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Shear wave elastography in hepatitis C patients before and after antiviral therapy

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Abstract

AIM: To investigate shear wave (SW) propagation velocity in patients with untreated hepatitis C and patients with sustained virological response (SVR).

METHODS: A total of 136 hepatitis C patients (85 patients who had not received antiviral therapy (naïve group) and 51 patients who had received antiviral therapy and subsequently achieved SVR of at least 24 weeks (SVR group)) and 58 healthy volunteers and outpatients without liver disease (control group) underwent evaluation of liver stiffness by SW elastography (SWE). Various parameters were evaluated in the chronic hepatitis C patients at the time of SWE.

RESULTS: SW propagation velocity (Vs) was 1.23±0.14 m/s in the control group, 1.56±0.32 m/s in the SVR group, and 1.69±0.31 m/s in the naïve group. Significant differences were seen between the control group and the SVR group (p=0.0000) and between the SVR group and the naïve group (p=0.01417). All four fibrosis markers were higher in the naïve group than in the SVR group. In the naïve group, Vs was positively correlated with alanine aminotransferase

(ALT) (r=0.5372), α feto protein (AFP) (r=0.4389), type IV collagen (r=0.5883),

procollagen III peptide (P-III-P) (r=0.4140), hyaluronic acid (r=0.4551), and

Mac-2 binding protein glycosylation isomer (M2BPGi) (r=0.6092) and

negatively correlated with albumin (r=-0.4289), platelets (r=-0.5372), and

prothrombin activity (r=-0.5235). On multiple regression analysis, Vs was the

most strongly correlated with ALT (standard partial regression std β=0.4039,

p=0.00000). In the SVR group, Vs was positively correlated with AFP (r=0.6977),

type IV collagen (r=0.5228), P-III-P (r=0.5812), hyaluronic acid (r=0.5189), and

M2BPGi (r=0.6251) and negatively correlated with albumin (r=-0.4283), platelets

(r=-0.4842), and prothrombin activity (r=-0.4771). On multiple regression

analysis, Vs was strongly correlated with AFP (standard partial regression std

β=0.5953, p=0.00000) and M2BPGi (standard partial regression std β=0.2969,

p=0.03363).

CONCLUSION: In hepatitis C patients, liver stiffness is higher in

treatment-naïve patients than in those showing SVR. SWE may be a predictor of

hepatocarcinogenesis in SVR patients.

Key words: Antiviral therapy; Hepatitis C; Hepatocarcinogenesis; Sustained virological response; Shear wave elastography

Core tip: This study is the first to compare liver stiffness in a group of hepatitis C patients in whom the virus was eliminated with antiviral therapy and a group of untreated hepatitis C patients using shear wave elastography. The liver stiffness value was higher in the untreated group than in the group in which the virus had been eliminated, which is thought to be due hepatitis activity. This study also suggests the possibility that liver stiffness measurements with shear wave elastography can be used as predictors of hepatocarcinogenesis in patients in whom the virus has been eliminated.

Suda T, Okawa O, Masaoka R, Gyotoku Y, Tokutomi N, Tamano M. Shear wave elastography in hepatitis C: Comparison of patients before antiviral therapy and patients with sustained virological response. World J Hepatology 2016; In press.

INTRODUCTION

Shear wave elastography (SWE) is a new technology that gauges liver stiffness by measuring the propagation velocity of shear waves generated in liver tissue. At the same time, images are observed in real time using a normal B-mode ultrasound probe. The velocity of laterally propagated shear waves (lateral waves) is measured. SWE is useful for a diagnosis of breast tumor[1], thyriod tumor[2], muscle stiffness[3] as well as liver stiffness. SWE resembles acoustic radiation force impulse[4], but it is new another technology,

Liver stiffness measurements with SWE are reported to be useful in diagnosing fibrosis in hepatitis C[5]. In studies using transient elastography, liver stiffness was affected not only by liver fibrosis but also by necroinflammatory activity[6, 7]. Therefore, the meaning of liver stiffness is predicted to differ in untreated patients with hepatitis activity and patients whose hepatitis has subsided with antiviral therapy.

The purpose of this study was to investigate the significance of SW propagation velocity in patients with untreated hepatitis C and patients with sustained virological response.

MATERIALS AND METHODS

Patients

This prospective study was reviewed and approved by the Ethics Committee of Dokkyo Medical University Koshigaya Hospital, and written, informed consent was obtained from all participants and healthy volunteers. This study conformed to the ethical guidelines of the 2008 Declaration of Helsinki.

