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Utility of Real-time Tissue Elastography in the Sequential Evaluation of Liver Fibrosis in Patients with Chronic Hepatitis C

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Abstract Liver fibrosis is related to hepatocarcinogenesis in patients with chronic hepatitis C. Real-time tissue elastography (RTE) is a useful tool for evaluating liver fibrosis. In this study, we investigated the clinical utility of RTE to sequentially evaluate liver fibrosis in 30 patients with chronic hepatitis C undergoing interferon-based therapy. We compared the change in liver fibrosis index measured by RTE before and after treatment with the change in aminotransferase-to-platelet ratio index. In addition, we compared them across patients who were divided into two groups based on pretreatment alanine transaminase (ALT) level. Liver fibrosis index at pretreatment and at achievement of sustained virological response were 2.27 and 1.94 ($p=0.0003$), respectively. The aminotransferase-to-platelet ratio index also decreased significantly from 0.78 to 0.40 ($p<0.0001$). The liver fibrosis index showed significant improvement in both the ALT elevated group (ALT≥31 IU/L) ($p=0.0265$) and the persistently normal ALT group (ALT<30 IU/L) ($p=0.0234$). By contrast, the aminotransferase-to-platelet ratio index did not show significant improvement in the persistently normal ALT group ($p=0.2813$). We consider RTE as one of the non-invasive modalities, which can replace liver biopsy for sequential evaluation of liver fibrosis in patients with chronic hepatitis C.

Key words: real-time tissue elastography, liver fibrosis, HCV, interferon

Introduction

Liver fibrosis is related to hepatocarcinogenesis in patients with hepatitis C virus (HCV)-related chronic liver disease.[1] The rate of carcinogenesis has been reported to be dependent on the fibrosis grade in HCV-related chronic liver disease. Hence, accurate evaluation of liver fibrosis over time is important in patients undergoing antiviral therapy. Moreover, planning imaging examinations at appropriate intervals is significant for early detection of cancer in patients with a high degree of fibrogenesis. Achievement of sustained virological response (SVR) with interferon (IFN)-based therapy in patients with chronic hepatitis C virus (CHC) improves liver fibrosis.[2] Liver biopsy is the gold standard for evaluating liver fibrosis, but it is invasive in nature.[3] Hence, it is difficult to perform in daily clinical practice to evaluate liver fibrosis sequentially in patients receiving antiviral therapy. Some serum markers of liver fibrosis and scoring systems for hepatic fibrosis are used in place of liver biopsy but are not well established.

Transient elastography (TE) (FibroScan) is the most popular elastography technique worldwide. Liver elasticity is correlated with liver fibrosis. FibroScan measures liver elasticity by measuring the speed with which the shear wave is conducted in the liver.[4] TE is also noninvasive and can be performed repeatedly over time. However, TE findings are influenced by hepatic inflammation, jaundice, and liver congestion.[5] Furthermore, Coco et al.[6] reported that using TE in patients with normal alanine transaminase (ALT) level by antiviral therapy or natural history, even if the same fibrosis stage, liver stiffness showed lower value than the patients with abnormal ALT level. Hence, real-time tissue elastography (RTE) has emerged as a useful tool for evaluating liver fibrosis over time. RTE is categorized as strain elastography, which can visualize relative strain. The strain images show progressively increasing patchiness as the degree of liver fibrosis increases.[7,8] Nine image feature values are extracted from each RTE image and have been reported to correlate with liver fibrosis.[7] In addition, the liver fibrosis index (LFI) calculated from RTE has been proposed as a method for evaluating liver fibrosis in chronic viral hepatitis.[8,9] TE findings may be affected by not only acute inflammation, but also chronic inflammation; on the other hand, RTE findings are not affected by them.[8] In particular, RTE has the advantage of combining direct visualization of liver parenchyma with liver stiffness measurements. This enables the operator to directly correlate the anatomical correspondence between tissue elasticity and B-mode display, thus avoiding the subcapsular region and reducing the variability between measurements. TE findings are correlated with RTE findings, and recent studies have shown a high level of accuracy in the diagnosis of liver cirrhosis with RTE.[10] In this study, we aimed to investigate the clinical usefulness of RTE in the sequential evaluation of liver fibrosis in patients with CHC receiving IFN-based therapy.

