Influence of Liver Inflammation on Liver Stiffness Measurement in Patients with Autoimmune Hepatitis Evaluation by Combinational Elastography

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Key Words

Combinational elastography · Shear wave imaging · Transient elastography · Strain imaging · Real-time tissue elastography · Autoimmune hepatitis

Abstract

Objective: In order to evaluate the influence of liver inflammation on liver stiffness measurement (LSM) by the simultaneous use of shear wave and strain imaging (combinational elastography), shear wave and strain imaging were compared before and after initial therapy for autoimmune hepatitis (AIH). **Methods:** Nine AIH patients initially treated with steroid were enrolled. Transient elastography and real-time tissue elastography were performed just before and 1 month after the start of initial steroid treatment. Blood samples, LSM, and the liver fibrosis index (LFI) were compared. **Results:** Aspartate aminotransferase ($p = 0.002$) and alanine aminotransferase (ALT) ($p = 0.015$) were significantly decreased after initial treatment. The LSM was 15.5 ± 9.6 kPa at baseline, decreasing to 7.2 ± 2.3 kPa after initial treatment ($p = 0.034$). The LFI was 1.67 ± 0.67 at baseline and 1.61 ± 0.66

after initial treatment; no significant change in LFI was recognized ($p = 0.842$). Between Δ ALT and Δ LSM, a significant regression equation could be calculated as follows: Δ ALT = $-0.55 + 0.654 \times \Delta$ LSM. **Conclusions:** Combinational elastography was useful in evaluating not only the degree of liver fibrosis, but also the degree of liver inflammation in AIH.

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Introduction

In recent years, ultrasound elastography has been attracting attention as a noninvasive diagnostic tool for liver fibrosis [1–7]. Evaluation of liver fibrosis is important in the prediction of hepatocellular carcinoma incidence risk and prognosis [8–10]. Ultrasound elastography can be classified into two groups depending on the measurement of physical quantities. Shear wave imaging measures the propagation speed of a shear wave, and strain imaging displays the relative strain of the tissue [11–14].

Autoimmune hepatitis (AIH) is one of the autoimmune diseases where steroids and immunosuppressants

are remarkably effective [15]. While shear wave imaging has been reported to be useful in the detection of fibrosis in the diagnosis of AIH, it has also been reported that liver stiffness varies greatly depending on the level of hepatic inflammation activity [16, 17]. In several liver diseases both of these elastography methods are useful to diagnose the degree of liver fibrosis, but it is said that only shear wave imaging is influenced by liver inflammation, jaundice, liver congestion, etc. [5, 15, 18–21]. However, the degree of influence on liver stiffness by liver inflammation has not been examined so far.

The objective of this study was to examine the influence of liver inflammation on the degree of liver stiffness measurement (LSM) with the simultaneous use of shear wave and strain imaging (combinational elastography).

Patients and Methods

Patients

This prospective study was performed at Kindai University Hospital (Osaka-Sayama, Japan). From July 2010 to May 2012, consecutive patients with AIH, who were initially treated with steroid, were enrolled. AIH was diagnosed using the Japanese diagnostic criteria for AIH published in 1996 [22]. Patients were excluded if they consumed >20 g alcohol per day. Patients with histories of viral hepatitis, drug-induced hepatitis, primary biliary hepatitis, primary sclerosing cholangitis, IgG4-related disease, hemochromatosis, α 1-antitrypsin deficiency, or Wilson's disease were also excluded. The study protocol conformed to the Declaration of Helsinki and was approved by the ethics committee at the Kindai University Faculty of Medicine. Each patient provided informed consent to participate in the study.

Clinical and Laboratory Assessments

Clinical data were collected at the start of initial therapy with steroid. The relevant clinical data recorded were age, sex, height, and weight. The body mass index was calculated as weight (in kg) divided by height (in m) squared. The presence or absence of jaundice, congestive liver, pleural effusion, and ascites and encephalopathy was confirmed by physical findings and inspection of various images. Blood samples were taken after overnight fasting on the same day as elastography was performed. Laboratory tests, including the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin levels, and platelet counts, were conducted using automated methods.

