Original Article

Normal Values of Spleen Stiffness in Healthy Volunteers and Effect of Meal on Liver Stiffness and Spleen Stiffness

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Abstract

Background: The portal hypertension leads to variceal bleeding. Spleen stiffness (SS) is useful in estimating esophageal varices risk and less invasive as compared with reference standard method (hepatic venous pressure gradient). Using different methods of ultrasound elastography as well as different novel system provide different value of SS. **Methods:** After the approval by the institutional review board, we enrolled volunteers aged over 18 years who had no obesity, excessive alcohol use, history of systemic disease, hepatobiliary disease, or malignancy. Spleen and liver stiffnesses were measured with LOGIQ E10 SWE by two radiologists. Volunteers ingested a 460 kcal liquid meal. The spleen and liver stiffnesses were performed at baseline and 60, 120 and 180 min after meal. **Results:** A total of 46 volunteers participate and two volunteers were excluded. The mean SWE SS was 12.6 ± 1.18 kPa. There was no statistically significant difference between sex, age and spleen size. SS trends to decrease with time. In first 2 h after meals, there was no significant difference from baseline. After 3 h, SS significantly decreased from baseline. In contrast, liver stiffness trend to increase with time and significant elevate from baseline at 3 h. Intraclass correlation coefficient between the two radiologists showed fair agreement for SS and substantial agreement for liver stiffness. **Conclusion:** Our outcomes may be a reference value for evaluating SS in patients with other illnesses in clinical setting the utilize SWE with LOGIQ E10. SS decreased with time after a meal in normal volunteers and significant difference at 3 h. In contrast, liver stiffness increases with time after a meal in normal volunteers and significant difference at 3 h. There is importance of the operator's expertise in SWE measurement should be considered for SS.

Keywords: Liver stiffness, shear wave elastography, spleenic stiffness

Introduction

Chronic hepatitis is common health problems among people in many regions worldwide including Thailand. A major cause of liver cirrhosis is viral hepatitis, especially hepatitis B virus and hepatitis C virus infection. Risk of cirrhosis and portal hypertension (PH) are increased in chronic hepatitis patient.

Esophageal varices (EV) are mainly induced by PH. Variceal bleeding is correlate with high mortality. In patients with cirrhosis, upper gastrointestinal endoscopy using for EV screening is highly recommended.[1] The hepatic venous pressure gradient measurement is now the best method to assess the presence and severity of PH. The both investigations are invasive, expensive, may be not well tolerated by patients, difficult to repeat and HPVG is not widely available.[2]

The splenic vein is connected with portal vein. PH cause splenic congestion, increase in spleen stiffness (SS) and fibrosis.[3] Recently, developing noninvasive methods to predict the presence and size of EV and their hemorrhage risk including measure SS are gaining more and more interest. One meta-analysis proved that the SS is superior to liver stiffness for identify the present of EV in chronic liver disease.[4]

Elastography of spleen could diagnose and evaluate other medical condition. Splenic stiffness in myelofibrosis is significantly higher than healthy population.[5-7]

Furthermore, SS measurement can monitor portal hemodynamics in patients undergoing transjugular intrahepatic portosystemic shunt (TIPS) placement and determining TIPS dysfunction.[8,9]

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SS can be evaluated through magnetic resonance elastography and ultrasound-based elastography (USE). The USE is widely use according to availability and less expensive. Currently available USE techniques can be categorized by the measured physical quantity (1) Strain imaging and (2) Shear wave imaging (SWI).

Currently, the SWI is more widely used. SWI employs a dynamic stress to generate shear waves in the parallel or perpendicular dimensions. Tissue elasticity is estimated by shear wave speed measurement. There are currently three technical approaches for SWI:

1. One-dimensional transient elastography (1D-TE), FibroScan™ (Echosens, Paris, France)
2. Point shear wave elastography (SWE), acoustic radiation force impulse (ARFI)
3. 2D SWE. Currently newest SWI method that uses ARFI.

