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Original Article

Influence of Food Intake on Measurement of Liver Stiffness by Transient Elastography in Patients with Chronic Viral Hepatitis B and C

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ABSTRACT

Background: Transient elastography (TE) is a non-invasive test for evaluation of fibrosis and cirrhosis in many chronic liver conditions including chronic viral hepatitis B (CHB) and C (CHC). Studies have shown that many factors other than fibrosis and cirrhosis influence the liver stiffness measurement (LSM) including high liver enzymes, cholestasis and conditions that increase liver blood flow such as congestive heart failure and possibly food intake. Aim of this study is to evaluate the influence of food intake on LSM by TE in patients with CHB and CHC

Methods: Forty-five patients with CHB and 37 patients with CHC, all without cirrhosis and flare hepatitis, underwent LSM after a 4-hour fast. Each patient then ate a standard Thai meal (500 kcal CHO: protein:fat 55:15:30) and the 2nd and 3rd LSM measurement were performed immediately and at 1-hr after finishing the meal. Changes in median values of LSM were compared across times and individual measurements fitted to a random-intercept, mixed-effects linear regression model to identify differences between CHB and CHC.

Results: LSM increased significantly at both postprandial times, by 0.33 kPa (95% CI 0.20-0.46) and 0.38 kPa (95% CI 0.25-0.51), respectively, in CHB and 0.81 (95% CI 0.63-0.99) and 0.56 (95% CI 0.38-0.75) in CHC. The immediate increase in LSM after meal was 0.5 kPa (95% CI 0.26-0.71) greater in CHC than in CHB. There were 73/82 patients (89%) and 74/82 (90.2%) who showed an increase in LSM immediately and at 1 hour after meal. The 31.7% and 32.9% of these patients had an increase in LSM ≥1 kPa.

Conclusions: Postpandrial LSM is significantly elevated compared with fasting LSM. The elevation remains up to at least 60 minutes. To standardize liver stiffness evaluation, we recommend that the measurement should be performed after at least 4 hour fasting.

Key words : Liver stiffness, transient elastrography, chronic viral hepatits B, chronic viral hepatitis C, food intake

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Liver biopsy is a gold standard method to evaluate grading and staging of liver fibrosis and cirrhosis in patients with chronic liver diseases[1]. However, there are some limitations in accuracy[2], size of liver samples, sampling error, intra-and interpersonal variability as assessment by pathologists. Moreover, it has small but significant chance of morbidity and mortality as 1-5% and 0.001-0.0001%[2-3], respectively.

Transient elastography (Fibroscan®, Echosens, Paris, France)[4] is a quick, noninvasive and high accuracy[5] test for liver stiffness (kiloPascal, kPa), which measures the transmission of a mechanical wave generated by vibration. This technique is aimedto evaluate liver stiffness. A strong correlation between liver stiffness measurements and liver fibrosis stages, assessed by simultaneous liver biopsies, has initially been reported in chronic hepatitis C[6-10] with area under the receiver operating characteristic curves (AUROCs) range from 0.79 to 0.83 for significant fibrosis[25]. Later, there were reported on other chronic liver diseases such as chronic viral hepatitis B[26-27], non-alcoholic fatty liver disease[11], HIV-HCV coinfection[28-29], cholestatic disease such as primary biliary cirrhosis and primary sclerosing cholangitis with AUROCs ranging from 0.74 to 0.93 and cut-offs ranging from 4.0 to 8.7 kPa.

However, failure to obtain valid LSM were reported in older age (> 75 year)[6], higher BMI, especially over 40 kg/m^2[12,19], patients with narrow rib spaces[14] and not possible in the presence of ascites[15]. Other factors may affect the liver stiffness value, reducing the diagnostic accuracy. One of the most important factors is with severe flares of hepatitis (ALT >10x ULN)[15-17] or patients with congestive heart failure[20], since congestive heart failure increases central vein pressure, and can increase LSM up to 6.1-51.3 kPa[20].