Histological Liver Assessment

Percutaneous ultrasound-guided liver biopsy was performed on the right lobe of the liver using a Tru-Cut semiautomatic 18-gauge needle apparatus (Monopty; CR Bard, Tempe, Ariz., USA) within 2 weeks before the start of the initial treatment. The liver biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin, and Masson's trichrome stain. All biopsy specimens were examined by pathologists who were blinded to the patient characteristics. Liver fibrosis was

Table 1. Patients' baseline characteristics

Age, years	66.6±2.2
Males	1 (11.1%)
Females	8 (88.9%)
Height, cm	154.6±8.5
Weight, kg	54.3±11.5
BMI	22.8±5.4
F stage	
0	0
1	1
2	4
3	4
4	0
Jaundice	0
Liver congestion	0
Pleural effusion	0
Ascites	0
Encephalopathy	0

Table 2. Elastographic and hematological data before and after initial treatment

	Before initial treatment	After initial treatment	p value
LSM, kPa	15.5±9.6	7.2±2.3	0.034
LFI	1.67±0.67	1.61±0.66	0.842
AST, IU/l	274.7±168.3	22.9±7.5	0.002
ALT, IU/l	360.6±319.5	29.8±13.5	0.015
Serum bilirubin, g/dl	1.1±0.4	0.9±0.3	0.418
Platelet count, ×10 ⁹ /l	18.8±7.6	20.0±6.5	0.733

The mean values of LSM, LFI, AST, ALT, serum bilirubin, and platelet count before and after initial treatment are given. There were significant decreases in LSM ($p = 0.034$), AST ($p = 0.002$), and ALT ($p = 0.015$). ALT = Alanine aminotransferase; AST = aspartate aminotransferase; LFI = liver fibrosis index; LSM = liver stiffness measurement.

scored using the New Inuyama classification. The stage of fibrosis was classified from F0 to F4 as follows: F0 = no fibrosis; F1 = fibrosis portal expansion; F2 = bridging fibrosis (portal-portal or portal-central linkage); F3 = bridging fibrosis with lobular distortion (disorganization); and F4 = cirrhosis [23].

Transient Elastography

Transient elastography (TE) was performed using a dedicated LSM device, namely a FibroScan 502 with the M-probe (Echosens, Paris, France). TE was carried out just before and 1 month after the start of the initial treatment. The procedure was performed on the right lobe of the liver through the intercostal space, with patients in the supine position. Examinations that achieved no successful measurements after ≥10 attempts were deemed failures. The median LSM value (in kPa) was considered as being representative of

Fig. 1. Line graph and box-and-whisker plot of LFI and LSM changes. The color of the line graph is the same in LFI (a) and LSM (b) in each case. The LSM was 15.5 ± 9.6 kPa at baseline and decreased to 7.2 ± 2.3 kPa after initial treatment ($p = 0.034$). The LFI was 1.67 ± 0.67 at baseline and 1.61 ± 0.66 after the initial treatment; there was no significant change in LFI ($p = 0.842$). LFI = Liver fibrosis index; LSM = liver stiffness measurement; BT = before treatment; AT = after treatment.

the elastic modulus of the liver. As an indicator of variability, the ratio of the interquartile range (IQR) of the LSM to the median value (IQR/M) was calculated. Examinations with <10 valid measurements or an IQR/M >30% or a success rate <60% were considered potentially unreliable [19].

Real-Time Tissue Elastography

Real-time tissue elastography (RTE) and TE were performed on the same day, just before and 1 month after the start of the initial treatment, and continued until the next TE. The procedure was performed after overnight fasting using ultrasound (EUB-8500 or HI VISION Ascendus; Hitachi, Tokyo, Japan) and an EUP-L52 linear type probe (3–7 MHz; Hitachi), by means of a previously reported method [4, 6]. The liver fibrosis index (LFI) was calculated using a multiple regression equation with feature values obtained from RTE images, for the diagnosis of liver fibrosis in patients with chronic hepatitis C [1]. An examiner who was unaware of the patient's background selected 10 high-quality images for the estimation of the median LFI. In the current study, the median LFI was used as an objective evaluation of RTE values.

Statistical Analysis

Descriptive statistics are shown as mean $\pm$ SD, median (range), or percentage, as appropriate. Correlation between the data was tested using nonparametric Spearman rank correlation analysis. Differences were considered statistically significant at p values <0.05. Analysis was performed using the SPSS Statistics 20 software (IBM, Armonk, N.Y., USA).

Results

Demographics and Baseline Features

A total of 9 cases were enrolled. The clinical characteristics and laboratory data are shown in table 1. Eight patients (88.9%) were women. The patients' mean age was 66.6 ± 2.2 years. In the pathological diagnosis, 1 case was

in F1, 4 cases were in F2, and 4 cases were in F3 (table 1). Jaundice, congestive liver, pleural effusion, ascites, and encephalopathy were not observed (table 1). The LSM and LFI were measurable in all cases. There was no significant correlation between elastographic and hematological data at baseline.

