⟨*Original Article*⟩

Relationship Between Liver Fibrosis Noninvasively Measured by Fibro Scan and Blood Test

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ABSTRACT

Recently, Fibro scan was developed as a non-invasive device for measuring liver fibrosis. Progression of liver fibrosis has been reported to increase the measurement value (elasticity). We have performed the Fibro scan for liver diseases.[;normal liver (n=33), hepatitis C (n=156), hepatitis B(n=36), non-alcoholic fatty liver disease (n=24), auto immune hepatitis (n=9), primary biliary cirrhosis (n=10), alcoholic liver disease (n=7), acute hepatitis (n=3), other liver disease (n=14), recipient of living donor liver transplantation (n=11)] Elasticity, blood test, and indirect fibrosis diagnostic score were comparatively investigated. In the hepatitis C group, the mean value for elasticity was 14.7 ± 12.0 kPa. Elasticity was found to be significantly correlated with the hepatobiliary enzymes, fibrosis markers and indirect fibrosis diagnostic score. When elasticity was divided into $\geqq 14.6$kPa(liver cirrhosis) and<14.6kPa(non liver cirrhosis) groups for the investigation, liver inflammation and fibrosis markers were correlated in the progress of fibrosis in non liver cirrhosis, and it could be speculated that if liver cirrhosis is reached, progression of fibrosis reflects decreased liver function.

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INTRODUCTION

In chronic hepatitis C, it is known that liver fibrosis progresses as the period of infection prolongs, and may reach liver cirrhosis. If it progresses to liver cirrhosis, the risk of onset of hepatocellular carcinoma increases. And, apart from periodical diagnostic imaging, measures against decrease in liver function and gastroeso-phageal varices also become necessary. Apart from chronic hepatitis C, the same also applies to chronic hepatitis B and auto immune hepatitis (AIH) etc. Furthermore, in hepatitis C(HC) and hepatitis B(HB), if antiviral therapy such as interferon is applied, viral elimination effect is decreased when there is liver cirrhosis, and side effects such as leucopenia and thrombocytopenia increase. Therefore, in order to decide on the treatment strategy, it is very important to assess the extent of liver fibrosis. In addition, in nonalcoholic fatty liver disease (NAFLD), a pathological image that includes necrosis and inflammation of parenchymal cells due to fat deposition in the liver in nonalcoholic subjects is notably similar to alcohol-induced hepatitis. Recently, attention is being paid to nonalcoholic steatohepatitis (NASH), which often progresses to liver fibrosis and liver cirrhosis[1]. In Japan, fatty liver is found in 20~30% of individuals that are examined. Saibara et al. estimate that there are approximately 1 million NASH patients[2]. The number of NASH patients is tending to increase due to elucidation of the clinical condition and change in lifestyle, and the assessment of fibrosis is also important in NAFLD[3].

Presently, liver biopsy is the most highly reliable method for assessing fibrosis, and apart from fibrosis, much information on the extent of inflammation can be obtained. However, it is an invasive procedure that may be complicated with pain, bleeding etc[4]. In addition, sampling error may have occurred because only 1/50000th of the organ is sampled. Moreover, there is the problem of the diagnosis being semi-quantitative[5]. Because of that, various serum fibrosis markers are being used as the daily clinical method for assessing fibrosis quantitatively and non-invasively. In Western countries, this method is combined with biopsy tests and so fibrosis is indirectly assessed[6~13]. In addition, the company EchoSens in France recently developed Fibro scan with which liver fibrosis can be measured non-invasively[14,15]. An elastic shear wave is generated from the probe of this device. And the speed of transmission of this wave in liver tissue is measured. Liver fibrosis is calculated from this speed. Thus, its usefulness in hepatitis C has been assessed[16]. We have comparatively investigated elasticity and fibrosis markers obtained with the Fibro scan against indirect fibrosis diagnosis methods in liver diseases focusing on hepatitis C. The present status and future issues in the diagnosis of liver fibrosis have been discussed based on the results of the investigation.