If venous pressure had influence on liver stiffness measurement, in the same method, food intake[21] may increase in liver stiffness measurement as well by increase portal pressure from post pandrial portal hyperemia. The aims of this study were to evaluate the influence of food intake on the liver stiffness measurement by transient elastography in chronic viral hepatitis B or C patients.

METHODS

Patients

We reviewed data from OPD card from our NKC Institute liver outpatient clinic, from October to December 2011. There were 77 patients with chronic viral hepatitis B (CHB) and 66 patients with chronic viral hepatitis C (CHC) that met inclusion criteria. Forty-nine patients with CHB and 41 patients with CHC were eligible. Eligible patients were men and women, 18 to 75 of age, who had been diagnosed chronic viral hepatitis B or chronic viral hepatitis C for more than 6 months before the enrollment. All of them had no evidences of cirrhosis, as documented by means of either physical examination, laboratory, radiology or pathology. All of them had been confirmed patency of hepatic artery and portal vein, absence of focal lesions (e.g. mass, cyst, abscess) and absence of ascites by ultrasonography within 3 months. Exclusion criteria included BMI more than 40 kg/m^2, coinfection with HIV, primary biliary cirrhosis, primary sclerosing cholangitis, or other chronic liver diseases, pregnancy women, patients with congestive heart failure, ALT above five times upper limit of normal within 4 weeks before enrollment. Written informed consent was obtained from all patients. The study was approved by the Ethical Committee of Prince of Songklanakarin University.

Study design

All of the eligible patients underwent liver stiffness measurements and had complete physical examination with laboratory tests on the same day. The following data were collected for each subject: age, gender, body weight, height, BMI, blood pressure, MAP, underlying disease, current medication. Laboratory tests included: Liver tests, fasting plasma glucose, serological tests for hepatitis B surface antigen (HBsAg), HBV-DNA, anti HCV, HCV-RNA. Then they were underwent first LSM after a 4-hour fast. After that each patient had intake a standard Thai meal, approximately 500 kcal, 55% carbohydrate, 15% protein, 30% fat, over a maximum period of 30 minutes. Second and third LSM were performed immediately and 1-hr after finishing the meal.

Determination of liver stiffness by transient elastography

Liver stiffness was determined using Fibroscan® (Echosens, Paris, France) Measurement technique as described by Sandrin. Liver stiffness measurements were performed by two experienced investigators (who had been trained and used the equipment successfully for more than 30 patients, each). These two investiga-

tors were validated and had low interpersonal variability. The patients were in the supine position, with their right arms behind their heads. A suitable point was chosen and marked in the intercostal spaces in mid-axilary line and the level of the right lobe of the liver. Afterward, the tip of the transducer was placed on the skin and measurements were performed. All measurements of each liver stiffness determination pre- and post-pandrial were performed at the same position on the skin in a similar angle. The depth of measurement was between 25-65 mm below the skin surface. The operators performed at least 10 valid measurement of LSM at each time. The results of a liver stiffness determination is expressed in kPa (values ranging from 2.5 to 75 kPa). Data were recorded if value of interquartile range/median (IQR) was less than 30% and success rate were more than 80%.

End points

The primary end point was changes in liver stiffness measurement (kPa) between fasting and at immediate and 1hour after meal in patient chronic viral hepatitis B or C. The secondary end points were the differences in LSM between chronic viral hepatitis B and chronic viral hepatitis C patients.

Statistical analysis

Sample size were calculated based on having a power of 80% to detect an increase in liver stiffness measurement after having a meal of at least 1 kPa as significant at an alpha of 0.05, assuming a standard deviation of change in liver stiffness of 2.0 kPa as reported[22]. The required sample size, allowing for ten percent unusable data, was at least 36 for each group.

The baseline characteristics of the patients were presented as percentage or mean ± SD, as appropriate.