Comparison of Hematological Laboratory Findings, Liver Stiffness, and LFI at Baseline and after Initial Treatment

AST ($p = 0.002$) and ALT ($p = 0.015$) levels were significantly decreased after the initial treatment with steroid. However, there was no significant change in serum bilirubin level ($p = 0.418$) or platelet count ($p = 0.733$) (table 2). The LSM was 15.5 ± 9.6 kPa at baseline and decreased to 7.2 ± 2.3 kPa after initial treatment ($p = 0.034$). The LFI was 1.67 ± 0.67 at baseline and 1.61 ± 0.66 after the initial treatment; there was no significant change in LFI ($p = 0.842$) (fig. 1; table 2).

Relationship between LFI and LSM

At baseline, LFI and LSM were not significantly correlated, but a strong correlation was observed between LFI and LSM after initial therapy (fig. 2).

Relationship between Δ LFI and Δ LSM

Δ AST, Δ ALT, and Δ LSM represent the amount of change in AST, ALT, and LSM, calculated as follows: (date after treatment – date at baseline)/date at baseline. The trend in Δ LSM correlated with the trend in Δ AST and Δ ALT. Between Δ ALT and Δ LSM, a significant regression equation could be calculated as follows: Δ ALT = $-0.55 + 0.654 \times \Delta$ LSM (fig. 3).

Fig. 2. Scatter diagram of the relationship between LFI and LSM before (a) and after initial treatment (b). A strong correlation was observed between LFI and LSM after initial therapy. LFI = Liver fibrosis index; LSM = liver stiffness measurement.

Fig. 3. Relationship between Δ ALT and Δ LSM. Δ ALT and Δ LSM represent the amount of change in ALT and LSM, calculated as follows: (date after treatment – date at baseline)/date at baseline. Between Δ ALT and Δ LSM, a significant regression equation could be calculated as follows: Δ ALT = $-0.55 + 0.654 \times \Delta$ LSM. ALT = Alanine aminotransferase; LSM = liver stiffness measurement.

LFI and LSM in the Various Fibrosis Stages

The LFI gradually increased in accordance with the progress of liver fibrosis. Baseline LSM also significantly increased in accordance with the progress of liver fibrosis (fig. 4).

Discussion

Since the mean LSM at baseline was very high (15.5 ± 9.6 kPa), in some cases it might be misdiagnosed as cirrhosis. After initial treatment the LSM was significantly reduced to 7.2 ± 2.3 kPa. The observation interval was only 1 month; liver fibrosis seemed not to have changed.

There were no jaundice or liver congestion cases in this study. Moreover, such a relationship between Δ ALT and Δ LSM could be established (Δ ALT = $-0.55 + 0.654 \times \Delta$ LSM), so the main cause of the reduction in LSM was considered to be an improvement in inflammation. Thus, at baseline the mean LSM seems to have been mainly influenced by inflammation and liver fibrosis. After treatment, the inflammation was assumed to be substantially healed, and liver stiffness after treatment could be assumed to reflect only the effect of hepatic fibrosis.

In the current study, we used TE for shear wave imaging; however, TE can be substituted by other shear wave imaging devices, because there is a strong correlation between LSM and the liver stiffness calculated by other shear wave imaging devices [7].

On the other hand, the LFI was not changed after initial treatment; it seems to be mainly influenced by liver fibrosis, not by inflammation. In my opinion, since the liver fibrosis level can be evaluated by the LFI, it is possible to predict the LSM corresponding to the liver fibro-

Fig. 4. LFI and LSM in the various liver fibrosis stages before and after initial treatment. The LFI (a) and the LSM (b) of each fibrosis stage were divided into before and after treatment. The LFI gradually increased in accordance with the progress of liver fibrosis. The baseline LSM also significantly increased in accordance with the progress of liver fibrosis. LFI = Liver fibrosis index; LSM = liver stiffness measurement; BT = before treatment; AT = after treatment.

sis. If the LSM at pretreatment is much greater than the predictive value using the LFI, it can be determined that the degree of inflammation is high. In this way, if there is a discrepancy of LSM and LFI with the simultaneous use of shear wave and strain imaging (combinational elastography), the other influence to accelerate the propagation speed of shear wave, such as inflammation, jaundice, or liver congestion, could be grasped.

Unfortunately the sample size of this study was too small, so the degree of predictive evaluation of inflammation could not be determined accurately. It is necessary to verify this by studying further cases, with other liver diseases or conditions. At least, combinational elastography was useful in evaluating not only the degree of liver fibrosis, but also the degree of liver inflammation in AIH.

Conclusions

Changes in LSM, LFI, and hematological features were examined before and after the initial treatment of patients with AIH. After successful treatment with steroid, AST and ALT levels promptly improved and the LSM also greatly decreased. However, there was no significant change in LFI. Using shear wave imaging and

strain imaging simultaneously (combinational elastography), it may be possible to determine not only the degree of liver fibrosis, but also the degree of severity of conditions such as liver inflammation in AIH.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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