Materials and Methods

This study was conducted in compliance with the ethical principles of the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Yamaguchi University Hospital.

Patients: Thirty patients with CHC undergoing liver biopsy at Yamaguchi University Hospital and receiving IFN-based therapy were enrolled between November 2011 and March 2015 to investigate the correlation between pathological liver fibrosis and various fibrosis parameters before and after therapy. In addition, we divided the 27 patients who achieved SVR into two groups based on pretreatment ALT level: the ALT elevated group (ALT≥31 IU/L, N=19) and the persistently normal ALT (PNALT) group (ALT<30 IU/L, N=8), to evaluate the influence of inflammation.

Fibrosis parameters: We analyzed various parameters related to liver fibrosis: RTE findings, fibrosis 4 (FIB-4) index,[11] aspartate aminotransferase-to-platelet ratio index (APRI),[12] and levels of type IV collagen and procollagen III peptide (P-3-P). We also compared ALT level to RTE and FIB-4 index, APRI, and P-3-P. In the 30 patients receiving IFN-based therapy, the difference in RTE findings before and after IFN therapy was evaluated, and compared with serum fibrosis markers, FIB-4 index, and APRI. Venous blood samples were obtained from patients after overnight fasting. We measured the serum levels of aspartate aminotransferase (AST), ALT, g-glutamyltransferase, albumin, total bilirubin, type IV collagen, P-3-P, and platelet count (Plt). The FIB-4 index was calculated as follows: age (years) × AST (IU/L)/Plt ($\times 10^9$/L) × $\sqrt{}$ALT(IU/L). The APRI was calculated as follows: (AST [IU/L]/upper limit of normal AST range [IU/L])/Plt ($\times 10^9$/L) × 100.

RTE: RTE was performed using HI VISION Ascendus (Hitachi Medical, Tokyo, Japan) with a linear probe (EUP-L52; central frequency, 5.5 MHz). The ultrasound probe was placed in the right intercostal space, with the patient in the supine position. We set the ROI within the liver, avoiding large vessels and artifacts. The LFI was measured three times, and the mean value was calculated. RTE was

performed on the day of liver biopsy by two specifically trained physicians.

Liver histology: Ultrasound-guided percutaneous liver biopsy was performed with a 16-G, 200-mm needle (SuperCore Biopsy Instrument; Argon Medical Devices, Texas, USA). The liver biopsy specimens were analyzed by us and a pathologist who was blinded to the clinical data. Liver fibrosis stages and activity grades were estimated according to the New Inuyama classification of chronic hepatitis.[13]

Statistical analysis: Statistical analysis was performed using JMP® 9.0.2 (SAS Institute Japan, Tokyo, Japan). Unpaired and paired between-group comparisons were performed using the Wilcoxon rank-sum test and the Wilcoxon signed-rank test, Tukey test, respectively.

Results

Patient characteristics

Thirty patients with CHC receiving IFN-based therapy are summarized in Table 1. Their median age was 60 (28-77) years, and treatments were as follows: pegylated interferon (PEG-IFN) monotherapy, 2 cases; PEG-IFN plus ribavirin (RBV), 8 cases; PEG-IFN plus RBV with telaprevir, 14 cases; and PEG-IFN plus RBV with simeprevir, 6 cases. Twenty-seven patients achieved SVR, 2 had a relapse, and 1 patient discontinued treatment due to adverse effects.

