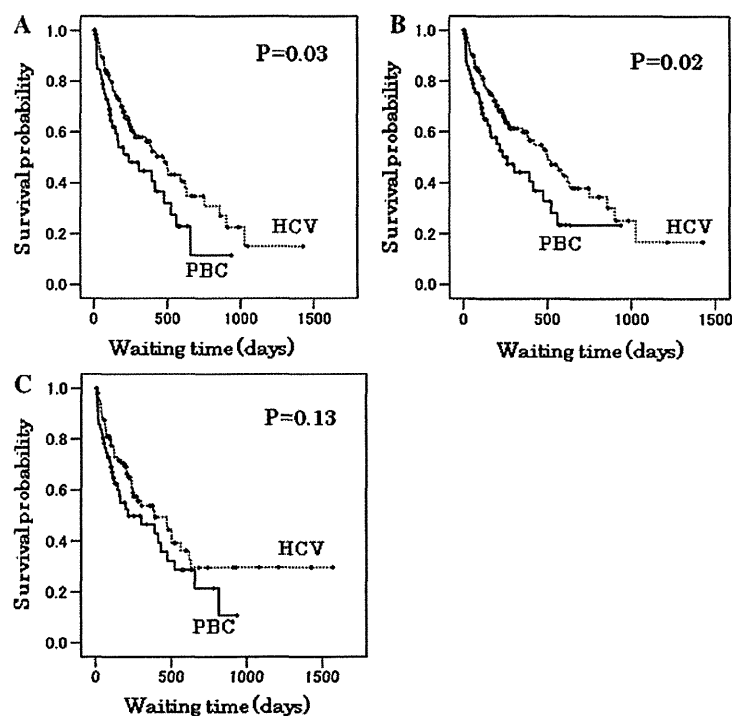


Fig. 4 Kaplan–Meier curves comparing the cumulative waiting list survival probability of patients with chronic hepatitis C (HCV) and primary biliary cirrhosis (PBC). Patients stratified medical point = 6 (a), and Child–Turcotte–Pugh score ≥ 10 (b), and Model of End-Stage Liver Disease (MELD) score ≥ 15 (c)



compared with HCV patients. In bivariate analysis, the medical point-adjusted HR of waiting list mortality of PBC patients was significantly higher than that of HCV patients (HR 1.58; 95 % CI 1.07–2.35, $P = 0.02$). The CTP score-adjusted HR also showed a significantly increased risk of waiting list mortality in PBC patients (HR 2.15; 95 % CI 1.42–3.25, $P < 0.001$). However, the MELD score-adjusted HR did not show a statistically significant risk of waiting list mortality in PBC patients (HR 1.29; 95 % CI 0.87–1.91, $P = 0.21$).

Waiting list survival of patients with HCV and PBC was compared with stratification by each of the disease severity indices (Fig. 4). Patients with medical point 6, for which most PBC and HCV patients were registered, showed a significantly shorter waiting list survival for PBC patients than of HCV patients (median 261 vs. 503 days, $P = 0.02$). In patients with CTP score ≥ 10 , the score classified as C, the shorter waiting list survival of PBC patients was also significant (median 235 vs. 475 days, $P = 0.03$). On the other hand, when they were selected by MELD ≥ 15 , the score indicating patients who can be expected to achieve improved survival with liver transplantation [12], there was no significant difference in the waiting list survival rate between them ($P = 0.13$).

Discussion

The result of this study clearly indicated that the most common reason for removal from the waiting list in Japan was “waiting list death”, which was a combination of

death and becoming too sick for transplantation. The waiting list death included 58.1 % of all the patients removed from the list. In the United States, a recent report indicated that waiting list death was the reason for removal from the list in 25.9 % of adult patients [1]. Although this report included patients with acute liver failure and retransplantation, high waiting list mortality in Japan was evident. Thus, the high mortality rate on the liver transplant waiting list is a major challenge in Japan. Moreover, severe donor organ shortage in Japan should contribute to the high waiting list mortality [13]; an improved organ allocation policy will be necessary to cause a decrease in waiting list death.

In this study, we found that PBC patients had a significantly higher risk of waiting list mortality compared with patients with other etiologies in the JOT registry. Since PBC is currently the third most common diagnosis in the JOT registry for liver transplantation, poor waiting list survival of PBC patients would contribute to the high waiting list mortality in Japan. PBC is a cholestatic liver disease that causes bile duct deterioration and progresses slowly to a terminal phase characterized by hyperbilirubinemia, signs of decompensated cirrhosis, ascites, and variceal bleeding. Only one type of medical therapy, involving the use of ursodeoxycholic acid (UDCA), is now widely recognized to improve the prognosis of PBC patients. Many studies have shown that UDCA therapy not only improves biochemical indices, but also delays histologic progression and improves survival without transplantation [14–16]. However, evidence has also accumulated that the

favorable effect of UDCA therapy is limited to patients with early-stage disease. In histologically advanced patients or biochemical non-responders, the transplant-free survival rate of UDCA-treated patients was not different from spontaneous survival [16, 17]. This means that PBC patients have no effective medical therapeutic option to prolong their survival when they have progressed to end-stage liver disease, and liver transplantation remains the only hope of a cure [18, 19]. PBC patients in our cohort also showed a consistently poor survival of a median period of 392 days.

The reason why PBC patients have a higher risk for waiting list mortality compared with patients with other etiologies of chronic liver disease is not clearly understood. Interestingly, PBC patients were younger, and their INR and serum creatinine levels were lower than for HCV patients at registration. This indicated that neither age nor liver and renal function at registration alone caused poor waiting list survival of PBC patients; the registration of PBC patients was not later than that for HCV patients. The rate of disease progression and lethal complications might be involved in their short waiting list survival rate. Moreover, the actual waiting list survival rate in PBC patients was not greater than the updated Mayo score-predicted spontaneous survival rate. This observation indicated that the PBC patients on the waiting list were refractory to the medical therapy and their waiting list survival suddenly deteriorated. Further analyses, particularly on the cause of death, are required to clarify the pathophysiology of PBC patients who have progressed to end-stage liver disease.