PATIENTS and METHODS

Patients

The subjects were 303 patients who had received a thorough explanation of the study and from whom informed consent was obtained prior to being subjected to abdominal ultrasono-graphy(US), blood tests, and Fibro scan test at this department from August 2005 to June 2006. We classified the subjects into a total of nine groups: normal liver group as well as HC group, HB group, NAFLD (including NASH) group, AIH group, primary biliary cirrhosis(PBC) group, alcoholic liver disease(ALCLD) group, acute hepatitis(AH) group, disease of unknown origin group (unknown origin group), and LDLT group, as the liver disease groups. The normal liver group consisted of patients testing negative for HBs antigen (HBsAg), and HCV antibody (HCVAb)in the blood tests, with no abnormal hepatobiliary enzymes, and no abnormality in the liver parenchyma as confirmed by abdominal US. The HC group consisted of patients that were HCVAb positive and the HB group consisted of those that were HBsAg positive regardless of the status of abnormalities in the blood tests and abdominal US. The NAFLD group were patients found to have a fatty liver by abdominal US, no history of alcoholism (including the chance drinking group), and HBsAg and HCVAb negative in blood tests. The AIH group consisted of patients with probable or definite diagnosis based on the AIH international standards[17,18], and the PBC group of patients that are anti-mitochondria antibody (AMA) positive and have variations in biliary system enzymes. The ALCLD group consisted of patients with liver damage due to alcoholism (a habitual drinker who drinks alcohol equivalent to $\geqq 60$ g of ethanol per day for $\geqq 5$ years). The unknown origin group consisted of patients found to have abnormal hepato enzymes but the origin is unclear. The AH group consisted of patients found to have acute liver damage

regardless of the cause. The causal factors for three patients in the AH group were AIH, HB, and unknown. However, these three patients were not included in the three respective disease groups above. In addition, the LDLT group consisted of patients who had received a liver transplant with no rejection at the liver surgery department of this hospital and are making outpatient visits. Due to the mechanical properties of Fibro scan, measurements are difficult in patients with ascites, therefore the subjects were restricted to those with compensated liver cirrhosis without ascites. Histopathological tests were performed for 21 patients in the HC group who gave their informed consent to do so.

Fibro scan

Fig. 1 shows the main unit of the Fibro scan and the measurement screen.Fibro scan is composed of a probe, a dedicated electronic system and control unit. The measurement principle is as follows: the speed of transmission of the audib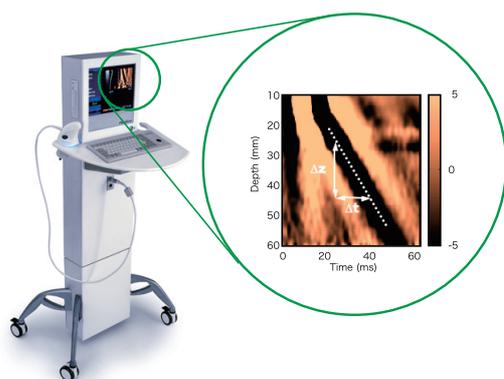le vibrations generated from the probe inside the liver is fast when liver fibrosis is advanced and slow when it is not. This principle is used to measure the extent of fibrosis in the liver by tracking the speed of transmission in the liver using ultrasound (3.5 MHz: A wave) and analyzing the change in speed. The speed of transmission ranging from 25 to 65 mm from the surface with the tip of the probe at right angles to the skin from the right intercostals is measured and converted to number value and used as the quantitative value.(Fig. 2) Result is expressed in kilo Pascal (kPa). The Δz shown in Fig. 1 is the distance and Δt is the time to transmit the distance. Speed (Vs) is expressed as $\Delta z / \Delta t$. ρ is the mass density, and elasticity (E)is determined using the formula $E = 3 \rho Vs^2$. The measurement was performed 10 times and the median value was assessed[15]. The measurement values that were obtained by Fibro scan are expressed as elasticity. Ziol et al. reported that liver cirrhosis in HC could be diagnosed by more than 14.6kPa level of elasticity[16].
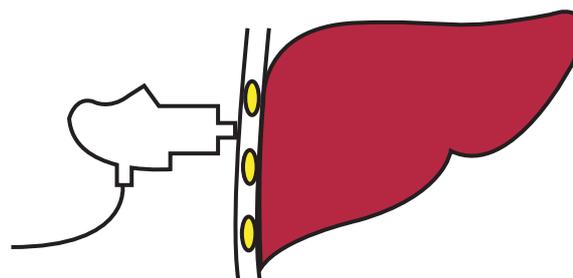

Fig. 1. Fibro scan and the measurement screen

Fig. 2. Fibro scan probe and its positioning on patient during examination

Liver Histology and Quantification of Liver Fibrosis

The 21 HC patients from whom pathological tissues were obtained, were 16 patients that underwent liver biopsy with interferon treatment as the prerequisite, and 5 surgical patients from whom tissues were obtained when the hepatocellular carcinoma was being excised. Percutaneous liver biopsy was performed using a 14G needle and the diagnosis was performed by two experienced pathologists. Fibrosis was assessed using the new Inuyama classification. However, in this study, the values were converted to the METAVIR scoring system and assessed[19]. Fibrosis was staged on a 0-4 scale: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.

Blood tests

white blood cell (WBC), platelets (PLT), albumin (Alb), total-bilirubin (T-Bil), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH),alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (γ-GTP), cholinesterase (Ch-E), iron (Fe), total-cholesterol (T-chol), triglyceride (TG), glucose (Glu), prothrombin time (PT), ammonia (NH3), zinc sulfate turbidity test (ZTT), thymol turbidity test (TTT) and fibrosis markers were measured as the blood test items.