Difference between pretreatment and post-treatment values of LFI and other parameters in patients who achieved SVR

The changes in LFI, FIB-4 index, APRI (pretreatment and at the time SVR was detected), type IV collagen, and P-3-P levels (pretreatment and posttreatment) are shown in Figure 1. The median LFI at pretreatment and at the time SVR was detected (6 months after therapy) were 2.27 and 1.94 ($p=0.0003$), respectively. The FIB-4 index changed from 2.13 to 1.93 ($p=0.0543$), and APRI decreased significantly from 0.78 to 0.40 ($p<0.0001$). Type IV collagen and P-3-P levels were measured in 6 patients before and at the end of the treatment. The median type IV collagen levels at pretreatment and at the end of the treatment were 6.00 ng/ml and 6.45 mg/ml, respectively. Thus, no significant improvement was found ($p=0.5000$). P-3-P levels changed from 1.05 U/ml to 0.77 U/ml ($p=0.0938$), which was again not a significant improvement. In addition, 18 patients were followed up from before treatment to 1 year after SVR (Figure 2). The LFI significantly improved from 2.24 to 2.01 with achievement of SVR ($p=0.0013$) and to 1.92 after 1 year ($p=0.0006$).

Changes in LFI and APRI according to ALT levels (pretreatment and post-treatment)

Both LFI and APRI showed improvement with SVR. However, in the ALT elevated group, both LFI and APRI decreased significantly from 2.26 to 2.08 ($p=0.0265$) and from 0.88 to 0.47 ($p<0.0001$), respectively (Figure

Table 1 Clinical characteristics and laboratory data of patients receiving interferon-based therapy.

Age (median min-max)	60(28-77)y.o.
Sex M:F	11:19(cases)
Genotype 1a:1b:2a:2b:3b	1:22:5:1:1(cases)
F stageF1:F2:F3:F4	22:5:1:2(cases)
Therapy PEG-IFN:PEG-IFN+RBV:TVR+PEG-IFN+RBV:SMV+PEG-IFN+RBV	2:8:14:6 (cases)
EfficancySVR:relapse:cassation	27:2:1 (cases)

PEG-IFN:PEG interferon, RBV: ribavirin,TVR: telaprevir, SMV: simeprevir, y.o.: years old