In general, deceased donor livers are allocated for transplantation on the basis of "sickest first", i.e., those who are more likely to die without a liver transplantation are assigned the highest priority. Therefore, the disease severity index used in the liver allocation system should consider the urgency of PBC patients for liver transplantation. However, our results have clarified the inability of the currently used Japanese allocation system to identify the risk of PBC patients. The medical point-adjusted HR of PBC patients revealed that they were at 58 % increased risk of waiting list mortality compared with HCV patients. In addition, the CTP score-adjusted HR showed that PBC patients were at 115 % increased risk for waiting list mortality. Thus, it is not only the current allocation system but also the CTP score-based allocation that cannot capture the risk for waiting list mortality in PBC patients. On the other hand, we found that the MELD score-adjusted HR of PBC patients lost statistical significance, and stratification by MELD score revealed comparable survival curves between patients with PBC and HCV. These results indicated that PBC patients had a similar risk of waiting list mortality compared with patients with other etiologies when they were stratified by MELD score. At the time of

registration, the patients with HCV and PBC had different characteristics; however, only the MELD score accurately evaluated their disease severity, and therefore, MELD-based allocation would adequately assign priority to the patients according to their risk of waiting list mortality. Thus, our results demonstrated that the MELD score was superior to both the current Japanese allocation and CTP score-based allocation for ranking patients in the JOT registry by their risk of waiting list mortality.

In addition, patients should be re-evaluated according to their chronological change of hepatic failure to improve allocation. However, most patients with chronic liver disease were waiting at medical point 6 as an upper limit, because the highest priority at medical point 9 was generally awarded to the patients with acute liver failure or early graft failure in the current Japanese allocation system. Therefore, the current allocation system did not completely reflect the chronological change in the degree of liver failure. Thus, the MELD score, which was expressed numerically as a continuous variable with a wide dynamic range in the evaluation of hepatic decompensation, would have an advantage over the medical point system for assessing the chronological change in patients' risk of death.

In conclusion, this study demonstrated that patients with PBC, the third most common indication for liver transplantation in Japan, have a high risk for waiting list mortality in the current Japanese allocation system. The allocation system should be changed to accurately prioritize the patients with a higher mortality risk; MELD-based allocation would be suitable for this purpose and could reduce the waiting list mortality of PBC patients.

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Conflict of interest The authors declare that they have no conflict of interest.

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A study of the effects of saliva stimulation by nizatidine on dry mouth symptoms of primary biliary cirrhosis

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Abstract

AIM: To elucidate the effect of saliva stimulation by nizatidine on oral symptoms of primary biliary cirrhosis (PBC) by administering it to PBC cases.

METHODS: From among 73 cases that had been definitively diagnosed as PBC at our hospital by February 2010, we selected 27 cases of PBC, 4 males and 23 females, as subjects. We obtained subjects' consent after giving them a full explanation of the administration of nizatidine. Nizatidine 150 mg was administered internally twice daily, after morning and evening meals. To observe changes in the quantity of saliva secreted, chewing gum tests were carried out four times: before the initial dose, and after 6 mo, 12 mo and 24 mo of administration. For subjective dry mouth symptoms, a visual analog scale (VAS) method was used to assess their feelings of oral dryness and eating difficulty, five times: before the initial dose, and after 1, 6, 12 and 24 mo of administration in 8 cases. The nutritional condition and the hepatic functional reserve were compared between before and after the nizatidine treatment.

RESULTS: The result of a chewing gum test on the subjects before the administration of nizatidine showed that 50% produced less than 10 mL of saliva, *i.e.*, the standard under which cases are considered to have hyposalivation. The results of these tests showed that the quantity of saliva secreted was 10.5 ± 6.8 mL before administration of nizatidine, 10.9 ± 6.0 mL after 6 mo, 10.6 ± 4.9 mL after 12 mo, and 11.8 ± 6.8 mL after 24 mo administration. Thus, there was a slowly increasing trend in the quantity of saliva in the whole group. The percentage of subjects with saliva production above 10 mL was 45.8% after 6 mo administration of nizatidine, that is, only a slight change from before its administration, but it was 64.3% after 12 mo, that is, a significant increase. The saliva secretion by subject patients was examined before the beginning of administration of nizatidine, 12 mo later, and 24 mo later, and Fisher's combined probability test was used to examine the results for increases in saliva secretion. The analysis yielded *P* values of 0.51 and 0.53 for 12 mo later and 24 mo later, respectively. Thus, although there was no statistically significant increase, it was confirmed that saliva secretion tended to increase. A VAS method was employed to study the intensities of subjective symptoms of oral dryness and eating difficulty. Almost every case indicated some improvement of subjective oral dryness on the VAS early in the administration, *i.e.*, one month after. We also studied the effects of the administration of nizatidine on nutritional condition, hepatic functional reserve, and long-term prognosis of PBC. No significant improvements in cholinesterase (ChE) level, albumin (Alb) level, or Child-Pugh score were found during the period of observation from the beginning to the end of administration of nizatidine, nor in comparison with the non-administration group. A comparative analysis between before administration and 24 mo later yielded *P* values of 0.41 for Alb, 0.56 for ChE, and 0.59 for the Child-Pugh scores.

CONCLUSION: It was confirmed that administering

nizatidine to cases of PBC with dry mouth increased the secretion of saliva and improved the symptoms.

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Key words: Primary biliary cirrhosis; Nizatidine; Dry mouth; Sicca syndrome; Visual analog scale

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INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic hepatic disease, in the onset of which an autoimmune mechanism is involved. This disease is characterized serologically by anti-mitochondrial antibody positivity and high serum immunoglobulin M values, histologically by chronic non-suppurative destructive cholangitis of the hepatic lobules, and the presence of florid duct lesions that involve severe inflammation and biliary epithelial inflammation^[1,2]. The condition develops gradually from the asymptomatic phase when no subjective symptoms are observed into the symptomatic phase when itching, jaundice, and other symptoms appear. A group of cases with poor prognosis may finally develop from cirrhosis to hepatic failure to death. There are no radical cures for this disease other than liver transplantation. Thus, it is one of a group of intractable liver diseases for which there are no established treatments^[3].

