measured weekly.

SWE detection

As with previous experiments, rats were injected intraperitoneally with pentobarbital sodium (50 mg/kg) and after good anesthesia, the supine position and right supine position respectively were taken, and the diagnostic apparatus (Supersonic Imagine Aixplorer, Aix-en-provence, France) was used with a frequency 5–15 MHz linear array probe [16]. After the two-dimensional ultrasound, oblique section of the intercostal area clearly shows the liver or spleen, the sampling frame needs to be placed in the parenchyma while trying to avoid visible duct structures and keeping the probe fixed and vertical. The spleen (SS) or liver stiffness (LS) were measured 5 times at the same site, and the average was taken (Fig. 1).

Detection of hemodynamic parameters

Color Doppler blood flow imaging was performed with a Mindray R70B ultrasonic diagnostic instrument using a 5–18 MHz high-frequency linear array probe. Measurements were made at the midpoint of the main portal vein, the splenic vein near the superior mesenteric artery, and the main portal vein and splenic vein in turn. The inner diameter (D) and peak velocity of blood (V) were observed, and the blood flow (Q) and congestion index (CI) were calculated using $Q = 0.57\pi D^2\ V/4 \times 60$, and $CI = \pi D^2/(4 \times 0.57 \times V)$, respectively [17].

Detection of portal pressure

The portal vein pressure (PVP) was measured by direct puncture through the main portal vein. The rat was taken in the supine position and after the limbs were fixed, the abdomen was depilated and disinfected. An incision was made about 3 cm along the midline of the abdomen to fully expose the hilar of the liver. A blunt glass minute needle was used to carefully dissociate the main portal vein and a 24 G indwelling needle was used to puncture the main portal vein. After the flow of blood begins, the other end of the indwelling needle is connected to the biological signal acquisition and analysis system through the blood pressure sensor. The PVP is recorded after the blood flow stabilizes.

SWE analysis of LS and SS in sacrificed rats

The SWE measurements were repeated after injection of a large dose of anesthetic and confirmation of sacrificed of the mice.

Pathological examination

The sampling operation was carried out after confirming the sacrificed of the mouse. The abdominal cavity was

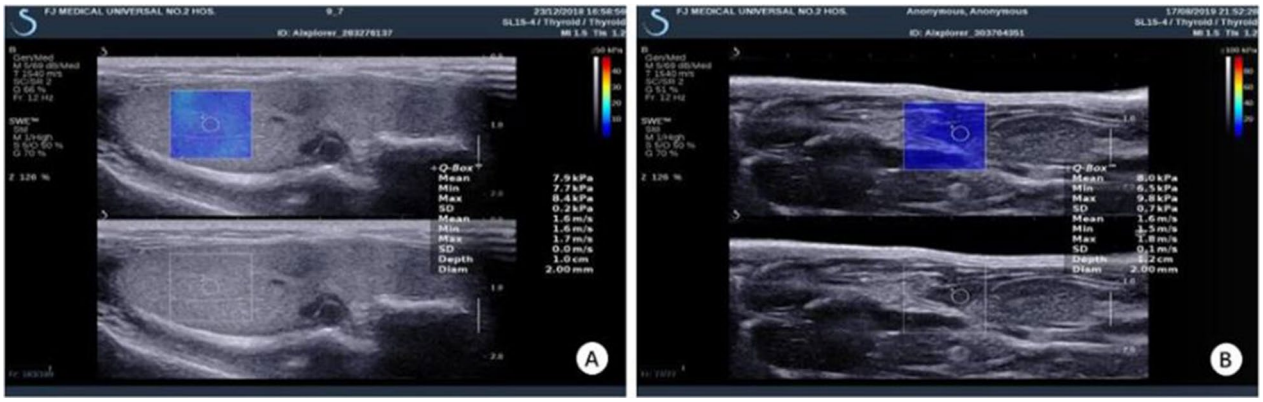

Fig. 1 SWE measurement diagram. The sampling frame needs to be placed in the parenchyma while trying to avoid visible duct structures and keeping the probe fixed and vertical. **a** SWE measurement of the elastic modulus of the spleen. **b** SWE measurement of liver elastic modulus

exposed and the morphology of the liver and spleen was observed. The organs were removed, fixed in formaldehyde, embedded in paraffin, serially sectioned, and HE staining, Masson staining, and electron microscopy were performed. According to the corresponding standards, the degree of liver fibrosis and splenic fibrosis are divided into 4 stages and 4 grades [18, 19].