Liver stiffness at different time point and the changes from fasting measurement to zero minutes and sixty minute after having a meal were compared across group using Student's *t*- test. Changes from fasting were also examined graphically. Variables potentially related to the change in liver stiffness, in addition to type of hepatitis group, were explored using a random-intercept mixed effect linear regression in which patient was the random element and other variables considered to have fixed effects. Statistical significance was set at < 0.05.

RESULTS

Patients

A total of 143 patients were reviewed for the study, Seventy-seven chronic viral hepatitis B and 66 chronic viral hepatitis C patients were enrolled from October 2011 to December 2011 (Figure 1). Forty-nine of the

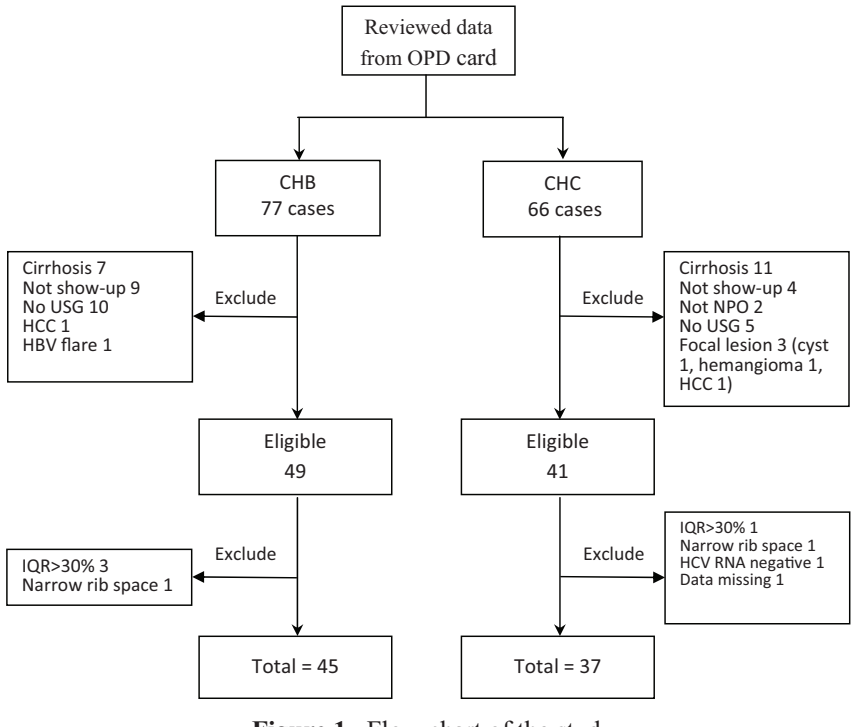

Figure 1. Flow chart of the study

first group and forty-one of the latter were met the inclusion criteria and successfully underwent liver stiffness measurement.

The patients with chronic viral hepatitis C were slightly more male predominate (67.6%) and younger than patients with chronic viral hepatitis B, (mean age were 39.7 years and 47.7 years, respectively, $p < 0.001$). Mean BMI and MAP were similar between the two groups. Their blood chemistries were shown in Table 1.

Influence of food intake on liver stiffness measurements

At least ten individual valids in every single patient at fasting state were compared with at least ten individual valids at both time points after meal. LSM increased significantly at both postprandial times in both groups (Table 2). Mean increase on LSM between fasting stiffness ($LS_{fasting}$) and immediately after meal (LS_{0min}), and fasting stiffness vs. 1-hour after meal (LS_{60min}), in chronic viral hepatitis B were 0.33 kPa (95% CI 0.20-0.46) and 0.38 kPa (95% CI 0.25-0.51) and in chronic viral hepatitis C were 0.81 kPa (95% CI 0.63-0.99) and 0.56 kPa (95% CI 0.38-0.75). The LSM immediately after meal increased in 39 out of 45 patients with chronic viral hepatitis B (86%) and 34 out of 37 (92%) in chronic viral hepatitis C. An increase of at least 1 kPa was found in 12 out of 45 (26.7%) in chronic viral hepatitis B and 14 out of 37 (37.8%) in chronic viral hepatitis C patients, as shown in Table 3. Changes in LSM from fasting to immediately after meal and from fasting to 1 hr after meal in each group are shown in Figure 2 and 3.