PBC shows a high rate of complication by Hashimoto's disease or collagen diseases, such as Sjogren syndrome^[4,5]. Sjogren syndrome includes dry mouth and dry eyes; dry mouth and/or dry eye symptoms of PBC are called sicca syndrome, and are found in approximately 70%, of patients with PBC^[6-8]. However, only 20% to 30% of PBC cases with these symptoms meet the diagnostic criteria for Sjogren syndrome^[9] and many such patients are not well treated for these symptoms.

It has been reported that nizatidine, an H₂ blocker, has a saliva stimulation effect, in addition to its effects of accelerating gastric juice release and increasing the pressure of the lower esophageal sphincter which suppress the onset of gastroesophageal reflux disease^[10].

We report here a study of whether administering nizatidine, which has a saliva stimulation effect, improves dry mouth as a subjective symptom of PBC.

MATERIALS AND METHODS

From among 73 cases that had been definitively diagnosed as PBC at our hospital by February 2010, we selected 27 cases of PBC as subjects, including 4 males and 23 females, giving them a full explanation of the administration

of nizatidine and thereafter obtaining their consent. Nizatidine 150 mg was administered internally twice daily, after morning and evening meals. In order to observe changes in the quantity of saliva secreted, chewing gum tests were carried out four times: before the initial dose, and after 6, 12 and 24 mo of administration. For subjective dry mouth symptoms, a visual analog scale (VAS) method was administered five times to assess patients' feelings of oral dryness and eating difficulty: before the initial dose, and after 1, 6, 12 and 24 mo of administration in 8 cases. The VAS scale ranged from 0 to 10 (0: no subjective symptoms; 1-3: mild; 4-6: moderate; 7-9: severe; 10: very severe).

In addition, nutritional condition and hepatic functional reserve of patients were checked in terms of albumin (Alb) levels, cholinesterase (ChE) levels, as well as Child-Pugh scores before and after the nizatidine treatment. Data were compared between the nizatidine administration group and the nizatidine non-administration group.

Statistical analysis

The obtained data were statistically analyzed using SPSS v.17 to perform Willcoxon signed-rank tests or paired *t* tests with a level of significance of *P* < 0.05.

RESULTS

The average age of the subjects was 66.7, and the female subjects accounted for 85% of the subject group. The aspartate aminotransaminase level was 63.7 IU/L and the alanine aminotransaminase (ALT) level was 69.2 IU/L, indicating that liver function was mildly impaired. In comparison, the alkaline phosphatase level was 679.1 IU/L and the γ -glutamyl transpeptidase level was 242 IU/L, thus indicating high biliary enzyme values. In the phase before administering nizatidine, any significance differences, in addition to ALT value, were identified between the administered and non-administered group (ALT: *P* = 0.04). The 12 cases on which liver biopsies were performed were histologically classified according to Scheuer's classification as 11 cases in stage 1 and 1 case in stage 2, with no case in which there was a high level of fibrosis. The M-2 antibody-positive rate was 67%. In 14 cases, 54% of the subjects, collagen disease complications were found, such as Sjogren syndrome, chronic thyroiditis, and/or rheumatoid arthritis (Table 1).

The changes in the quantity of saliva secreted that were observed in the chewing gum tests were 10.5 \pm 6.8 mL (2.2-30 mL) before the start of administration of nizatidine, 10.9 \pm 6.0 mL after 6 mo, 10.6 \pm 4.9 mL after 12 mo, and 11.8 \pm 6.8 mL after 24 mo of administration. Thus, they showed a slowly increasing trend (Figure 1A). In order to further analyze the changes in the quantity of saliva secreted, we divided the subject group into one sub-group with less than 10 mL before the start of administration of nizatidine and another with 10 mL or more before administration of the drug. The sub-group with initial secretion of large quantities of saliva, of 10 mL or more, did not show a significant increase after 6 mo of administration. On the other hand, the sub-group

Table 1 Characteristics of the patients at baseline

	All PBC cases	Cases with nizatidine	Cases without nizatidine	P value
No. of cases	73	27	46	
Age (mean \pm SD)	65.6 \pm 12.2	68.2 \pm 11.8	64.1 \pm 12.3	0.32
Sex (male/female)	9 (18%)/64 (88%)	4(%) /23(%)	5 (18%)/41 (88%)	0.72
Histological classifications	24/8/3/2	11/1/0/0	13/7/3/2	0.12
Scheuer (1/2/3/4)	65%/22%/8%/5%	92%/8%/0%/0%	52%/26%/12%/8%	
Alb (g/dL)	4.0 \pm 0.3	4.1 \pm 0.3	4.0 \pm 0.4	0.50
ChE (IU/L)	288.3 \pm 66.4	302.2 \pm 68.5	277.9 \pm 64.0	0.24
AST (IU/L)	55.6 \pm 47.3	64.6 \pm 44.5	50.3 \pm 48.5	0.10
ALT (IU/L)	58.2 \pm 64.6	75.7 \pm 67.0	48.0 \pm 61.5	0.04
γ GTP (IU/L)	183.8 \pm 205.6	221.0 \pm 254.7	162.0 \pm 169.8	0.10
ALP (IU/L)	638.7 \pm 446.8	661.0 \pm 616.3	625.7 \pm 315.5	0.12
T-Bil (mg/dL)	0.69 \pm 0.37	0.71 \pm 0.33	0.67 \pm 0.39	0.33
Plt ($\times 10^4/\mu$ L)	21.7 \pm 8.8	23.2 \pm 10.9	20.7 \pm 7.3	0.74
M2 antibody (< 5/5)	17 (23%)/56 (77%)	9 (33%)/18 (67%)	8 (17%)/38 (83%)	0.15
ANA (< 40/40)	23 (32%)/50 (68%)	7 (26%)/20 (74%)	16 (35%)/30 (65%)	0.60
Collagen disease complication (presence/absence)	45 (62%)/28 (38%)	13 (48%)/14 (52%)	32 (70%)/14 (30%)	0.08

PBC: Primary biliary cirrhosis; Alb: Albumin; ChE: Cholinesterase; AST: Aspartate aminotransaminase; ALT: Alanine aminotransaminase; γ GTP: γ -glutamyltranspeptidase; ALP: Alkaline phosphatase; T-Bil: Total bilirubin; Plt: Platelet; ANA: Anti-nuclear antibody.