Serum fibrosis markers

A liver fibrosis markers that use the extracellular matrix metabolism-related substances have been developed. In this study, we measured three markers: procollagen III N-terminal propeptide (PIIIP), collagen type III, and hyaluronic acid[20~22].

Assay for serum concentration of PIIIP: The solid phase method was used. In this method, the specimen was added to a tube with PIIIP antibodies as the solid phase. After being washed, 125I-labeled PIIIP antibody was added. After discarding the unreacted substances, the radioactivity of the antibody-PIIIP-125I labeled PIIIP bound substances was measured. PIIIP concentration was then determined from the calibration curve of the known serum concentrations.

Assay for serum concentration of Collagen type IV: Latex agglutination turbidimetric method was used. In this method, the specimen and mouse anti-human type IV collagen monoclonal antibody sensitized latex were made to react under fixed conditions to form an agglutinate of type IV collagen and latex in the specimen. Measurement of this agglutinate as the difference in turbidity (amount of change in absorbance) gives the amount of type IV collagen in the specimen.

Assay for serum concentration of Hyaluronic acid: Latex agglutination immuno-turbidimetric method was used. In this method, hyaluronic acid binding protein sensitive latex that specifically binds to hyaluronic acid and the hyaluronic acid in the specimen were made to undergo latex agglutination reaction. The change in turbidity was treated as the amount of change in absorbance to assay the concentration of

hyaluronic acid in the specimen.

The indirect fibrosis diagnosis score

The methods whereby we could relatively simply evaluate a combination of the items examined in the blood test were measurement using the formulas by Forns et al.[11], Wai et al.[12], and the HALT-C Study on liver cirrhosis[13]. These are the reported methods for the indirect diagnosis of fibrosis in hepatitis C. However, we also used these formulas to investigate the hepatitis B group.

Formula by Forns et al.(FORNS)[11]:

Forns et al. examined the relationship between laboratory test values and liver fibrosis in 351 chronic hepatitis C patients. It became evident that PLT, γ-GTP, age, and T-chol are closely related with liver fibrosis. They determine the formula below based on this.

$$Score = 7.811 - 3.131LN(PLT) + 0.781LN(γ\text{-}GTP) + 3.467LN(age) - 0.014(T\text{-}chol)$$

Using a best cut-off score of more than 6.9, the presence of significant fibrosis (F2 to F4) could be included with high accuracy (positive predictive value of 78%)

Formula by Wai et al.(WAI)[12]:

Wai et al. examined the relationship between laboratory test values and liver fibrosis in 192 chronic hepatitis C patients. They reported that the ratio between the multiple of the upper limit of normal of GOT and PLT is useful for assessing liver fibrosis.

$$Score = GOT / upper\ limit\ of\ normal\ for\ GOT / PLT(10^9/L) \times 100$$

Using a cut off score of more than 1.5, the presence of significant fibrosis (F2 to F4) could be included with high accuracy (positive predictive value of 88%). And using a cut off score less than 2.0, liver cirrhosis (F4) could be predicted with high accuracy (negative predictive value of 93%)

HALT-C study(HALT-C)[13]:

Lok et al. examined 1145 chronic hepatitis C patients including 429 liver cirrhosis patients. This was the prediction of liver cirrhosis rather

than a graded assessment of fibrosis. The usable para-meters are PLT, GOT/GPT, and PT/international normalized ratio (PT-INR) values.

Log odds (predicting cirrhosis)=-5.56-0.0089× PLT(×10³/mm³)+1.26×GOT/GPT+5.27×PT-INR.
The formula to calculate predicted probability is : exp (log odds)/1+exp (log odds)

When the cut-off value is 0.8~0.9, the positive predictive value for liver cirrhosis was 86%.

Statistical analysis

The test results are expressed as the mean ± standard deviation (SD). Windows Xp Stat View Version 5 was used for the statistical analysis. For gender differences and comparison of the various groups, Mann-Whitney's U test was used and $p<0.05$ indicated a significant difference. For the comparison of the correlation between elasticity and the various types of blood tests, age, and body mass index (BMI), Spearman's rank correlation was used and a correlation coefficient $|\delta|$ of $\geqq 0.40$ when $p<0.05$ indicated a significant correlation.

RESULTS

(1) Overall investigation

Table 1 presents the patients' background of each disease groups. Fig. 3 shows the distribution of elasticity.

Table 1. Number of patients, age, sex, and BMI of the normal liver group and various liver disease groups.