Kjærgaard *et al*. reported postmeal value of SS increases significantly from baseline in 36 patients with liver disease including METAVIR F0-F4 evaluated by 1D-TE and 2D-SWE.[10]

The different novel platforms gave different value of liver stiffness as the study of Mulabecirovic *et al*. included 100 healthy volunteers to measure liver stiffness. The result of the study showed that liver stiffness was significantly higher for GE s8 2D-SWE compared to Samsung RS80A despite the same technique of elastography.[11]

This study measured the value of SS in healthy participant without obesity, excessive alcohol use, history of systemic disease, hepatobiliary disease, or malignancy using LOGIQ E10 SWE in our institution (2D-SWE) and investigate any correlation with sex, age, spleen dimension or liver stiffness and to evaluate the repeatability. Another aim of this study was to investigate the effect of food intake on spleen and liver stiffness. Our results probably are a reference value for SS evaluation in patients with many diseases such as PH, EV, as well as myelofibrosis.

Materials and Methods

Patient population

The study was conducted in accordance with the Declaration of Helsinki. After institutional review board approval (approval no. 784/61), we enrolled healthy volunteers aged over 18 years who had no history of systemic disease, hepatobiliary disease, or malignancy. The study included 46 volunteers (16 men and 30 women). The study was performed in the detail of the study was described to the volunteers, and informed consent was acquired in each case.

Exclusion criteria was: Obesity (body mass index >25 kg/m^2), excessive alcohol use ≥30-g alcohol daily intake for men and ≥20 g for women, splenomegaly, splenic nodule or mass, hepatic nodule or hepatic mass, and ascites.

Abdominal ultrasonography was done for measure longitudinal dimension, transverse dimension of spleen and rule out liver lesion, spleen lesion, or ascites. Two volunteers were excluded according to incidental spleen lesion and liver lesion.

The final study group included 44 volunteers. The characteristics of healthy volunteers are revealed in Table 1. Volunteers ingested a 460-kcal liquid meal (ensure Abbott, containing 17.1 g of protein, 15.6 g of lipids and 61.74 g of carbohydrates). The test meal was administered in 10 min. The spleen and liver stiffness were performed at baseline after at least 6 h fast, 60, 120, and 180 min after the meal. The spleen and liver stiffnesses were measured with LOGIQ E10 SWE using a 1–6 MHz convex probe. Two radiologists independently measure of the liver and spleen stiffnesses. The first radiologist had 4 years of experience in USE examinations, whereas the second radiologist had 1 year of experience in USE examinations. Volunteers lie down on back or lateral decubitus position with arm abduction. The volunteer was requested to hold their breath at a maximal expiration, or in relaxed mid-breath hold to minimize breathing motion.

The region of interest (ROI) was placed in right lobe of liver at 1 cm or more from the hepatic surface not include large vessels, bile ducts, and rib shadows for liver stiffness as shown in Figure 1a. The ROI was placed at parenchyma of spleen with homogeneous elasticity and not include blood vessels as shown in Figure 1b. Stiffness was measured in kilopascals (kPa). The interquartile range (IQR) was defined as an index of intrinsic variability of organ stiffness. The median value was considered representative of the organ elasticity. At least 10 valid measurements and an IQR-to-median ratio <30% were considered reliable.[12]

We use average SS of two values from two radiologists. If the values were different more than 25%, the values of first radiologist were used for analyzing.

Statistical analysis

To verify the normal distribution of variables, the Shapiro–Wilk test was performed. From the test, only age and transverse dimension of spleen and liver stiffness are not normally distribution. Data were expressed as mean ± standard deviation.

The Mann–Whitney *U*-test was performed to analyze SS associate with sex. The Spearman's rank correlation coefficient was used to measure the strength and direction of the linear relationship between SS with age, spleen dimension, and liver stiffness.