Influence of underlying chronic viral hepatitis B and C on liver stiffness measurements

Table 1. Patients' baseline characteristics.

Characteristics of patients	HBV (n=45)		HCV (n=37)		*p* value
	Mean	Range	Mean	Range	
Male sex - n(%)	23 (51.1%)		25 (67.6%)		
Age (yrs)	47.7	24-72	39.7	19-64	< 0.001
BMI (kg/m2)	24.2	13-32	23	18-31	
MAP (mmHg)	94.5	73-114	91.7	67-115	
Laboratory results	**Median**	**Range**	**Median**	**Range**	
Direct bilirubin (mg/dL)	0.14	0-1	0.15	0-1	
Total bilirubin (mg/dL)	0.51	0-2	0.41	0-1	
AST (mg/dL)	26	15-104	26	13-91	
ALT (mg/dL)	26	7-148	25	6-146	
ALP (mg/dL)	68	29-135	71	47-108	
Total protein (g/dL)	7.5	5-9	7.7	7-9	
Albumin (g/dL)	4.5	2-5	4.6	4-5	
FPG (mg/dL)	93	62-233	87	69-149	

Abbreviations: BMI, body mass index; MAP, mean arterial blood pressure; AST, aspatate aminotransferase; ALT, alanine aminotransferase; FPG, fasting plasma glucose

Table 2. Mean liver stiffness at fasting, immediately after meal and 1-hr after meal.

Group	N	Mean $LS_{fasting}$ (kPa)	Mean LS_{0min} (kPa)	Mean increase LS_{0min}-$LS_{fasting}$	95% CI	Mean LS_{60mim} (kPa)	Mean increase LS_{60min}-$LS_{fasting}$	95% CI
CHB	45	5.4±1.7	5.7±1.8	0.33	0.20-0.46	5.7±1.8	0.38	0.25-0.51
CHC	37	6.1±2.8	6.9±2.9	0.81	0.63-0.99	6.7±2.6	0.56	0.38-0.75
Both	82	5.7±2.3	6.2±2.4	0.55	0.44-0.66	6.2±2.2	0.46	0.35-0.57

Table 3. Number and percentage of the patients that has changes in LSM at immediately and 1-hr after meal.

Group	Increase	Increase ≥1 kPa	Decrease ≥1 kPa
CHB LS_0-$LS_{fasting}$	39/45 (86.7%)	12/45 (26.7)	6/45 (13.3%)
CHB LS_{60}-$LS_{fasting}$	40/45 (88.8%)	11/45 (24.4%)	4/45 (8.9%)
CHC LS_0-$LS_{fasting}$	34/37 (91.9%)	14/37 (37.8%)	3/37 (8.11%)
CHC LS_{60}-$LS_{fasting}$	33/37 (89.1%)	15/37 (40.5%)	4/37 (10.8%)
Both groups LS_0-$LS_{fasting}$	73/82 (89%)	26/82 (31.7%)	9/82 (11%)
Both groups LS_{60}-$LS_{fasting}$	74/82 (90.2%)	27/82 (32.9%)	8/82 (10%)