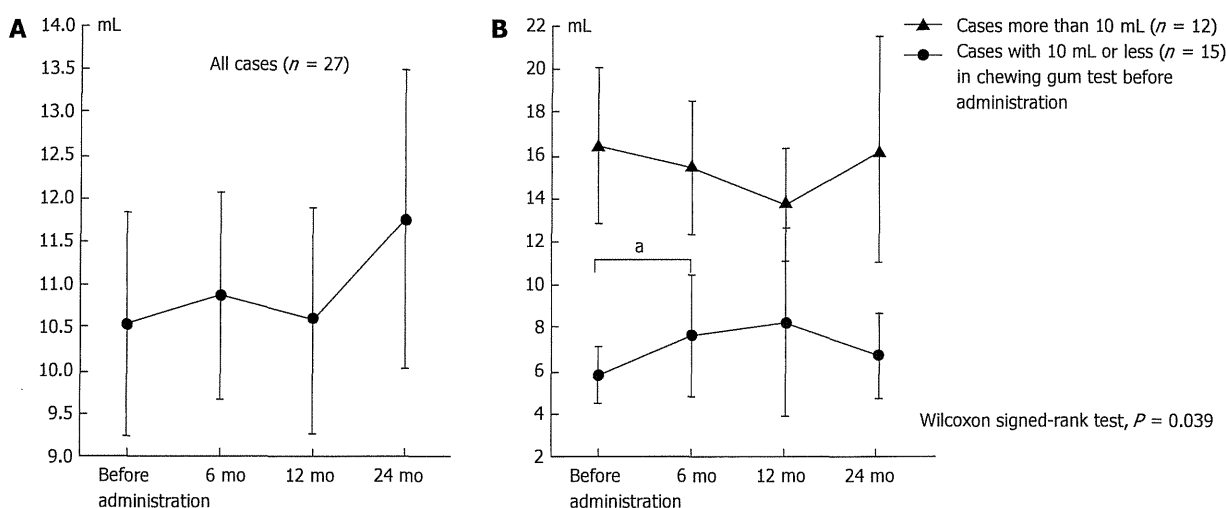


Figure 1 Course of saliva secretion before and after the administration of nizatidine in all cases (A) and in cases with 10 mL or less of saliva in chewing gum test before administration (B). * $P < 0.05$.

with initial secretion of small quantities of saliva, less than 10 mL, showed a statistically significant increase after six months of administration, with $P = 0.039$ in the Wilcoxon signed-rank test (Figure 1B). The percentage of all subject patients with saliva secretion of 10 mL or more was 48.1%, before the start of administration, and 45.8% after 6 mo of administration, thus indicating no large changes. However, this increased significantly to 64.2% after 12 mo of administration. The saliva secretion by patients was examined before the beginning of administration of nizatidine, 12 mo later, and 24 mo later, and Fisher's combined probability test was used to examine the results for increases in saliva secretion. The analysis yielded P values of 0.51 and 0.53 for 12 mo later and 24 mo later, respectively. Thus, although there was no statistically significant increase, it was confirmed that saliva secretion tended to increase (Figure 2).

A VAS method was employed to check the patients in terms of their subjective feelings of oral dryness and eating difficulty. In almost every case, feelings of oral dryness improved according of the VAS evaluation after 1 mo of administration of nizatidine (Figure 3A). In general, this showed a continuing modest increase after 12 and 24 mo of administration although, in some cases, it was seen to fall back to the level before the start of administration. Feelings of eating difficulty were also improved after one month of administration in some cases, according to the VAS evaluation. However, this parameter improved less and in a smaller number of cases than feelings of oral dryness. In addition, while the symptoms continued to improve in the long-term in some cases, no improvements were observed at all in other cases. Indeed, in some cases, even long-term administration of nizatidine not only failed to produce a significant positive

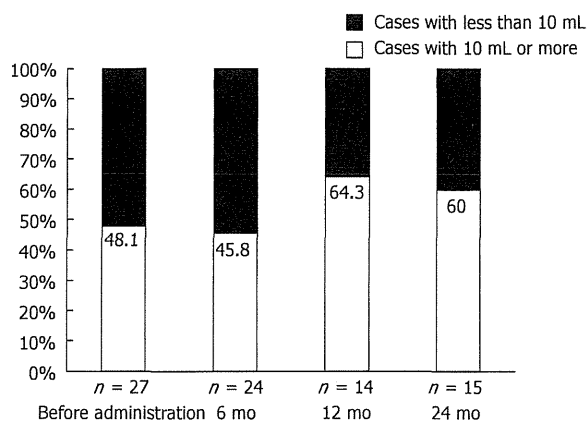


Figure 2 The percentage of all patients with saliva secretion of 10 mL or more before and after the administration of nizatidine.

effect, but the symptoms actually worsened, back to the levels before the start of administration (Figure 3B). The VAS evaluation results showed that, over time, while both feelings of oral dryness and of eating difficulty improved with the administration of nizatidine by a statistically significant difference, feelings of oral dryness improved by a more significant amount.

The ChE and Alb values were compared between before and after the administration of nizatidine. ChE before administration was 302.2 IU/L, 297.5 IU/L after 1 mo of administration, 300.5 IU/L after 6 mo of administration, 304.0 IU/L after 12 mo of administration, and 314.0 IU/L after 24 mo of administration. No significant improvements in either ChE or Alb were found over the period of observation. A comparative analysis between values before administration and 24 mo later yielded *P* values of 0.41 for Alb, 0.56 for ChE, and 0.59 for the Child-Pugh scores. Thus, no significant improvements were found. These values were also compared between the nizatidine administration group and the nizatidine non-administration group of patients with PBC, yielding *P* values of 0.67 for Alb and 0.73 for ChE. Thus, no statistically significant differences were found (Table 2).