Table 1: Basic data of the healthy volunteer

Parameter	Value
Number of participants	44
Number of men[c]	14 (31.8)
Number of women[c]	30 (68.2)
Age[a]	27.0 (21–46)
Men's age[a]	27.0 (22–29)
Women's age[a]	27.0 (21–46)
Longitudinal spleen diameter[c]	9.62±0.944
Transverse spleen diameter[a]	5.43 (4.1–7.2)

[a]Median, range in brackets, [b]Average±SD, [c]Percentage parentheses

ANOVA test was used to compare between multiple groups. Bland–Altmann test and calculation of correlation coefficient were analyzed to estimate the repeatability of spleen and liver stiffness measurement.

The interobserver agreement of spleen and liver stiffness measurements was obtained by comparing the results of two sets of 176 measurements acquired from 44 patients. According to Landis and Koch[13] (1977), the intraclass correlation coefficient (ICC) was classified as slightly agreement (0.01–0.02), fair agreement (0.21–0.40), moderate agreement (0.41–0.60), substantial agreement (0.61–0.08), and almost perfect agreement (0.81–0.99).

Data were analyzed using SPSS IBM (International Business Machines Corporation), is an American multinational technology company headquartered in Armonk, New York (Statistical Package for the Social Sciences) version 26.0 and Jamovi version 1.2 for Windows. Two-sided statistical significance was defined as $P < 0.05$.

Results

Spleen and liver stiffnesses and their dependences on sex

The mean value of SWE SS was 12.6 ± 1.18 kPa. Ninety-five percent confidence interval was 12.23–12.95 kPa.

In men, it was 12.81 ± 1.23 kPa, and in women, it was 12.49 ± 1.17 kPa. There was no statistically significant difference between two groups ($P = 0.811$).

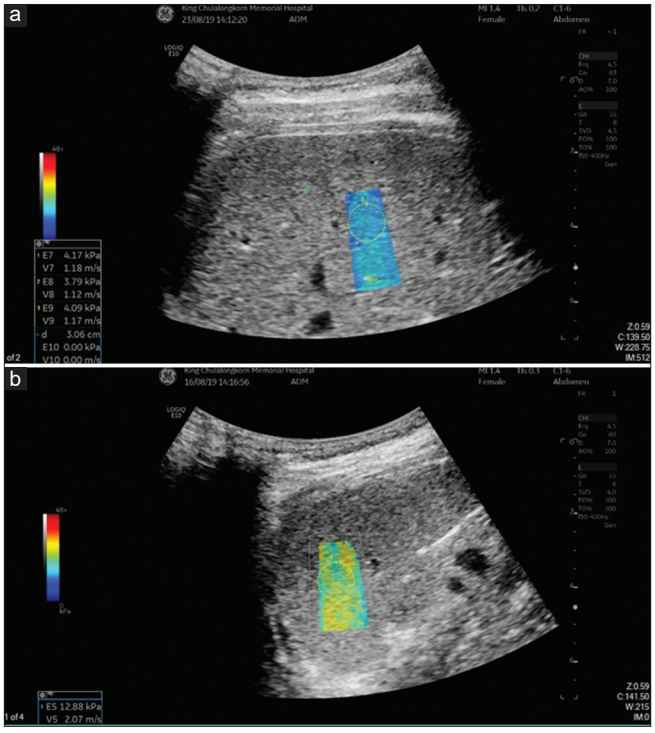

Figure 1: (a) Shear wave elastography liver view. Region of interest put in homogeneous elasticity in right lobe of liver. (b) Shear wave elastography spleen view. Region of interest put in homogeneous elasticity

The mean value of SWE liver stiffness was 4.23 ± 0.67 kPa. Ninety-five percent confidence interval was 4.03–4.43 kPa.

In men, it was 4.32 ± 0.46 kPa, and in women, it was 4.18 ± 0.74 kPa. There was no statistically significant difference between two groups ($P = 0.579$) [Table 2].

Correlation between spleen stiffness and age

Low correlation between SS and age. Pearson's correlation (r) = 0.173 ($P = 0.230$).

Correlation between spleen stiffness and size of spleen

No correlation between SS and transverse dimension. Pearson's correlation (r) = 0.094 ($P = 0.546$). There was no correlation between SS and longitudinal dimension. Pearson's correlation (r) = 0.049 ($P = 0.753$).