The subjects were 136 chronic hepatitis C patients who were diagnosed in the Department of Gastroenterology of Dokkyo Medical University Koshigaya Hospital from April to October, 2015. The 136 patients included 85 patients in a naïve group who had not received antiviral therapy and 51 patients who had received antiviral therapy, either interferon-based therapy or direct-acting antiviral agent therapy (daclatasvir/asunaprevir), and subsequently achieved sustained virological response (SVR) of at least 24 weeks (SVR group). Patients with decompensated liver cirrhosis, hepatocellular carcinoma, autoimmune disease, collagen disease, or chronic heart disease were excluded. Patients with a history of drinking ≥20 g alcohol per day and those diagnosed with obvious fatty liver on abdominal ultrasound were also excluded.

To obtain a standard liver stiffness value, SWE was performed in a total of 58

people including healthy volunteers and outpatients without liver disease (control group).

Measurement of shear wave elastography

Measurement of liver stiffness by shear wave elastography was performed using a LOGIQ E9 (GE Healthcare, Milwaukee, WI). The right lobe of the liver was visualized through an intercostal space while the patient was lying in a supine position with the right arm in maximum abduction. Measurements were taken while subjects held their breath during spontaneous breathing. The visual depth of the system was fixed at 8 cm, and the region of interest (ROI) was 1-2 cm below the surface of the liver. The system was adjusted so that sample volume depth (SVD) was 4 cm or less. Liver stiffness was automatically calculated by the apparatus, and the results are expressed as the velocity of shear wave (Vs (m/s)). Measurements were performed by two investigators (Suda T and Tamano M) who have measurement experience of SWE more than 100 patients. They shot 10 to12 times on liver segment 5, and the result was considered reliable only when 10 successful shots and a measurement success rate >80% were obtained.

Clinical parameters

The following clinical parameters were determined in chronic hepatitis C patients at the time SWE was performed: age; aspartate aminotransferase (AST); alanine aminotransferase (ALT); total bilirubin, serum albumin; white blood cell (WBC) count; platelet count; prothrombin activity; α fetoprotein (AFP); hyaluronic acid; type IV collagen; procollagen III peptide (P-III-P); Mac-2 binding protein glycosylation isomer (M2BPGi) measurements; and the Fib-4 Index.

Statistical analysis

Continuous data for Vs and other clinical parameters are expressed as means ± standard deviation (SD). The Mann-Whitney U test was used for between-group comparisons. Correlations between Vs and other parameters were assessed using Spearman's rank correlation coefficient and multiple regression analysis. Values of $p<0.05$ were regarded as statistically significant.

RESULTS

Figure 1 shows Vs (m/s) measured by SWE in each group. Vs was 1.23 ± 0.14 m/s in the control group, 1.56 ± 0.32 m/s in the SVR group, and 1.69 ± 0.31 m/s

in the naïve group. Significant differences were seen between the control group and the SVR group (p=0.0000) and between the SVR group and the naïve group (p=0.01417).

Table 1 shows the characteristics of the naïve group and the SVR group. Compared with the SVR group, the naïve group had significantly higher AST and ALT values (p=0.00001) and a significantly lower serum albumin value (p=0.01049). No significant differences were seen between the two groups in total bilirubin, WBC, platelet count, or prothrombin activity. AFP was significantly higher in the naïve group than in the SVR group (p=0.00773). All four fibrosis markers were higher in the naïve group than in the SVR group. No significant differences were seen in type IV collagen or hyaluronic acid, but significant differences were seen in P-III-P (p=0.00215) and M2BPGi (p=0.00546). The FIB-4 index tended to be higher in the naïve group than in the SVR group, but the difference was not significant (p=0.37443).

Correlation between Vs and each parameter in the naïve group (n = 85)

Vs was positively correlated with ALT (r=0.5372), AFP (r=0.4389), type IV collagen (r=0.5883), P-III-P (r=0.4140), hyaluronic acid (r=0.4551), and M2BPGi (r=0.6092). Vs was negatively correlated with albumin (r=-0.4289), platelets (r=-0.5372), and prothrombin activity (r=-0.5235). A multiple regression analysis

was performed with the five parameters of ALT, platelets, prothrombin activity, type IV collagen, and M2BPGi that had correlation coefficients (r) ≥0.5, and the results showed that Vs was the most strongly correlated with ALT in the naïve group (standard partial regression std β=0.4039, p=0.00000) (Table 2).

Correlation between Vs and each parameter in the SVR group (n = 51)

Vs was positively correlated with AFP (r=0.6977), type IV collagen (r=0.5228), P-III-P (r=0.5812), hyaluronic acid (r=0.5189), and M2BPGi (r=0.6251). Vs was negatively correlated with albumin (r=-0.4283), platelets (r=-0.4842), and prothrombin activity (r=-0.4771). A multiple regression analysis was performed with the five parameters of AFP, type IV collagen, P-III-P, hyaluronic acid, and M2BPGi that had correlation coefficients (r) ≥0.5, and the results showed that Vs was strongly correlated with two parameters, AFP (standard partial regression std β=0.5953, p=0.00000) and M2BPGi (standard partial regression std β=0.2969, p=0.03363) (Table 3).