Statistical analysis

Measurement data was expressed as mean ± SD and SPSS (version 19.0; SPSS) was used for all analysis. Single-factor analysis of variance was used for comparison between groups, LSD test was used for pairwise comparison within groups, count data were expressed as cases or rates, and $\chi 2$ test was used for comparison between groups. A group t test was used to analyze the SS between live and sacrificed rats in the PH and NC groups, and the LS between live and sacrificed rats in the NC group. However, the subtraction of the value of LS between live and sacrificed rats in the PH group did not satisfy the normal distribution, and Wilcoxon signed rank test of paired samples was used. Spearman correlation analysis was used to analyze the correlation between the elastic modulus and the pathology of the liver and spleen, and a Pearson correlation analysis was used for the other correlation analyses. Multiple linear regression analysis was used to analyze factors affecting PH. $P<0.05$ indicates a statistically significant difference.

Results

Portal pressure and pathological conditions

The PVP was 11.38 ± 1.63 mmHg and 5.82 ± 0.65 mmHg in the PH and NC groups, respectively, and the difference between the groups was statistically significant ($P<0.05$). The PH group showing a significantly higher PVP indicates that the model was successfully established. The pathological examination results of the liver and spleen are shown in Tables 1 and 2. The pathological grading of the spleen in the PH group was biased to grade II (Fig. 2A), and the pathological staging of the liver in the PH group was biased to stage F4 (Fig. 2B).

Analysis of elastic modulus of liver and spleen

The measurement results of the elastic modulus of the liver and spleen are shown in Table 3. (1) There was a statistically significant difference between the alive and post-sacrifice SS in the PH group ($t=11.513$, $P<0.05$). (2) There was a statistically significant difference between the alive and post-sacrifice LS in the PH group ($Z=-5.358$, $P<0.05$). (3) There was a statistically significant difference between the alive and post-sacrifice SS in the normal group ($t=3.829$, $P<0.05$).

Table 1 Pathological classification of spleen

Group	0	I	II	III	IV	Total
Spleen in PH group	0	7	34	4	0	45
Spleen in NC group	13	2	0	0	0	15
Total	13	9	34	4	0	60

PH portal hypertension, *NC* normal control

Table 2 Pathological staging of liver

Group	S0	S1	S2	S3	S4	Total
Liver in PH group	0	2	3	12	28	45
Liver in NC group	15	2	3	0	0	15
Total	15	4	6	12	28	60