Furthermore, in order to study the effect of nizatidine on hepatic functional reserve, changes in Child-Pugh scores were checked and analyzed. Before the start of administration of nizatidine, one case in the subject group showed a Child-Pugh score of 6 points in class A, one case 7 points in class B, and the remaining 25 cases 5 points in class A. After an average of 58.5 mo (15-100 mo) of observation, the equivalent results were: one case with 7 points in class B; one case that developed into symptomatic PBC progressing to liver failure to death during the observation period; and the remaining 25 cases with 5 points in class A. In the 46 cases of PBC in the nizatidine non-administration group, 42 were checked in terms of Child-Pugh scores when their illness was diagnosed as PBC: 41 of them showed 5 points in class A, and one showed 6 points in class A. The Child-Pugh scores after an average of 46.3 mo (10-98 mo) of observation of this group were: one case with 6 points in class A; one

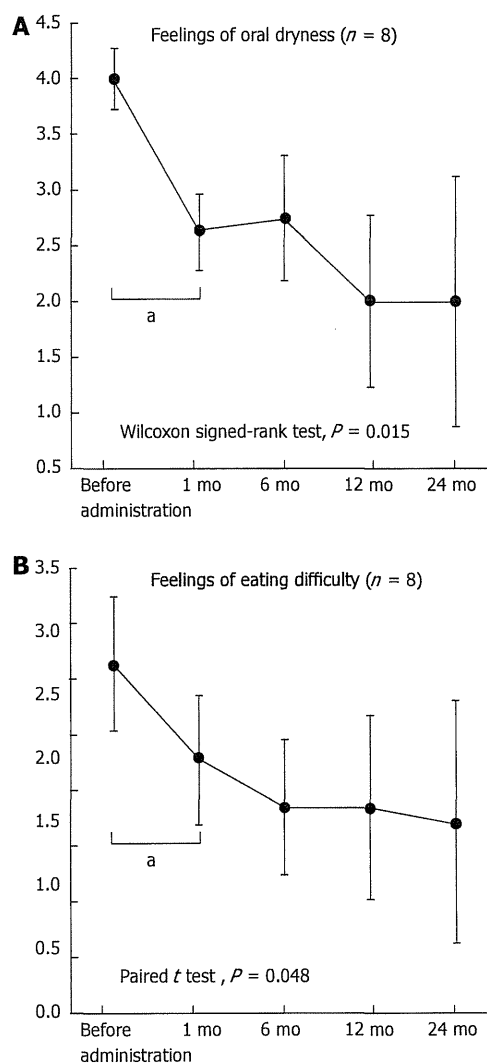


Figure 3 Visual analog scale method employed to check the subjects in terms of their feelings of oral dryness (A), and eating difficulty (B). ^a*P* < 0.05.

case of death; and the remaining 40 cases with 5 points in class A. A comparative analysis between the nizatidine administration group and the nizatidine non-administration group indicated no significant differences (Table 2).

DISCUSSION

Dry mouth and dry eye symptoms are frequently found in cases of PBC. It is assumed that these common symptoms are associated with a high rate of autoimmune disease complications with PBC. The likelihood that PBC has at least one autoimmune disease complication is 84%, and is 41% for the presence of at least two complications^[11-13]. Sjogren syndrome, rheumatoid arthritis, and scleroderma are the most frequent complications of PBC, with cross-sectional study on more than 5000 Japanese cases of PBC indicating that they were found in 13.5%, 7.3% and 2.0% of PBC cases, respectively^[14-16]. Another report showed and an even higher frequency of Sjogren syndrome as a complication of PBC, at 26% to 72%^[15,17-19]. PBC is char-

Table 2 The effects of the administration of nizatidine on the nutritional condition, the hepatic functional reserve, and the long-term prognosis of primary biliary cirrhosis

	Nizatidine administration group (<i>n</i> = 27)			Nizatidine non-administration group (<i>n</i> = 46)		
	Before administration	24 mo	<i>P</i> value	Before treatment	24 mo	<i>P</i> value
Alb	4.1 ± 0.3	4.2 ± 0.3	0.41	4.0 ± 0.4	4.1 ± 0.3	0.67
ChE	302.2 ± 68.5	314.0 ± 62.3	0.56	277.9 ± 64.0	286.5 ± 71.1	0.73
Child-Pugh score	5.1 ± 0.4	5.1 ± 0.4	0.59	5.0 ± 0.2	5.0 ± 0.2	0.99

Alb: Albumin; ChE: Cholinesterase.

acterized by high rates of complications of other autoimmune diseases and by slow progression of the disease in many cases^[20,21]. In many cases of PBC, patients are in the asymptomatic phase, without subjective symptoms, even after onset, and complications in these cases are found only during detailed examinations after a definitive diagnosis of PBC is made, or after patients enter the symptomatic phase. The way in which PBC develops has yet to be well elucidated. Some reports indicate that PBC is associated with medical history of the patient and their family as well as their lifestyle. Various environmental factors such as infections including urinary tract infection as well as cigarette smoking, and a history of administration of estrogen, can cause immune tolerance failures, which lead to the onset of PBC^[22-25]. It is well conceivable that immune tolerance failures due to such environmental factors may lead to the onset of not only PBC but also other autoimmune diseases. In this study, Sjogren syndrome was found as a complication in 25% of the cases, rheumatoid arthritis in 14.8%, and CREST syndrome in 7.4%. Thus, the frequency of Sjogren syndrome as a complication appeared to be relatively low. This may be partially because this study was based on chewing gum tests, which could not detect cases with dry eye symptoms but without oral symptoms, and partially because a definitive diagnosis of the syndrome might not have been made in some cases. In many cases, salivary secretion disorder due to secondary Sjogren syndrome as a PBC complication is milder than primary Sjogren syndrome as a clinical symptom, and there were many asymptomatic PBC cases in the subject group with a mild complaint of dryness. Another report indicated that hepatic dysfunctions were found in 38.2% of cases of collagen diseases, and that 15.9% of cases with such disorders were found to have PBC on diagnosis^[26]. It is therefore necessary for various medical departments to cooperate closely to detect such complications of PBC early and exactly, in order to begin the most appropriate treatment.

Saliva has an essential role in functions such as cleaning the oral cavity, inhibiting the proliferation of bacteria and fungi in the mouth, protecting oral mucosa, and helping swallow food^[27]. In PCB patients aged 60 or more, an age group with a predilection for PBC, subjective symptoms of oral dryness are found in 60%-70% of cases, and more than 20% of these cases have oral candidiasis, which is likely to damage their quality of life^[28]. Sicca syndrome with PBC has been treated with immunomodulators, with-

out substantial clinical effects. Our study demonstrated that administering nizatidine to cases of PBC with subjective symptoms of oral dryness and eating difficulty increased the quantity of saliva secreted, leading to improvements in the subjective symptoms.