DISCUSSION

The extent of hepatic fibrosis has classically been evaluated by histological procedures. However, the accuracy of this evaluation of hepatic fibrosis is limited by both sampling variability and inter-observer variability between

pathologists[8, 9]. In addition, liver biopsy is associated with patient discomfort and a risk of serious complications[10].

Transient elastography (TE) has attracted attention as a noninvasive, objective diagnostic tool, and liver stiffness measured by TE is reported to be useful in diagnosing fibrosis in hepatitis C[11]. TE is useful in diagnosing non-alcoholic fatty liver disease[12] and in predicting carcinoma development in viral hepatitis patients[13]. However, TE is a test that is done blindly using a special probe in the right hepatic lobe confirmed with B mode, as a result of which measurement results are imprecise if vessels or other structures are present in the measured region. SWE is built into ultrasonic diagnostic equipment, and reliable measurements are possible in a short time under observation with normal B mode[14].

The results of SWE measurements are expressed as the SW propagation velocity Vs (m/s). In this investigation, the Vs was 1.23 ± 0.14 m/s in healthy livers, 1.69 ± 0.31 m/s in the naïve group, and 1.56 ± 0.32 m/s in the SVR group. The naïve group had a significantly higher Vs than the SVR group, suggesting that Vs decreases with virus elimination in hepatitis C patients. In this study, however, the naïve group and SVR group were different populations, and Vs

measurements over time in the same population will be needed to accurately

compare Vs before and after treatment.

Vs is determined not only by tissue elasticity (fibrosis), but it is also affected

by viscosity. Thus, in cases of active hepatitis, propagation is expected to

become faster due to increased tissue viscosity from increased exudate into the

interstitium and cell infiltration, and in acute hepatitis that trend is marked[15, 16].

In the naïve group, Vs was most strongly correlated with ALT. This is thought

to be because the naïve group included many patients with active hepatitis with

high ALT levels. Good positive correlations were seen between Vs and the liver

fibrosis markers of hyaluronic acid, type IV collagen, P-III-P, and M2BPGi in the

naïve group. Thus, in the naïve group, Vs is thought to reflect both hepatic

activity (viscosity) and fibrosis (elasticity).

In the SVR group, hepatitis had subsided for six months or more, and, in fact,

the correlation between Vs and ALT in the SVR group in this study was very

low. Therefore, Vs in the SVR group is presumed to almost purely reflect liver

fibrosis (elasticity), and thus it is thought to have better correlations with

fibrosis markers. Among the four different fibrosis markers, M2BPGi had the

strongest positive correlation with Vs. M2BPGi is a new liver fibrosis marker

that quantitatively measures changes in the carbohydrate structure of Mac-2

binding protein[17], and it is also considered useful in predicting carcinogenesis

in hepatitis C patients[18-20].

In hepatitis C patients, AFP is a useful indicator of hepatocarcinogenesis

following interferon therapy[21]. Although AFP had positive correlations in both

the naïve group and the SVR group, a stronger correlation was seen in the SVR

group. In the naïve group, AFP reflects inflammation and necrosis of

hepatocytes and the accompanying hepatocyte regeneration. In the SVR group,

on the other hand, AFP has a strong element as a surrogate marker of

hepatocellular carcinoma, as mentioned previously. A very interesting finding

in the SVR group in the present study was the strong correlations between Vs

and AFP and between Vs and M2BPGi, which suggest the possibility that liver

stiffness measurements with SWE may be used as predictors of

hepatocarcinogenesis in hepatitis C patients following SVR.

CONCLUSIONS

In hepatitis C patients, liver stiffness with SWE was higher in the naïve group

than in the SVR group, presumably due to hepatitis activity. In the SVR group,

liver   stiffness   measurements   with   SWE   may   be   a   predictor   of

hepatocarcinogenesis.

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Table 1 Clinical characteristics in the naïve and SVR groups

Characteristic	Naïve group (n=85)	SVR group (n=51)	P-value
Age (years)	63.5±13.7	64.9±10.2	0.9726
Sex (male/female)	43/42	27/24	0.79039
AST (IU/L)	55.4±38.7	29.2±16.8	0.00001
ALT (IU/L)	61.9±50.1	23.9±18.0	0.00001
Total bilirubin (mg/dL)	0.89±0.36	0.95±0.33	0.14736
Serum albumin (g/dL)	4.10±0.46	4.32±0.44	0.01049
WBC ($\times 10^3$/mm^3)	5.21±1.83	5.24±1.56	0.79015
Hb (g/dL)	13.9±1.6	14.1±1.5	0.47879
Platelets ($\times 10^4$/mm^3)	14.5±6.4	14.5±5.7	0.89078
Prothrombin activity (%)	97.5±12.1	96.9±13.1	0.98341
AFP (g/dL)	9.6±10.6	5.1±3.3	0.00773
Type IV collagen (ng/ml)	180.5±71.8	179.2±71.6	0.62913
P-III-P (U/ml)	0.92±0.27	0.81±0.31	0.00215
Hyaluronic acid (ng/ml)	191.5±290.3	102.1±93.5	0.24908
M2BPGi (C.O.I)	3.67±4.41	2.03±2.49	0.00546
FIB-4 index	4.44±4.21	3.39±2.63	0.37443