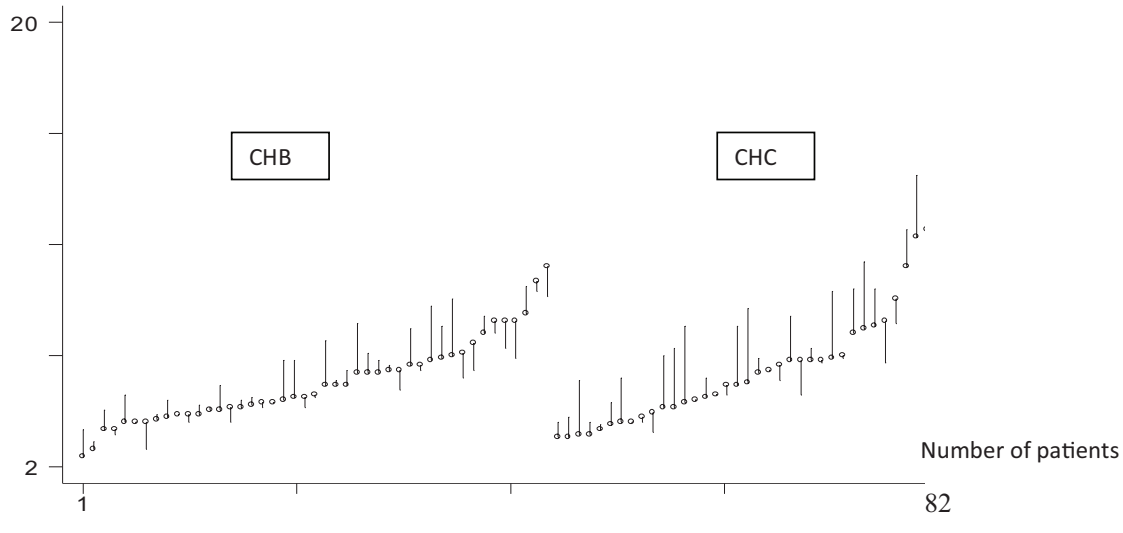

Figure 2. Food intake-associated change on LSM between fasting and immediately after meal.

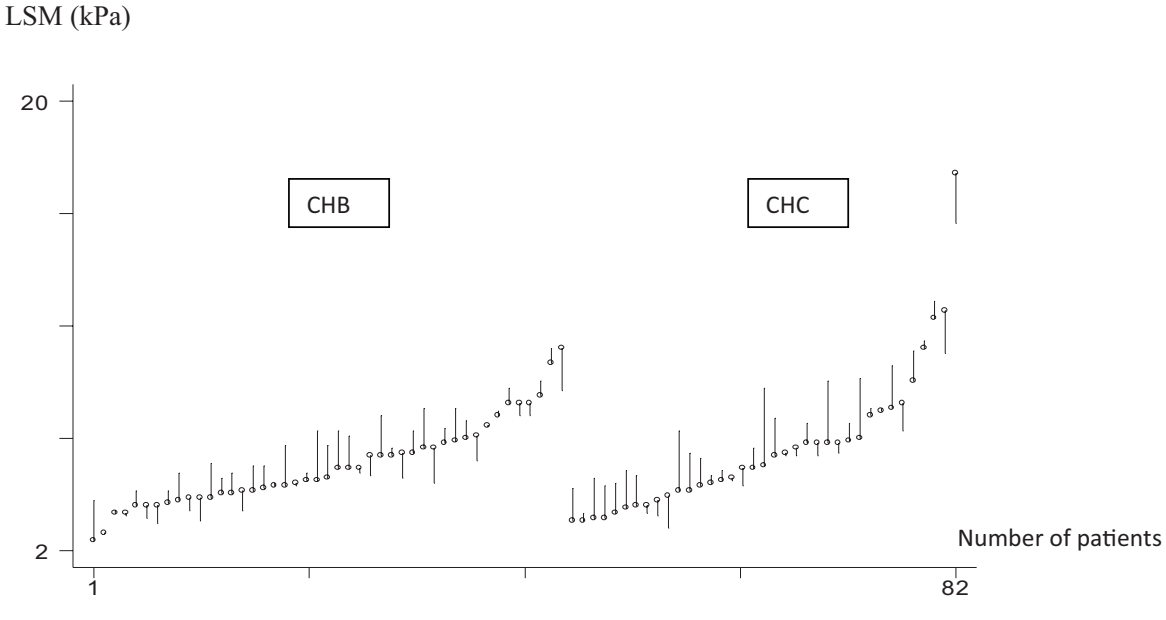

Figure 3. Food intake-associated change on LSM between fasting and 1-hr after meal.