	Subject(n)	Age(yrs)	Sex(♂/♀)	BMI(kg/m²)
NORMAL	33	63.2±14.5	12/21	20.9±2.6
HC	156	64.3±11.8	70/86	22.7±3.1
HB	36	53.4±13.9	16/20	22.9±2.7
NAFLD	24	61.5±12.0	11/13	23.7±2.8
AIH	9	61.4±12.4	0/9	22.8±3.1
PBC	10	58.4±14.7	1/9	21.5±2.1
ALCLD	7	65.9±5.9	5/2	23.3±4.0
OTHER	14	67.4±9.0	5/9	22.4±2.3
AH	3	45.3±21.9	2/1	21.8±2.6
LDLT	11	60.0±7.8	7/4	22.8±2.5
All	303	62.2±12.8	129/174	22.5±2.9

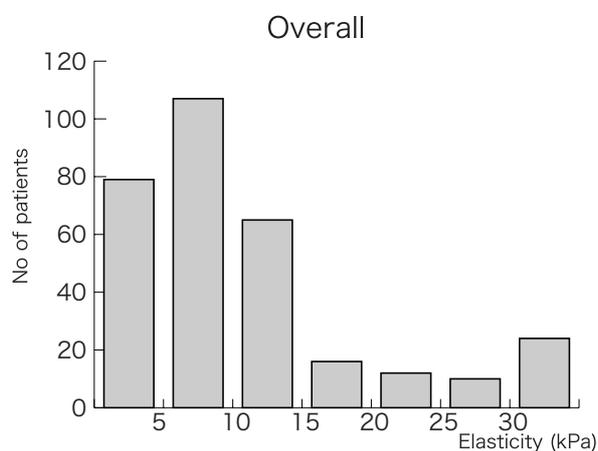※BMI(Body Mass Index)=weight(kg)/height(m)/height(m)

Fig. 3

(2) *Investigation of the normal liver group*

The mean value for elasticity was 4.6±1.9 (2.6-12.4)kPa. Elasticity did not significantly correlate with age, BMI, and the various types of blood tests. And there was no significant difference for elasticity between male and female.

(3) *Investigation of the HC group*

The mean value for elasticity was 14.4±12.0 (2.8-59.3)kPa. **Fig. 4** shows the distribution of elasticity. **Table 2** shows the correlation between elasticity and the various test items, and fibrosis score. A significant correlation was seen with PLT, Alb, GOT, GPT, ALP, γ-GTP, Ch-E, T-chol, PT, hyaluronic acid, collagen type IV, PIIIP, ZTT, TTT,

NH3, FORNS, WAI, and HALT-C.

In this study, we divided the HC patients into two groups according to elasticity that was set by Zoil et al., liver cirrhosis groups is ≧14.6kPa and non liver cirrhosis group is <14.6 kPa. And we investigated the correlation between elasticity and age, BMI, the various test items, and fibrosis score. In non liver cirrhosis group (N=110), a significant correlation was found with GOT, GPT, γ-GTP, Ch-E, hyaluronic acid, collagen type IV, FORNS, WAI and .In liver cirrhosis group (N=46), a significant correlation was seen with PT, collagen type IV, TTT, NH3, and HALT-C. (**Table 3**)

Table 2.   Correlations in the hepatitis C and hepatitis B groups.

	Hepatitis C		Hepatitis B	
	δ value	p value	δ value	p value
Age	0.145	0.072	0.245	0.148
BMI	0.09	0.266	0.077	0.65
WBC	−0.337	<0.001	−0.357	0.035
PLT	**−0.512**	<0.001	**−0.505**	0.003
Alb	**−0.429**	<0.001	**−0.561**	0.001
T−bil	0.343	<0.001	**0.505**	0.003
GOT	**0.646**	<0.001	**0.56**	0.001
GPT	**0.444**	<0.001	0.34	0.044
LDH	0.219	0.006	0.25	0.139
ALP	**0.409**	<0.001	**0.415**	0.014
γ−GTP	**0.44**	<0.001	**0.446**	0.008
Ch−E	**−0.63**	<0.001	**−0.656**	0.001
Fe	0.09	0.269	0.26	0.13
T−chol	**−0.416**	<0.001	**−0.468**	0.006
TG	−0.009	0.915	−0.237	0.813
Glu	0.202	0.012	−0.223	0.187
PT	**−0.585**	<0.001	**−0.62**	0.002
Hyalurinic acid	**0.619**	<0.001	**0.656**	0.001
Collagen type IV	**0.673**	<0.001	**0.645**	0.002
PIIIP	**0.527**	<0.001	**0.668**	<0.001
ZTT	**0.473**	<0.001	**0.49**	0.006
TTT	**0.4**	<0.001	0.368	0.041
NH3	**0.45**	<0.001	0.428	0.078
Forns	**0.608**	<0.001	**0.54**	0.01
Wai	**0.688**	<0.001	**0.774**	0.002
HALT−C	**0.513**	<0.001	**0.54**	0.01