PH portal hypertension, *NC* normal control

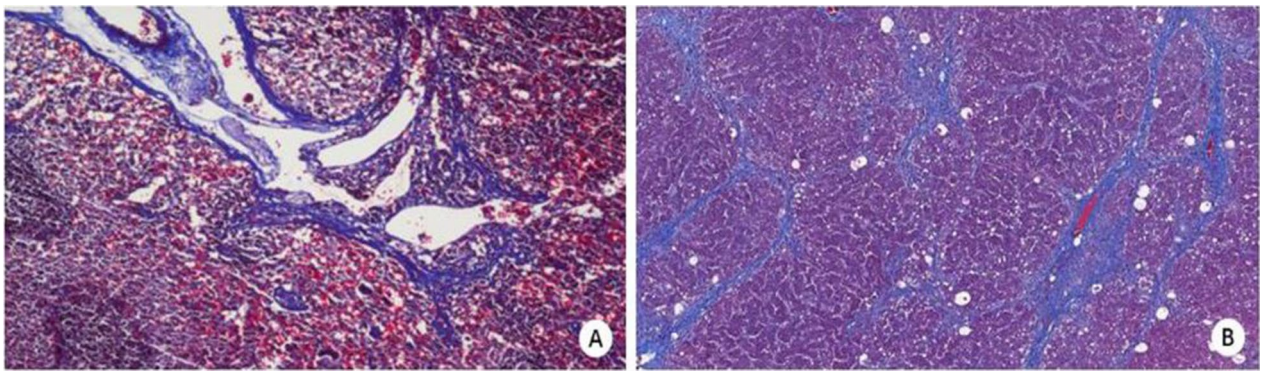

Fig. 2 **a** Most splenic fibrosis is classified as Grade II (× 100). **b** Most stages of liver fibrosis are F4 (× 100)

Table 3 Rat liver and spleen elastic modulus (kPa)

Group	Living SS (X ± SD)	Post-sacrifice SS (X ± SD)	Living SS (Q1, Q3)	Post-sacrifice LS (X ± SD)
PH group	14.46 ± 2.95	10.92 ± 1.55	(7.95, 10.95)	9.25 ± 1.98
NC group	8.75 ± 1.73	8.21 ± 1.36	(7.70, 10.10)	5.24 ± 0.39

SS spleen stiffness, *LS* liver stiffness, *PH* portal hypertension, *NC* normal control

(4) There was no statistically significant difference between alive and post-sacrifice LS in the normal group ($t = 3.238$, $P > 0.05$).

Analysis of hemodynamic parameters

Table 4 shows the hemodynamic measurement results of portal vein and splenic vein.

Correlation analysis

SS, LS, and PVP were all positively correlated, and the correlation between SS and PVP ($r = 0.746$, $P < 0.001$) was higher than that between LS and PVP ($r = 0.624$, $P < 0.001$). (2) SS was positively correlated with splenic vein congestion index ($r = 0.764$, $P < 0.001$) and also positively correlated with splenic vein blood flow ($r = 0.751$, $P < 0.001$). (3) LS was positively correlated with portal vein congestion index ($r = 0.724$, $P < 0.001$), and weakly correlated with portal vein blood flow ($r = 0.361$, $P < 0.05$). (4) SS and LS were positively correlated with pathological grading, but the correlation between SS and pathological grading of spleen ($r = 0.633$, $P < 0.001$) was lower than that between LS and liver pathological staging ($r = 0.905$, $P < 0.001$).

Multiple linear regression analysis

The original measurement of portal pressure (continuous variable) was used as the dependent variable, and

Table 4 Hemodynamic parameters and vessel diameter ($X \pm SD$)

Group	Portal vein (PV)				Splenic vein (SV)			
	D (cm)	V (cm/s)	Q(mL/min)	CI(cm ms)	D (cm)	V (cm/s)	Q (mL/min)	CI (cm ms)
PH group	0.18 ± 0. 02*	13.15 ± 2.28*	11.80 ± 3.02*	3.55 ± 0.75*	0. 12 ± 0. 02*	13.05 ± 2.09*	5.10 ± 1.45*	1.60 ± 0.56*
NC group	0.13 ± 0.01	12.23 ± 1.25	5.65 ± 0.87	1.96 ± 0.29	0.09 ± 0.01	10.17 ± 1.12	2.14 ± 0.36	1.08 ± 0.21

Comparison of hemodynamic parameters and vessel diameter in PH group with those in normal group *$P < 0.05$. D inner diameter, V peak velocity of blood, Q blood flow, CI: congestion index, PH portal hypertension, NC normal control

10 factors including SS, LS, portal vein inner diameter (PVD), splenic vein inner diameter (SVD), portal vein blood flow velocity (PVV), splenic vein blood flow velocity (SVV), portal vein blood flow (PVQ), splenic vein blood flow (SVQ), portal vein congestion index (PVCI), and splenic vein congestion index (SVCI) were used as independent variables. Multiple linear regression analysis (stepwise method) showed that SS, PVD, and SVV were significantly associated with PH (Table 5). The regression model was significant and could explain 69.2% of the total variation ($R^2 = 0.692$, $F = 42.023$, $P < 0.001$).