Correlation between spleen stiffness and liver stiffness

There was low correlation between SS and liver stiffness. Pearson's correlation (r) = −0.138 ($P = 0.372$) [Figure 2].

The effect of meal on spleen stiffness

SS trend to decrease with time and decrease significantly from baseline at 180 min after meal ($P = 0.001$), [Table 3 and Figures 3 and 4].

The effect of a meal on liver stiffness

Liver stiffness trends to increase with time and increase significantly from baseline at 180 min after meal ($P = 0.019$). For more details, [Table 4 and Figure 5].

Reliability of spleen and liver shear wave elastography examination

ICC of spleen examination was calculated between the measurement of the first and the second radiologist = 0.293, fair agreement.

ICC of liver examination was calculated between the measurement of the first and the second radiologist = 0.693, substantial agreement.

There was no systematic overestimation or underestimation between the two radiologists in measurement of stiffnesses.

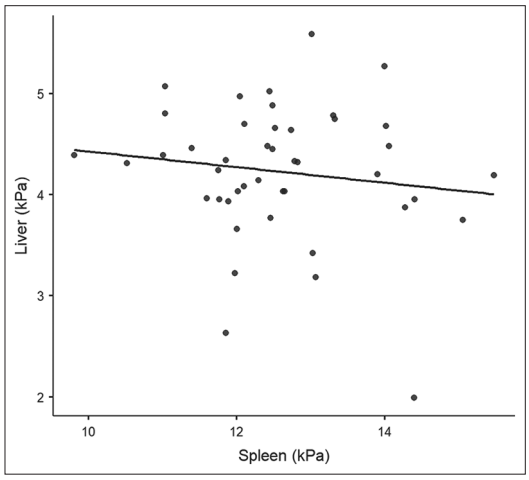

Figure 2: Relationship between spleen and liver stiffnesses is plotted ($P = 0.372$)

The mean difference for SS was −0.315, with 95% limits of agreement between −3.838 and 3.208. The mean difference for liver stiffness was −0.307, with 95% limits of agreement between −1.561 and 0.948 [Figure 6].

Discussion

In patients with PH, SS is better detected EV comparing to liver stiffness and other noninvasive method (such as platelet count to spleen diameter ratio). By anatomy, portal vein connected with the spleen vein, directly. Disorder in portal blood flow may lead to spleen congestion, tissue hyperplasia, fibrosis, and increased SS.[14]

The different novel platforms gave different value of liver stiffness as the study of Mulabecirovic *et al*.[11] There is also different value of SS obtained from different technique approach for SWI as shown in Table 5. The mean value of SS obtained in our study was 12.6 ± 1.18 kPa, and it was different from study of Leung *et al*.[15] and Pawluś *et al*.[17] which using the same elastography technique but the different novel platform [Table 5].

Table 2: Spleen and liver stiffnesses and their dependences on sex

	Sex		Men and women
	Women (n=30)	Men (n=14)	
Spleen stiffness (kPa)			
Mean±SD	12.49±1.17	12.81±1.23	P=0.811
Median	12.49	12.43	
Minimum–maximum	9.81–15.1	11.4–15.5	
Liver stiffness (kPa)			
Mean±SD	4.18±0.74	4.32±0.46	P=0.579
Median	4.31	4.35	
Minimum–maximum	1.99–5.59	3.22–5.02	

SD: Standard deviation

Multiple researchers have tried to determine spleen and liver stiffness values in healthy individuals. There was no correlation between sexes.[17-21] Our results proved no correlation between SS and age, sex, and spleen dimension.

In the study, we reported that SS decreased at 180 min after a meal about 8.17 ± 6.78% (P = 0.001). The result different from the study of Kjærgaard *et al*. that reported value of SS increases significantly from baseline in patients with liver disease after 625 kcal intake 17 ± 55% which time to peak value is 90 ± 120 min.[10] The SS should be always measured in fasting conditions in both healthy population and liver disease to guarantee reliable values.