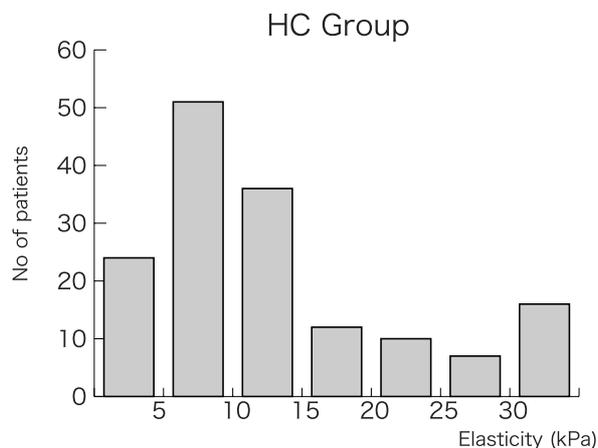※under line; correlation coefficient |δ|of ≧0.40   (p<0.05)

HC Group

Fig. 4

Table 3. Various types of correlations in the non-liver cirrhosis and liver cirrhosis subgroups of the hepatitis C group.

	Non-liver cirrhosis group (N=110)		Liver cirrhosis group (N=46)	
	δ value	p value	δ value	p value
Age	0.189	0.049	−0.017	0.91
BMI	−0.046	0.634	0.337	0.025
WBC	−0.187	0.051	−0.337	0.024
PLT	−0.349	<0.001	−0.311	0.037
Alb	−0.068	0.48	−0.251	0.092
T−bil	0.343	<0.001	0.116	0.438
GOT	**0.61**	<0.001	0.127	0.395
GPT	**0.481**	<0.001	−0.08	0.593
LDH	0.072	0.453	0.28	0.061
ALP	0.308	0.001	−0.051	0.731
γ−GTP	**0.402**	<0.001	0.092	0.537
Ch−E	**−0.432**	<0.001	−0.316	0.034
Fe	0.247	0.011	−0.205	0.174
T−chol	−0.271	0.005	−0.138	0.354
TG	−0.018	0.851	0.167	0.264
Glu	0.182	0.057	0.16	0.284
PT	−0.363	0.001	**−0.479**	0.003
Hyalurinic acid	**0.444**	<0.001	0.226	0.129
Collagen type IV	**0.436**	<0.001	**0.412**	0.006
PⅢP	0.334	0.001	0.347	0.02
ZTT	0.334	0.014	0.332	0.038
TTT	0.255	0.013	**0.419**	0.01
NH3	0.118	0.417	**0.497**	0.023
Forns	**0.469**	<0.001	0.276	0.064
Wai	**0.573**	<0.001	0.318	0.033
HALT−C	0.197	0.078	**0.506**	0.002

※under line; correlation coefficient｜δ｜of ≧0.40 (p<0.05)

(4) Investigation of the HB group

The mean value for elasticity was 10.5±9.6 (2.2-54.2)kPa. **Fig 5** shows the distribution of elasticity. **Table 2** shows the correlation between elasticity and the various test items, and fibrosis score. A significant correlation was seen with PLT, Alb, T-Bil, GOT, ALP, γ-GTP, Ch-E, T-chol, PT, hyaluronic acid, collagen type IV, PIIIP, ZTT, NH3, FORNS, WAI, and HALT-C.
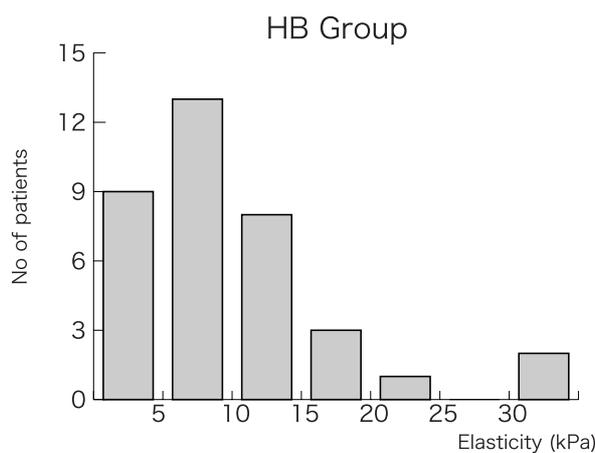

Fig. 5

Table 4.　Various types of correlations in the NAFLD group.