Actions of both sympathetic and parasympathetic nerves are involved in the secretion of saliva^[29,30]. Nizatidine simulates parasympathetic cholinergic nerves, to accelerate the gastrointestinal motility by inhibiting acetylcholine esterase. In addition, it increases the quantity of acetylcholine secreted by inhibiting acetylcholine esterase at the endings of cholinergic nerves, stimulating muscarinic receptors in the salivary glands to increase saliva secretion^[30-32]. Other H2-receptor antagonists such as ranitidine and cimetidine cause increases in acetylcholine concentration at cholinergic nerve endings, but do not increase saliva secretion as a result. Cevimeline, an anticholinergic agent, was found to be effective for dry mouth symptoms in Sjogren syndrome but produced side effects and its administration is now limited^[10,33-35]. In view of the foregoing, other H2-receptor antagonists are not expected to produce similar effects to those of nizatidine. In this study, nizatidine exhibited a stronger effect in accelerating saliva secretion in cases where saliva secretion in the chewing gum test was under 10 mL before the start of internal administration. The subjects were an average of 66.7 years old, and older people generally have reduced salivary gland secretion and more frequent occurrence of dry mouth symptom. However, the internal administration of nizatidine was thought to stimulate muscarinic receptors in the salivary glands and revitalize salivary gland cells, leading to increased saliva secretion.

Measured with the VAS method, both feelings of oral dryness and feelings of eating difficulty improved after only one month of internal administration of nizatidine. This may have been partially because the patients were sensitive to changes in the oral cavity and easily sensed such changes. The VAS results for both symptoms improved in almost every case after one month of administration, although in some cases they returned after 6 to 12 mo of admto where they were before the start of administration inistration. It is assumed that the cases involving worsening of symptoms may initially have felt subjective improvements in the symptoms because of psychological factors in addition to increased saliva secretion, but grew accustomed to the ongoing symptoms

over time. Another possibility is that nizatidine's salivary gland stimulation effect on the salivary secretion ability of older patients where salivary secretion had previously become poor may be only transient, finally resulting in the reduction in saliva secretion and the worsening of symptoms in some cases.

In this study, the H2-receptor antagonism of nizatidine stimulated appetite and increased saliva secretion, making the oral mucosa moister thus making it easier to masticate food. We studied how these effects of nizatidine improved the intake of food, and the effect on nutritional condition, hepatic functional reserve, and the long-term prognosis of PBC. The ChE and Alb values did not significantly improve after the administration of nizatidine, and the values in the nizatidine administration group, before or after administration, were not significantly different from the nizatidine non-administration group. The increased saliva secretion and improved dry mouth symptoms did not directly lead to improvements in nutritional condition in this short observation period. We also studied whether increased saliva secretion affects hepatic functional reserve by checking the Child-Pugh scores before and after the administration of nizatidine. The results showed no significant improvements in hepatic functions between before and after administration of the drug. Furthermore, no significant differences in hepatic functions were found between the nizatidine administration group and the nizatidine non-administration group. These results are partially because the cases of PBC in the control group were all in the asymptomatic phase, and partially because the general prognosis of PBC is relative good. PBC has a 5-year survival rate of 91% for men and 92% for women, and a ten-year survival rate of 81% for men and 85% for women, while the disease itself has an extremely long asymptomatic phase^[36]. It is therefore possible that while this study found no significant difference made by nizatidine with respect to hepatic functional reserve, a longer observation period might reveal changes in hepatic functional reserve due to the administration of nizatidine. To determine whether increased saliva secretion caused by the administration of nizatidine may affect the long-term prognosis of PBC, it will be necessary to administer the drug for a longer time to a greater number of cases of PBC in both the symptomatic phase and in the asymptomatic phase, and to observe various changes in these cases.

In this study, we actually started to administer nizatidine to more than 27 of the 73 cases that had been definitively diagnosed as PBC at our hospital. Some of the patients, however, could not continue to come to the hospital for regular examinations. Only the 27 cases continued to undergo regular chewing gum tests and VAS interviews every six months for two years. In selecting patients for the nizatidine administration group and for the non-administration group, we did not take any particular action to avoid bias, but we determined that there was no bias between the two groups because there was no statistically significant difference between the groups in terms

of their hepatic functions (without ALT: $P = 0.04$).

Strictly speaking, as the control group, a placebo should have been administered to the nizatidine non-administration group. One reason this was not done was that many cases dropped out and could not continue to take nizatidine, or undergo VAS interviews or chewing gum tests. This aspect will be improved in any future study.

In this study, we confirmed that administering nizatidine to cases of PBC with dry mouth increased saliva secretion and improved dry mouth symptoms. However, we were unable to show that this improvement led to improvement in the nutritional condition and long-term prognosis of the patients. The prognosis of PBC is generally good. However, it can be inferred that there is a group of PBC cases with poor prognoses, and a further extensive study is needed to establish effective treatments for such a group.

COMMENTS

Background

Dry mouth and/or dry eye symptoms of primary biliary cirrhosis (PBC) are called sicca syndrome, and are found in approximately 70% of patients with PBC. The authors have investigated whether administering nizatidine, which has a saliva stimulation effect, improves dry mouth as a subjective symptom of PBC.

Research frontiers

It was confirmed that administering medicines for gastric ulcers to patients with hepatic problems improves their symptoms. Further, readily available medicines such as H2 Blockers were found to have a significant value in treatment if they are internally used.

Innovations and breakthroughs

It was noted that administering nizatidine not only increased the saliva secretion but also improved xerostomia, a subjective symptom.

Applications

When nizatidine are administered to PBC patients with PBC who have dietary intake difficulties, increase of saliva secretion and improvement of subjective symptoms are expected. However, the influence of dietary intake on the improvements of their liver function and their prognosis should be closely monitored.

Terminology

Sicca syndrome: Dry mouth and dry eye symptoms of PBC are called sicca syndrome, and are found in a high percentage of patients with PBC. Commonly, it is not diagnosed precisely and left without any effective medical treatment.