Multiple studies show liver stiffness increases after food intake in patients with cirrhosis or chronic hepatitis.[22,23] One study included 19 controls or normal healthy volunteers and shows increase liver stiffness immediately after meal and 1 h after breakfast.[24] Contrarily, to what study of Silva *et al*., SS not significantly increase at 30 min after food intake in 22 healthy volunteers (P = 0.106).[25] In our study, no significant increase liver stiffness after meal at 60 min but significant increased value at 180 min or 3 h. To standardize liver stiffness

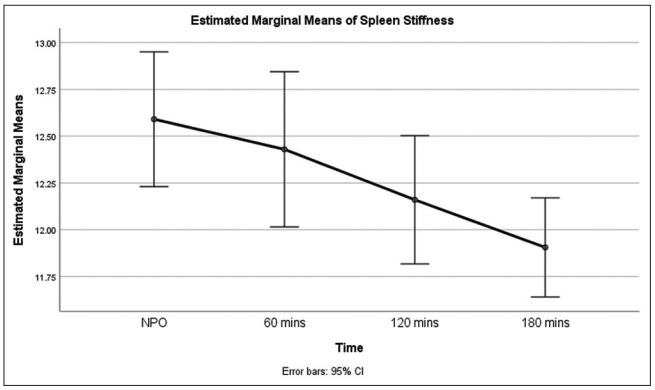

Figure 3: Spleen stiffness at different time points

Table 3: The effect of a meal on spleen stiffness

	Time	Mean difference	SE	Significant[b]	95% CI for difference[b]	
					Lower bound	Upper bound
NPO	60 min after meal	0.161	0.261	0.541	−0.366	0.688
	120 min after meal	0.431	0.233	0.071	−0.039	0.901
	180 min after meal	0.685*	0.185	0.001	0.311	1.059

*The mean difference is significant at the 0.05 level, [b]Adjustment for multiple comparisons: Least significant difference (equivalent to no adjustments). Based on estimated marginal means. SE: Standard error, CI: Confidence interval, NPO: Nothing per oral

Table 4: The effect of a meal on liver stiffness

	Time	Mean difference	SE	Significant[b]	95% CI for difference[b]	
					Lower bound	Upper bound
NPO	60 min after meal	−0.070	0.111	0.530	−0.295	0.154
	120 min after meal	−0.115	0.087	0.191	−0.290	0.060
	180 min after meal	−0.258*	0.105	0.019	−0.471	−0.045

*The mean difference is significant at the 0.05 level, [b]Adjustment for multiple comparisons: Least significant difference (equivalent to no adjustments). Based on estimated marginal means. SE: Standard error, CI: Confidence interval, NPO: Nothing per oral