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase;

WBC, white blood cells; P-III-P, Procollagen III peptide; M2BP,

Mac-2 binding protein; C.O.I, Cut-off index

Table 2 Multiple regression analysis in the naïve group

	Coefficient (β)	SE(β)	stdβ	*t*-value	df	P
ALT	0.00261	0.00052	0.4069	5.02998	62	0.00000
Plt	-0.0121	0.00436	-0.27300	0.27300	62	0.00740
PT %	-0.0044	0.00245	-0.1712	1.79923	62	0.07685
Type IV Collagen	0.00061	0.00040	0.1578	1.51030	62	0.13605
M2BPGi	0.00844	0.00709	0.1361	1.19098	62	0.23820

Table 3 Multiple regression analysis in the SVR group

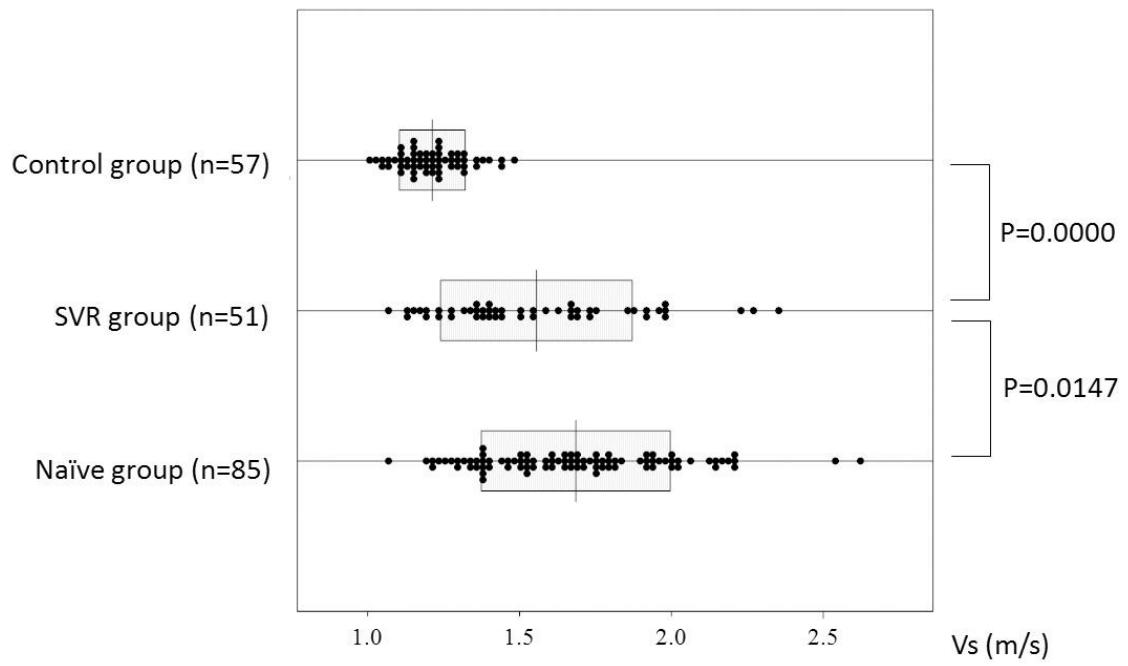|	Coefficient (β)	SE(β)	stdβ	t-value	df	P
AFP	0.05564	0.0098	0.5953	5.6797	28	0.00000
Type IV Collagen	-0.0003	0.00066	-0.0626	0.42483	28	0.67420
P-III-P	0.13859	0.17013	0.146	0.81462	28	0.42217
Hyaluronic acid	0.00053	0.00053	0.1658	0.99713	28	0.32724
M2BPGi	0.03554	0.01591	0.2969	2.23421	28	0.03363

Figure 1

Figure Legends

Figure 1: Vs based on SWE is shown for each group. Vs is 1.23 ± 0.14 m/s in the control group, 1.56 ± 0.32 m/s in the SVR group, and 1.69 ± 0.31 m/s in the naïve group. Significant differences are seen between the control group and the SVR group (p=0.0000) and between the SVR group and the naïve group (p=0.01417).