Peer review

The paper investigated the effects of saliva stimulation by nizatidine on dry mouth symptoms of PBC. It's well designed and written.

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Association of Visceral Obesity with High Viral Load and Histological Findings in Elderly Patients with Genotype 1 Chronic Hepatitis C

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Abstract

Objective Genotype 1 chronic hepatitis C (G1CHC) is generally accompanied by metabolic disturbances related to visceral obesity, such as insulin resistance, steatosis, or dyslipidemia. Because these abnormalities negatively influence the clinical course of G1CHC, we sought to clarify the effect of visceral obesity on the pathophysiology of G1CHC.

Methods We evaluated 180 G1CHC patients for the presence of visceral obesity on the basis of computed tomography findings. Multivariate analysis was performed to estimate the relationship between visceral obesity and demographic, viral, and biochemical characteristics of patients. The associations of visceral obesity with histological findings and serum adipokine levels were also analyzed.

Results Multiple logistic regression analysis revealed that visceral obesity was independently associated with metabolic syndrome, platelet count, high-density lipoprotein level, and serum viral load in elderly patients (≥ 65 years). Multiple linear regression analysis confirmed the association between visceral obesity and high viral load. However, visceral obesity was not correlated with viral load in non-elderly patients (< 65 years). Histological data (160 patients) demonstrated the significant association between visceral obesity and steatosis. Furthermore, patients with visceral obesity showed increase in the severity of fibrosis with advancing age. However, age-associated fibrosis progression was not evident in patients without visceral obesity. The serum adiponectin level was significantly low in patients with visceral obesity, whereas those of leptin, tumor necrosis factor- α , and interleukin-6 were not affected significantly.

Conclusion Visceral obesity was associated with high viral load and histological damage in elderly patients with reduced adiponectin levels.

Key words: chronic hepatitis C, visceral obesity, viral load, steatosis, fibrosis, adiponectin

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Introduction

Infection with hepatitis C virus (HCV) is a major cause of chronic liver disease, with almost 170 million persons being affected worldwide (1). Chronic HCV infection is responsible for a range of diseases, including minimal to severe chronic hepatitis, cirrhosis, and hepatocellular carcinoma (2, 3). Several studies reported thus far have focused

on the factors influencing the heterogeneous clinical course of HCV infection.

A cluster of insulin resistance (IR)-associated metabolic risk factors, such as obesity, dyslipidemia, glucose intolerance, hypertension, and hepatic steatosis, is called the metabolic syndrome. Patients with genotype 1 chronic hepatitis C (G1CHC) generally present with these metabolic factors, especially steatosis and IR (4-6). The detrimental effect of these metabolic risk factors on the course of HCV infection

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is increasingly being recognized. Both IR and hepatic steatosis are associated with the histological grade of portal inflammation and progression of liver fibrosis (6-8). Poor response to interferon-based antiviral therapy is observed in patients with these metabolic risk factors (9, 10). In addition, our recent report revealed that hypertriglyceridemia and glucose intolerance, both of which are components of the metabolic syndrome, had a significant correlation with high serum HCV viral load in patients with G1CHC (11).

Obesity, which is characterized by the excessive deposition of adipose tissue, is closely correlated to the metabolic syndrome. Especially, growing attention has been paid to particular patterns of adipose tissue distribution. Individuals with a selective excess of intra-abdominal or visceral adipose tissue are at a substantially higher risk of developing IR and other features of the metabolic syndrome than those without. Visceral adipose tissue has been reported to act as an endocrine organ that secretes a large number of bioactive substances regulating metabolism and inflammatory and immune responses, and excess visceral adipose tissue accumulation induces metabolic disturbances, which are induced by the dysregulation of bioactive molecules derived from adipose tissue (12-14). Therefore, visceral obesity is recognized not only as a marker of a dysmetabolic profile but also as a causal factor of IR. In the light of the clinical importance of metabolic factors in patients with G1CHC, it would be interesting to study the role of visceral obesity in the pathogenesis of genotype 1 HCV infection.

In this study, we evaluated patients for the presence of visceral obesity by measuring visceral fat area (VFA) on abdominal computed tomography (CT) and attempted to clarify the correlation between visceral obesity and the biochemical, viral, and histological characteristics of patients with G1CHC. We stratified the patients by age because insulin sensitivity is known to decrease with aging, while the prevalence of metabolic syndrome and type 2 diabetes mellitus, both of which involve IR, increases with advancing age (15-17).

Materials and Methods

Patients

We prospectively assessed 180 consecutive patients with G1CHC who visited the Department of Gastroenterology and Hepatology at Juntendo University Shizuoka Hospital, Shizuoka, Japan, between February 2006 and April 2011. Eligibility was defined by the detection of serum HCV-RNA of genotype 1. Exclusion criteria were (a) positivity for hepatitis B surface antigen; (b) presence of liver disease caused by mixed etiologies, including alcohol intake greater than 30 g/day, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease; (c) evidence of hepatocellular carcinoma by ultrasonography or CT; and (d) histology of liver transplantation.

The study protocol was approved by the Ethical Commit-

tee of Juntendo University Shizuoka Hospital and was in accordance with the Helsinki Declaration. Written informed consent was obtained from all the patients participating in the study.

Clinical and laboratory examinations

All patients underwent abdominal CT scan for the evaluation of visceral obesity (18). VFA was measured at the umbilical level and calculated using the Fat Scan[®] software (N2 System Osaka Japan). Visceral obesity was defined as VFA ≥ 130 cm² for male patients and VFA ≥ 90 cm² for female patients, as defined previously (19). All clinical, anthropometric, and laboratory data were collected at the time of obtaining the abdominal CT scan. The patients were classified into 2 groups according to their age: non-elderly (<65 years) and elderly (≥ 65 years) according to the previous report (17).