The changes in LSM immediately after meal were significantly higher in chronic viral hepatitis C population than in chronic viral hepatitis B population by 0.5 kPa (95% CI 0.26-0.71) (Table 2).

Influence of other variables to the change of LSM

Among all the variables, BMI and direct bilirubin were showed statistically significant to the changes of LSM at immediately after meal by increases of 0.7 ± 0.2 kPa per 1 kg/m^2 (95% CI 0.03 - 0.10) and 7.7 ± 1.0 kPa per 1mg/dL (95% CI 5.79 -9.60), respectively.

DISCUSSION

Liver fibrosis evaluation is important in clinical practice for identifying patients who will benefit from treatment in chronic viral hepatitis or for assessment of their response to treatment. Liver biopsy is a gold standard to determine the stage of fibrosis but patients may reject the procedure since it has small but significant risk of morbidity and mortality. Thereby leaving their possible fibrosis undiagnosed. Transient elastography is becoming more available as a non-invasive tool for the assessment of liver fibrosis. However, no clinical guideline has been specific mentioned about fasting before.

Our study showed that diet can significantly increase LSM. Over 80% of them had increase in LSM at postprandial LSM. Many studies had proved that diet had influence on splanchnic and portal circulation. The increase in LSM may result from increase in portal blood flow which might cause rigidity to the liver tissue. A study in healthy persons performed by infusion a liquid meal over a duodenal feeding tube and measured liver circulation by a doppler ultrasound showed marked increase in portal blood flow after start feeding to the maximum in 30 minute.[30] Which might explain why the increase in LSM at 60 min was less than at immediate after meal in patient with chronic viral hepatitis C group, thus, may be due to the returning of portal blood flow towards baseline.

Our study was not designed to demonstrate whether how long the LSM will return to normal state, but according to a pilot study in Germany[22], performed LSM by transient elastography in eight patients with chronic viral hepatitis C at fasting for at least 8 hour, and serial every 30 minutes up to 180 minutes after intake a standardized continental breakfast (including two rolls, ham, cheese, butter and jam with approximately 600 Kcal, 54% carbohydrates, 26% fat and 20% protein) over a maximum period of 30 minutes. They demonstrated a significant increase in liver stiffness immediately after finishing food intake up to 1 hour later and normalized 3 hour after finishing the meal. Whether difference in calories intake and difference food impact the portal pressure or LSM, will leave some room for further investigation.

Noticing that in patient with chronic viral hepatitis C had mean increase in LSM at immediately after meal more than in patient with chronic viral hepatitis B though their baseline characteristic shown in table 1 were no statistical difference between the two groups. Many factors known to be associated with chronic viral hepatitis C are steatosis, insulin resistance. Does steatosis has impact factor on LSM too? Though, we didn't performed liver biopsy in every cases but steatosis of chronic hepatitis C had been studies, steatosis did not appear to affect liver stiffness values[5]. Even in a study of nonalcoholic fatty liver disease, liver stiffness correlated with fibrosis but not steatosis[11]. However, in healthy subjects, the presence of metabolic syndrome is associated with slightly higher levels of liver stiffness[12]. In non-diabetic patients with genotype 1 chronic hepatitis C, insulin resistance also contributed to liver stiffness independent of liver fibrosis.

The different additional factors influencing liver stiffness in this study were BMI and direct bilirubin which had similar results as the other studies[12,18].

However, our study had some limitations. First of all, the direct measurement of portal and splanchnic blood flow correlate to the LSM was performing in this study. Secondly, we had two operators performed the LSM, but the second operators did performed only three of the patients (4%) and TE values showed low interpersonal variation when we validated between the two operators.

In conclusion, food intake has significant influence on liver stiffness measurement. To standardize liver stiffness evaluation, we recommend that the measurement should be performed after fasting for at least 4 hrs.

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