	ρ value	p value
Age	0.072	0.729
BMI	0.386	0.064
WBC	−0.236	0.257
PLT	−0.394	0.059
Alb	−0.242	0.246
T−bil	0.29	0.165
GOT	**0.675**	0.001
GPT	**0.621**	0.003
LDH	0.181	0.385
ALP	−0.101	0.628
γ−GTP	**0.542**	0.009
Ch−E	−0.138	0.517
Fe	−0.05	0.81
T−chol	−0.363	0.082
TG	−0.238	0.254
Glu	−0.287	0.169
PT	**−0.677**	0.015
Hyalurinic acid	**0.425**	0.042
Collagen type IV	**0.433**	0.038
PⅢP	**0.442**	0.034
ZTT	0.28	0.222
TTT	0.217	0.344
NH3	0.482	0.128

※under line; correlation coefficient |δ|of ≧0.40　(p＜0.05)

(5) Investigation of the NAFLD group

The mean value for elasticity was 9.7 ± 14.7 (3.3-75)kPa. **Table 4** shows the correlation between elasticity and the various test items, and fibrosis score. A significant correlation was seen with GOT, GPT , γ-GTP, PT, hyaluronic acid, collagen type IV, and PIIIP.

(6) Investigation of the AIH group

In the investigation of 9 patients, the mean value for elasticity was 10.4 ± 9.6 (3.7-34.3)kPa. Elasticity correlated significantly with BMI (δ =0.736)and ZTT (δ =0.767).

(7) Investigation of the PBC group

In the investigation of 10 patients, the mean value for elasticity was 6.1 ± 3.0 (3.3-11.7)kPa. There was no significant correlation between elasticity and any of the items.

(8) Investigation of the ALCLD group

In the investigation of 7 patients, the mean value for elasticity was 29.7 ± 22.9 (4.8-72)kPa. Elasticity correlated significantly with age (δ =0.964), T-Bil (δ =0.830), Glu (δ =0.893), and hyaluronic acid (δ =0.929).

(9) Investigation of the AH group

Elasticity of the 3 patients with acute hepatitis (AIH, HB, unknown cause)in the acute phase was 22.0KPa, 18.5kPa, and 39.3KPa, respectively, which were high values compared to the values in the normal liver group.

(10) Investigation of the other liver disease group

In the investigation of 11 patients, the mean value for elasticity was 8.1 ± 5.7 (3.4-26.3)kPa. There was no significant correlation between elasticity and any of the items.

(11) Investigation of the LDLT group

Table 5 shows the list of post-transplant patients. The mean value for elasticity was 6.7 ± 3.1 (3.4-26.3)kPa. There was no significant correlation between elasticity and any of the items.

(12) Comparison between the normal liver group and the NAFLD group

Among the patients in the NAFLD group, one patient diagnosed as liver cirrhosis by NASH had a high value of 75kPa for elasticity. This patient was excluded in the comparison with the normal liver group. The value for elasticity in the normal liver group was 4.6 ± 1.9KPa, and in the NAFLD group 6.8 ± 4.7KPa. In the Mann- Whitney's U test that was used for testing between 2 groups, a significantly higher value was shown in the NAFLD group (p=0.0083). In addition, when the other test were comparatively investigated, the NAFLD group showed significantly higher values for BMI (p<0.001), GOT (p<0.001), GPT (p<0.001), γ-GTP (p<0.001), and Ch-E (p=0.03).

(13) Comparison between the normal liver group and the LDLT group

In the LDLT group, we excluded the one patient (case 3) from this statistical analysis because who had postsurgical stricture of the bile duct and showed a high value for elasticity. Elasticity was significantly higher value (P=0.027) in the LDLT group than normal liver group. In addition, when the other test items were compared, the LDLT group showed significantly higher value for BMI (p=0.017), GPT (p=0.001), γ-GTP (p=0.003), TG (p=0.015), and PIIIP (p=0.001), and significantly lower value for PLT (p=0.012).

Table 5.　Liver transplant patients' background.

Case	Age(y) sex	Disease	Elasticity(kPa)	T-bil(mg/dl)	GOT(U/l)	GPT(U/l)	ALP(U/l)	γ GTP(U/l)	Hyaluronic (ng/ml) acid	Collagen (ng/ml) type IV	P-III-P(U/ml)
1	59♂	LC(HBV),HCC	12	0.6	20	17	203	43	102	118	0.64
2	55♀	LF(PBC)	6.5	1.1	19	20	153	26	13	125	0.59
3	59♀	FH(HAV)	13.4	1.4	52	64	804	570	84	277	1.5
4	55♂	LF(HCV)	5.9	1.1	30	33	957	402	50	74	0.66
5	63♂	LC(HCV), HCC	4.3	0.5	14	14	269	14	62	98	0.81
6	69♂	LF(HBV)	5.1	0.4	57	44	639	303	51	160	1.3
7	72♂	LC(HCV), HCC	4.3	1.1	21	27	197	21	9	111	0.59
8	66♀	LF(AIH)	5.3	0.1	20	16	284	14	162	181	0.98
9	43♂	LF(HBV)	4.8	1.1	17	19	320	52	34	73	0.72
10	61♂	LC(HBV),HCC	6.4	0.6	33	44	531	80	69	183	0.82
11	58♀	FH(unkno origin)	5.3	0.4	21	26	379	28	32	155	0.72

Abbreviation: LC; liver cirrhosis. HCC; hepatocellular carcinoma. LF; liver failure. FH; fulminant hepatitis. HAV(HBV,HCV); hepatitisA(B,C).