Discussion

The use of SWE to detect portal hypertension has become a critical research area in recent years [20, 21]. However, due to clinical conditions, the elastic modulus of liver and spleen cannot be directly studied with the increase in portal pressure, and there are few studies on the corresponding mechanism of increased spleen stiffness. The previous studies showed that the LS of isolated pig liver detected by SWE increased as hepatic venous pressure increased [22]. Giunta found that spleen stiffness was significantly positively correlated with the portal and hepatic venous pressure gradients [23, 24]. Their studies have shown that the stiffness of related organs increases with the increased vascular pressure, and SWE can indirectly reflect the vascular pressure gradient. Consistent with previous studies [12, 20, 25], this study shows that both the SS and LS in rats with portal hypertension are elevated, and that they are positively correlated with the severity of portal vein pressure. What's more, this study further demonstrate that 2D-SWE detection of SS can better reflect the severity of portal hypertension. We speculate that this may be related to the portal hyperdynamic blood flow state [26] and the inconsistency between the pathological changes of the spleen and liver caused by portal hypertension.

Blood flow and congestion index can reflect hemodynamic changes in patients with portal hypertension [27, 28] and are the main indicators of hyperdynamic blood flow theory. In this study, the portal vein blood flow (PVQ), splenic vein blood flow (SVQ), and congestion index of rats in the portal hypertension group were higher than those in the control group [29, 30]. The ratio of SVQ to PVQ in the portal hypertension group also increased, indicating that the simulation of the hyperdynamic circulatory state dominated by splenic circulation was successful [31, 32]. In this study, SS was positively correlated with splenic vein congestion index and SVQ. The hyperdynamic circulatory state disappeared after the rats were sacrificed, and the SS of the normal and portal hypertension groups decreased compared with the living body, but the SS of the portal hypertension group decreased significantly. The SS is greater in the PH group compared to the NC group, so we speculate that the high dynamic circulation state during portal hypertension may be an important factor in the increase in spleen stiffness. However, it is worth noting that portal vein blood flow velocity (PVV) was increased in our study, but the degree of increase was not large and other effective indicators were basically consistent with the previous models [33]. We speculate the possible reasons are as follows: Firstly, it may be due to the error of the measurement method during the experiment. Secondly, we observed that some model rats have ascites, abdominal cavity stickiness and other symptoms during the experiment, which may be due to the abdominal cavity injection molding. We speculated that these manifestations may have an effect on the elevation of PVV.

Some scholars found that HVPG decreased after intrahepatic portal shunt via internal jugular vein. At the same time, SS decreased significantly, but LS had no statistical significance [34]. In this study, after sacrifice, the hyperdynamic circulatory state disappeared, and the change of LS was not as obvious as that of SS. The correlation between LS and portal vein congestion index and PVQ was lower than that between SS and splenic vein congestion index and SVQ. There are several clinical studies which suggest that SS measurements can more accurately evaluate the treatment of portal hypertension, and esophageal varices [35–37]. This suggests that SS may be more sensitive to the hyperdynamic circulatory state caused by portal hypertension than LS which can be explained by Poiseuille's law [38]. As the portal venous system is further away from the liver, the blood flow resistance decreases

Table 5 Multiple linear regression analysis of influencing factors of PH

Items	B	SE	β	*t*	*P*	B (95% CI)
Constant term	−3.816	1.492	–	−2.557	0.013	−6.806 to −0.826
SS	0.389	0.076	0.505	5.149	< 0.001	0.238–0.541
PVD	33.774	12.407	0.304	2.722	0.009	8.919–58.629
SVV	0.246	0.109	0.198	2.252	0.028	0.027–0.466

SS spleen stiffness, *PVD* portal vein inner diameter, *SVV* splenic vein blood flow velocity, *PH* portal hypertension

and the hyperdynamic circulation becomes more pronounced. Compared to the portal vein, the splenic vein is further from the liver, the resistance is smaller, and the hyperdynamic circulation becomes more obvious [39].