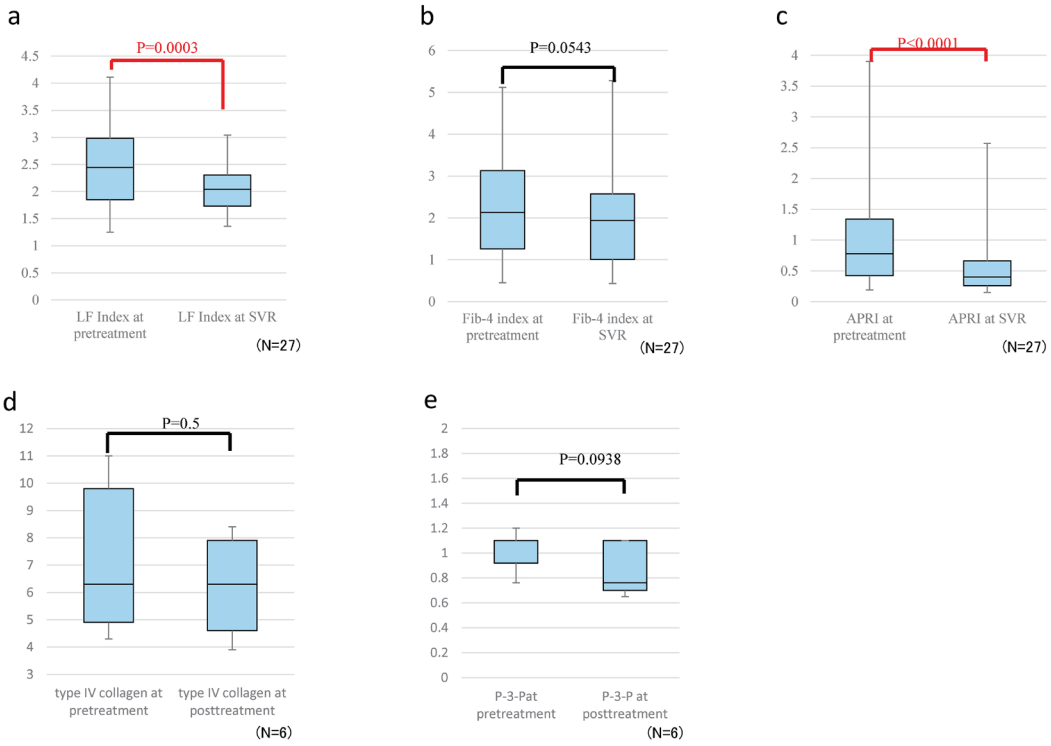

Fig. 1 Transition of fibrosis makers from pretreatment to SVR or to post-treatment
(a) The median LFI significantly declined after achieving SVR (p=0.0003). (b) The median FIB-4 index was not significantly different after achieving SVR (p=0.0543). (c) The median APRI significantly declined after achieving SVR (p<0.0001). (d) The median type IV collagen 7S domain was not significantly different after IFN therapy (p=0.5). (e) The median P-3-P was not significantly different after IFN therapy (p=0.0938).

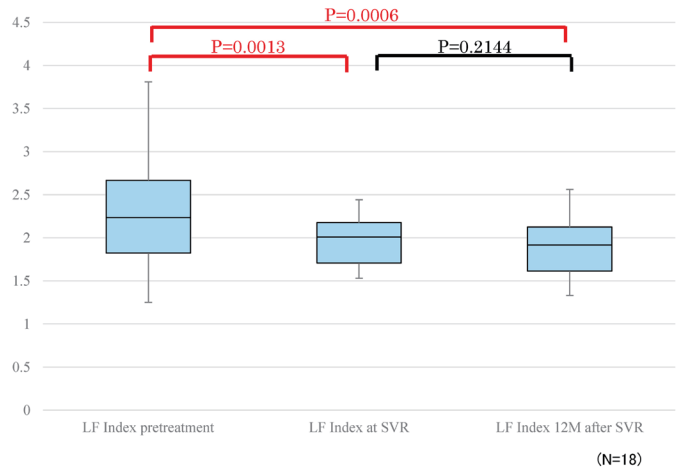

Fig. 2 Long-term transition of LFI before and after treatment of IFN-based therapy
The median LFI significantly declined after achieving SVR (p=0.0013). After one year (P=0.0006), the median LFI declined, but was not significant (p=0.2344).