(14) Comparison between the HC group and the HB group

When HC and HB were compared as viral hepatitis, the items which were correlated with elasticity were the same with no notable difference. Elasticity in the HC group was 14.4± 12.0 (2.8-59.3)KPa, and in the HB group 10.5±9.6 (2.2-54.2)KPa. Elasticity was significantly higher value(P=0.024) in the HC group.

(15) Investigation of the liver biopsy, and liver resection cases

The fibrotic stage of the 21 patients from whom pathological tissues were obtained is as follows: 8 patients were F1, 4 were F2, 1 was F3, and 8 were F4. **Fig. 6** shows elasticity by fibrotic stage. The mean value for elasticity in each stage was 6.5±2.5 (2.8-9.8)kPa, 9.5±1.2 (8-10.4)kPa, 27kPa, 26.4±10.9 (13.9-45.0)kPa, respectively. As there was only one F3 patient, the investigation of elasticity between 2 groups could not be performed. However, when comparisons were made between F1 and F2, and between F1 to F3 and F4, a significantly higher value was shown by F2 in the former and by F4 in the latter comparison (p=0.041) and (p=0.001).
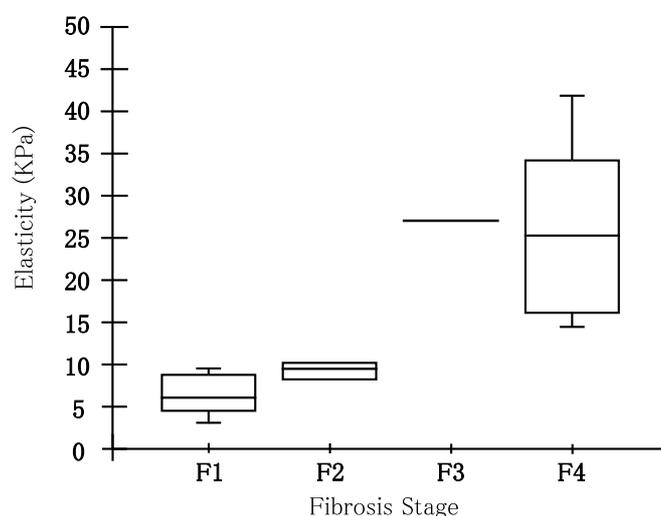

Fig. 6 Liver stiffness values for each fibrosis stage. The top and bottom of the boxes are the 1st and 3rd quartiles. The length of the box thus represents the interquartile Range (IQR) within which are located 50% of the values. The lines through the middle of the boxes represent the median. The error bars are the minimum and maximum values (measurement range).

DISCUSSION

Past studies have shown liver fibrosis in liver biopsy to be correlated with elasticity, fibrosis markers, and platelets. Murawaki et al[20]. comparatively investigated the diagnostic accuracy for liver fibrosis using PIIIP, type IV collagen, hyaluronic acid and PLT in chronic hepatitis C. They reported that it was somewhat possible to differentiate F1 between F2 or higher using type IV collagen and hyaluronic acid. Specifically, when type IV collagen is ≧110 ng/mL or hyaluronic acid is ≧50 ng/mL, the possibility of the stage having progressed to F2 or higher is high. The respective rates for positive predictive value were 72 and 77%. With PIIIP and PLT, the diagnostic accuracy in differentiating F1 and F2 or higher was slightly poor. Hyaluronic acid has been reported to be the most useful marker for diagnosing liver cirrhosis. In addition, Ziol[16] and Castera[23] et al. comparatively investigated liver fibrosis and elasticity in chronic hepatitis C and reported that they are closely related. Ziol investigated 251 chronic hepatitis C patients and made the following report.; F0-1:5.5 (4.1-7.1)kPa ;F2:6.6 (4.8-9.6)kPa ;F3:10.3 (7.6-12.9)kPa ;F4:30.8 (16.3-48)kPa (fibrosis classification based on the METAVIR scoring system[19]). However, in

all of these studies, liver fibrosis was determined by liver biopsy findings. Presently, liver biopsy in interferon treatment is no longer essential and so the number of cases of liver biopsy has decreased. In addition, unnecessary invasive tests are being shunned. We actually obtained liver biopsy tissue specimens from 21 patients within the present investigation period. A comparison of the results on elasticity with the data by Zoil et al. showed no contradiction. There was only one F3 patient and this patient showed a high value, however a comparison was difficult as the number of patients was too few. With the decrease in the number of liver biopsy cases, if it is possible to understand the disease state by measurements using the noninvasive Fibro scan or fibrosis markers, it will be very useful. So, we have comparatively investigated various types of test items and fibrosis markers with measurement of elasticity as the core in order to examine the usefulness.