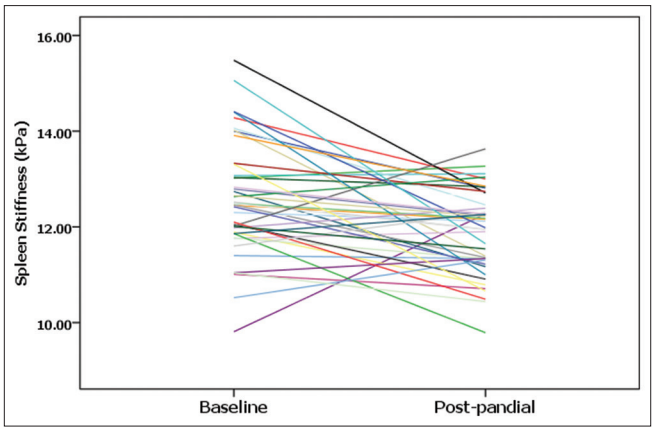

Figure 4: Individual change in spleen stiffness 180 min after a liquid test meal

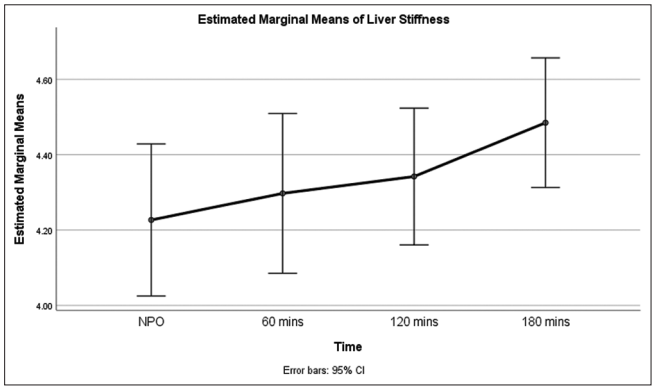

Figure 5: Liver stiffness at different time points

evaluation, we recommended measurement in the fasting condition.

In our study, the ICC form SS was **0**.293, which lower than the one obtained with liver stiffness (0.693). Spleen elastography is relate to its unfeasibility in patients with normal sized spleen.[26] The interobserver variability in our study may be affected by 3 years different experience of the two radiologists and inadequate training in SS measurement.

Concordance of organ stiffness values between different companies can give different reference values. Hence, our findings can provide as a comparison to determine normal SS value in the utilize SWE with LOGIQ E10.

Conclusion

Our outcomes may be a reference value for evaluating SS in patients with other illness in clinical setting the utilize SWE with LOGIQ E10. SS decreased with time after a meal in normal volunteers and significant difference at 3 h. In contrast, liver stiffness increases with time after a meal in normal volunteers and significant difference at 3 h. To standardize spleen and liver stiffnesses measurement, we recommended measurement in the fasting condition. The operator expertise in SWI measurement is important for SS.

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Nil.

Conflicts of interest

Dr. Natthaporn Tanpowpong , an editorial board member at

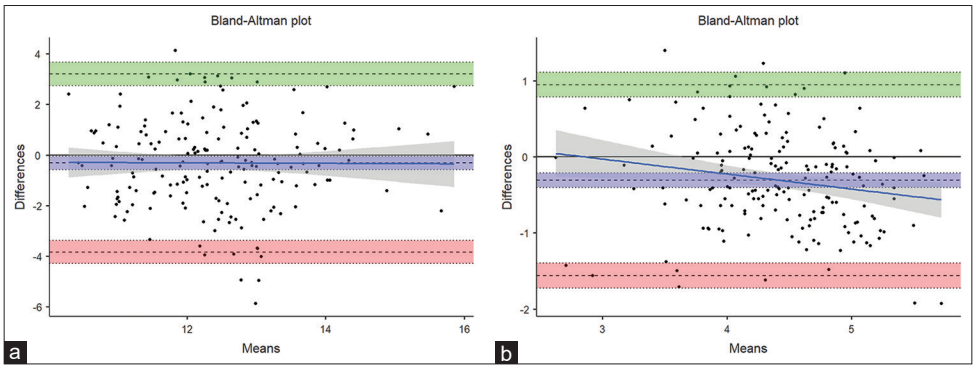

Figure 6: Bland–Alman's plot of spleen stiffness. (a) And liver stiffness value (b) The diagrams show no systematic overestimation or underestimation between the two radiologists

Table 5: Review of spleen stiffness in healthy volunteers with different elastographic techniques and devices

Reference	Elastography technique	Device	Number of volunteers	Spleen stiffness (kPa)
Leung *et al*., 2013[15]	2D-SWE	Aisplorer	171	17.3±2.6
Rewisha *et al*., 2016[16]	1D-TE	FibroScan™	40	19.41±3.63
Pawluś *et al*., 2016[17]	2D-SWE	Aisplorer	59	16.6±2.5
Giuffrè *et al*., 2019[18]	pSWE	Philips Affiniti 70	100	18.14±3.08
Arda *et al*., 2011[19]	SWE	SuperSonic Imagine	127	2.9±1.8

SWE: Shear wave elastography, pSWE: Point SWE, 2D-SWE: Two-dimensional SWE, 1D-SWE: One-dimensional SWE, 1D-TE: 1 dimensional transient elastography

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