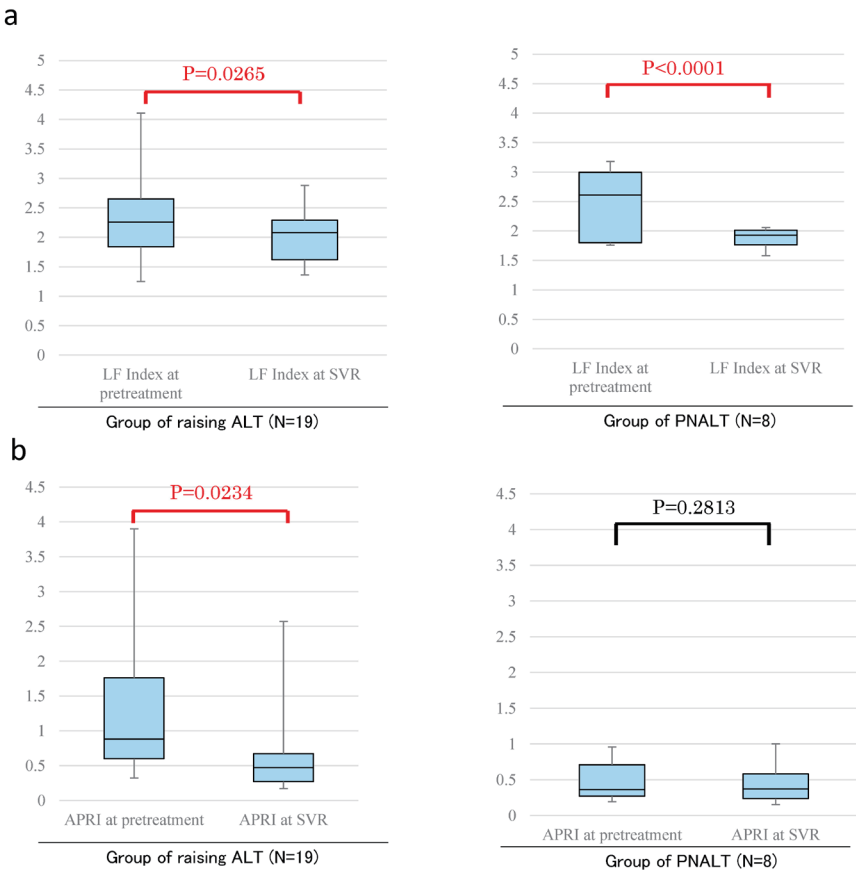

Fig. 3 Transition of LFI from pretreatment to SVR
(a) Group of raising ALT: The median LFI significantly declined after achieving SVR (p=0.0265). (b) Group of raising ALT: The median APRI significantly declined after achieving SVR (p=0.0234). (c) Group of PNALT: The median LFI significantly declined after achieving SVR (p<0.0001). (d) Group of PNALT: The median APRI was not significantly different after achieving SVR (p=0.2813).

3). In the PNALT group, the LFI at pretreatment and at the time SVR was detected were 2.61 and 1.88, showing a significant improvement (p=0.0234). However, the APRI did not change significantly (0.46 to 0.37; p=0.2813).

Discussion

After achieving SVR, hepatocellular carcinoma development is suppressed,[14] but severe fibrosis is the risk factor for carcinogenesis.[15] Sequentially evaluating liver fibrosis is also important to make a reasonable period of cancer screening after achieving SVR. In this study, both LFI and APRI showed significant improvement in patients who achieved SVR. RTE findings showed significant improvement regardless of the ALT levels. However, APRI showed improvement only in the group with elevated pretreatment ALT levels. APRI is influenced by inflammation because it takes into account AST levels. Therefore, the diagnosability for the patients after achieving SVR is low because it was devised as the marker for evaluating fibrosis in patients with chronic hepatitis. We consider RTE as the best modality for sequentially evaluating liver fibrosis without being influenced by inflammation. In our study, the LFI correctly reflected the transition of fibrosis with SVR in patients who received IFN-based treatment and achieved SVR.

The FIB-4 index, and type IV collagen and P-3-P levels could also reflect liver fibrosis in untreated patients with CHC, but they did not show significant improvement after

treatment. FIB-4 index was simple and easy, but could not diagnose fibrosis in patients who achieved SVR because it was devised as the marker for evaluating fibrosis in patients with chronic hepatitis. In addition, it is influenced by physiological factors, such as Plt and transaminase; thus, it cannot reflect liver fibrosis alone. Serum P-3-P levels are elevated when inflammation in the liver causes fibrogenesis. They are a better marker of active fibrogenesis and inflammation than an indicator of the extent of fibrosis.[16] However, serum P-3-P levels are influenced by not only liver fibrosis but also other organic fibrogenesis.[17] Type IV collagen is one of the major constituents of the basement membrane and is deposited in the fibrotic liver. The serum levels of type IV collagen increase in patients with chronic viral liver disease and correlate with the histological degree of liver fibrosis. Therefore, the measurement of serum fragment of type IV collagen is useful as an indicator of liver fibrosis.[18] However, it is influenced by not only liver fibrosis but also hyperthyroidism, not specific to liver fibrosis.[17]

Antiviral therapy has advanced dramatically, and with the advent of direct-acting antivirals, eliminating virus at a high rate, even if cirrhosis of the liver, has become possible. However, patients with CHC for antiviral treatment aged, and their livers have a high carcinogenic risk with severe fibrosis. The biggest advantage of RTE over liver biopsy is its noninvasiveness and ability to be performed sequentially. We consider that sequentially performing RTE for patients with CHC under treatment and after achieving SVR is useful when planning screening tests corresponding to the risk of carcinogenesis.

Conclusions

RTE accurately evaluates liver fibrosis and is not influenced by inflammation. Hence, it is useful in the sequential evaluation of liver fibrosis in a noninvasive manner in patients with CHC and has the potential to replace liver biopsy for this purpose.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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