In the overall investigation of the HC group, elasticity significantly correlated with hepato-billiary enzymes, fibrosis markers, and indirect fibrosis diagnostic score. Zoil et al. reported that to diagnose liver cirrhosis, when elasticity was set at a cut-off of $\geqq 14.6$kPa, the positive predictive value was 78%. So, using this data as reference, we performed a statistical analysis where the <14.6kPa elasticity group was taken as the non-liver cirrhosis group, and the $\geqq 14.6$kPa elasticity group as the liver cirrhosis group. In the non-liver cirrhosis group, elasticity correlated with hepatic enzymes and fibrosis markers, whereas in the liver cirrhosis group it correlated with PT and NH3. From these, it was confirmed that in chronic hepatitis C, inflammation of the liver affects the progression of fibrosis and the fibrosis markers are useful in assessing progression of fibrosis. If fibrosis stage progresses grade 3 (F3), we should decide immediately whether we perform antiviral therapy or not. As usual, fibrosis was assessed by liver biopsy or fibrosis markers. From now on, Fibro scan which is non invasive procedure, is useful to diagnose fibrosis stage. The increase in elasticity rather becomes related with synthetic capability of coagulating factors and ammonia metabolism, which are related with decreased liver function. In chronic hepatitis, liver function doesn't decrease. Although, in liver cirrhosis, it is important to assess liver function. We choose treatment strategy for hepatocellular carcinoma in liver cirrhosis according to liver function. Fibro scan will be useful tool for decide on treatment strategy. Due to the properties of Fibro scan,

measurements are difficult for patients with ascites, thus making it impossible to investigate patients with uncompensated liver cirrhosis. Investigations on patients with increased Bil and decreased Alb are few and so the correlation between these items and elasticity has not been elucidated. In uncompensated liver cirrhosis, the treatment is primarily symptomatic. Few treatments are based on the measurement of elasticity. In addition, even in uncompensated liver cirrhosis, there are patients with ascitic fluid retention. Therefore, the control of ascites obviously takes priority. Thus, not being able to measure elasticity in patients with ascitic retention is not very important. If we can measure elasticity of patients with ascites, elasticity may be very high.

In the HB group, elasticity was found to be correlated with the same items as in the HC group with no notable difference.

When elasticity was compared between HC and HB groups, the former showed significantly higher (P=0.024). However, the HC group showed higher value for fibrosis markers and hepatic enzymes and lower value for PLT compared to the HB group. Due to these differences in patients' background, elasticity of HC group may be higher than HB group.

In the NAFLD group, it showed a higher value for elasticity than the normal liver group. However, there is the possibility that NASH patients were also included among those in the NAFLD group. As such, one cannot judge whether there is an increase in elasticity simply by fat deposition only. Henceforth, histopathological examination and the change of elasticity before and after drug or diet therapy will be necessary. In the NAFLD group, BMI was high compared to that of the normal liver group and measurement by Fibro scan was difficult in many patients with high amount of subcutaneous fat and those with narrow intercostals. Therefore, the device needs to be improved upon hereafter.

In addition, for other liver disease groups, the same tests are being performed when the patients are few. In other liver disease group, there was a high correlation coefficient between similar items as in the case of viral hepatitis. In our study, there was no finding that was peculiar to any patient group. Fibro scan is useful in assessing the disease state, but it is difficult to analyze the pathological condition based on elasticity only. Although we cannot diagnosis the liver disease using Fibro scan, but elasticity is effective in understanding

the disease state.

There were only 3 patients with acute hepatitis, however all of them showed a high value for elasticity. Due to the decreased hemostatic funcion, liver biopsy could not be performed, however, fibrosis does not progress rapidly. It was thus demonstrated that liver inflammation and swelling of the liver likely affect elasticity.

In this investigation, there were 11 liver transplant recipients, 2 of which showed a high value for elasticity. In case 3, postsurgical stricture of the bile duct occurred and even in the blood tests there was increased biliary enzymes. Cholestasis is considered to affect elasticity. Case 1 progressed without any particular postsurgical complication and the blood data was stable and so it was difficult to explain.

However, in the comparison between the normal liver and the LDLT groups, the LDLT group showed significantly higher value for elasticity than the normal liver group, and even in the blood test findings, some of the hepatic enzymes and PIIIP, a fibrosis marker, were significantly higher. This data may be affected by the liver damage during and post operation.

CONCLUSION

The measurement of elasticity by Fibro scan was confirmed to be a very useful tool for the non-invasive assessment of fibrosis. In hepatitis C, liver inflammation and fibrosis markers are correlated with progression of fibrosis in chronic cases. And, if there is progression to the state of liver cirrhosis, the progression of fibrosis reflects a decrease in liver function.

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