Liver fibrosis is one of the initiating factors leading to the increase of portal system resistance and aggravating PH [40, 41]. In this study, LS and SS are positively correlated with fibrosis classification, indicating that fibrosis is one of the reasons for the increase of LS and SS [42–45]. However, most splenic fibrosis can only reach level II, while liver fibrosis can reach stage F4. The pathological changes in the liver and spleen are not consistent and the correlation between LS and pathological changes is greater than that of SS, indicating that fibrosis is more significant for the elevation of LS. In comparison, hyperdynamic circulation is more relevant when considering the increase in SS.

In summary, SS and LS are both closely related to hyperdynamic circulation and fibrosis. We speculate that the obvious increase of SS compared to LS during portal hypertension is closely related to the state of hyperdynamic circulation. The spleen is the "elastic heart" of the portal venous system, acting as a pressure regulating hub to protect the liver and portal venous system from excessive overflow. Therefore, the spleen is more sensitive to increased pressure in the portal system and hyperdynamic circulatory states [46]. The liver is also strong in terms of self-regulation and anatomical factors such as ligaments on the surface weaken the influence of hemodynamics. Therefore, the correlation between SS and PVP determined by SWE is higher than LS in this study. In addition, our study also found that SS was significantly associated with PH based on multiple linear regression analysis. Combined with previous studies, we speculate that SS may be a more suitable method to supplement or replace HVPG than LS, especially in the follow-up of high-risk patients with portal hypertension [47, 48].

Conclusions

In conclusion, this study shows that, as determined by SWE, the correlation between SS and PVP is higher than that of LS and PVP. This study also shows that the hemodynamic changes of the highly dynamic circulation with the splenic circulation as an important component, leads to a higher correlation between portal pressure and SS than LS. Fibrosis may play a more important role in the increase of liver stiffness rather that an increase of spleen stiffness in regard to portal hypertension. Moreover, SS may be a more effective noninvasive predictor of PH than LS.

Abbreviations

PH: Portal hypertension; HVPG: Hepatic venous press gradient; LS: Liver stiffness; SS: Spleen stiffness; 2D-SWE: Two-dimensional shear wave elastrography; CSPH: Clinically significant portal hypertension; TE: Transient elastography; PVD: Portal vein inner diameter; SVD: Splenic vein inner diameter; PVV: Portal vein blood flow velocity; SVV: Splenic vein blood flow velocity; PVQ: Portal vein blood flow; SVQ: Splenic vein blood flow.

Acknowledgements

Not applicable.

Author contributions

YC and GL conceived and designed the experiments; YC, JL and QZ performed the experiments; GL and SL corrected the manuscript. All authors read and approved the final manuscript.

Funding

This project was financially supported by Quanzhou City Science & Technology Program of China (2019N103S).

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly due to privacy restrictions but available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The experimental procedures in this study were complied with the NIH (National Institutes of Health publication 86-23, 1985) Guidelines for Use of Laboratory Animals. This study was also approved by the Ethics Committee of the Second Affiliated Hospital of Fujian Medical University (reference number: 2019-223). The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Ultrasound, The Second Affiliated Hospital of Fujian Medical University, No. 34 North Zhongshan Road, Licheng District, , Quanzhou 362000, Fujian, China. [2]Maternal and Child Health Service Application Technology Collaborative Innovation Center, Quanzhou Medical College, Quanzhou, Fujian, China.

Received: 22 November 2021 Accepted: 28 March 2022
Published online: 13 April 2022

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