Fasting blood samples were obtained from all subjects, and the following laboratory parameters were measured using commercially available assays: blood cell count; serum levels of aspartate transaminase (ALT), total cholesterol, and high-density lipoprotein (HDL); plasma triglyceride (TG); and blood levels of glucose, insulin, hemoglobin A1c, and alpha-fetoprotein (AFP). The level of low-density lipoprotein (LDL) was calculated using the Friedewald formula: [LDL (mg/dL)] = [total cholesterol]-[HDL]-([TG] \div 5). If the plasma TG level exceeded 400 mg/dL, LDL was measured directly. IR was determined using the homeostasis model assessment (HOMA-IR) method. The following equation was used: HOMA-IR = fasting insulin (μ IU/mL) \times fasting glucose (mg/dL) \div 22.5 and was defined as HOMA-IR > 2.7 . Serum HCV viral load was quantified by quantitative reverse transcription polymerase chain reaction performed using the COBAS TaqMan HCV Test (Roche Diagnostics, Branchburg). The HCV genotype was determined by polymerase chain reaction with the HCV Genotype Primer Kit (Institute of Immunology Co., Ltd., Tokyo, Japan) and classified according to Simmonds' classification system. Serum levels of adiponectin, leptin, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) were determined in thawed serum samples after storage at -20 $^{\circ}$ and measured in a commercial laboratory (SRL Inc. Tokyo, Japan).

Body mass index (BMI) was calculated by dividing the body weight (kg) by the square of the height (m²). Metabolic syndrome was diagnosed according to criteria defined by The Japanese Society of Internal Medicine (JIM) (20). According to the JIM, metabolic syndrome is diagnosed if the patient has central obesity (measured by ethnicity-specific thresholds for waist circumference for a population of Japanese origin: ≥ 90 cm in male patients and ≥ 80 cm in female patients) and any 2 of the following 3 components: (a) plasma TG of ≥ 150 mg/dL (1.7 mmol/L) and/or serum HDL cholesterol level of < 40 mg/dL (< 1.03 mmol/L) for both men and women, or taking lipid-lowering medications (b) systolic blood pressure of ≥ 130 mm Hg, diastolic blood pressure ≥ 85 mm Hg, or receiving antihypertensive medications; and (c) fasting plasma glucose level of ≥ 110 mg/dL

Results

(6.1 mmol/L) or treatment with oral hypoglycemic medications or insulin. The diagnosis of arterial hypertension was based on the following criteria: systolic blood pressure of ≥ 140 mm Hg and/or diastolic blood pressure of ≥ 90 mm Hg or use of blood pressure-lowering agents. The diagnosis of type 2 diabetes was based on the World Health Organization criteria (21). In the cases of previously diagnosed type 2 diabetes, current therapy with insulin or oral hypoglycemic agent was documented.

Histological evaluation

One hundred sixty of the 180 patients underwent ultrasound-guided percutaneous liver biopsy within 4 weeks of the abdominal CT scan. Liver biopsy specimens were embedded in paraffin and stained with hematoxylin-eosin, Azan-Mallory, and reticulin silver impregnation. The specimens were evaluated by an experienced pathologist who was blind to the clinical data of the patients. Histological evaluation was based on the METAVIR criteria reported previously (22). Hepatic fibrosis was defined as follows: F0, no fibrosis; F1, periportal fibrous expansion; F2, portal fibrous widening with bridging fibrosis; F3, bridging fibrosis with lobular distortion; and F4, liver cirrhosis. On the basis of the degree of lymphocyte infiltration and hepatocyte necrosis, inflammation was classified with scores A0 to A3, with higher scores indicating more severe inflammation. Steatosis in the biopsy specimens was quantitatively assessed by computer-assisted morphometric image analysis. The average percentage of the total area of macrovesicular fat droplets to the liver parenchyma was measured in 2 independent fields at 100 \times magnification, by using the Lumina Vision 2.4 Bio-imaging software (Mitani Corporation, Tokyo, Japan).

Statistical analysis

Continuous variables were summarized as median (range) values, and categorical variables, as frequency and percentage. Mann-Whitney *U* test and chi-square test were used as appropriate. Multiple logistic regression analysis was used to identify factors that were independently associated with visceral obesity. As candidate factors, we selected gender, BMI, type 2 diabetes, arterial hypertension, metabolic syndrome, platelet count; levels of blood AFP, plasma TG, serum HDL, serum hemoglobin A1c; IR, and HCV viral load. Multiple linear regression analysis was performed to assess the relationship of serum HCV viral load with the demographic and biochemical characteristics of the patients. As candidate factors, we selected gender, serum ALT level, platelet count, blood AFP, plasma TG, serum HDL, blood hemoglobin A1c, IR, and visceral obesity. The Spearman's rank correlation coefficient was used to analyze the correlation between 2 variables. All analyses were conducted using IBM SPSS version 19 (IBM SPSS, Chicago, IL, USA), and *p* value below 0.05 was considered to be statistically significant.

Characteristics of patients

The clinical, anthropometric, and laboratory data of the patients enrolled in this study are summarized in Table 1. The 180 patients (91 male and 89 female) had a median age 60 years (range, 20-85 years). The prevalence of arterial hypertension, type 2 diabetes mellitus, and metabolic syndrome were 51.4%, 13.3%, and 16.8%, respectively. The median VFA of all patients was 66.9 cm² (range, 2.4-298.6 cm²), with 41 of 180 patients (23%) having visceral obesity.

According to their age, 101 of 180 patients (56%) were classified as non-elderly (<65 years) and 79 patients (44%), as elderly (≥ 65 years). Comparison of the variables of non-elderly and elderly patients showed that the latter had a higher prevalence of arterial hypertension (*p*=0.002), lower platelet count (*p*=0.003), and higher blood AFP levels (*p*=0.003). No significant difference was noted between elderly and non-elderly patients in VFA or prevalence of visceral obesity.

Factors associated with visceral obesity

Data from the 180 patients were analyzed by logistic regression analysis to examine the correlation between visceral obesity and the demographic and biochemical variables stratified by patient age (Table 2). For non-elderly patients, BMI (*p*<0.001), presence of metabolic syndrome (*p*<0.001), plasma TG level (*p*=0.018), and IR (*p*=0.046) were associated with visceral obesity in univariate analysis. Multivariate analysis revealed that BMI (*p*=0.011) and presence of metabolic syndrome (*p*=0.003) were significantly associated with visceral obesity. For elderly patients, univariate analysis showed that BMI (*p*=0.03), metabolic syndrome (*p*=0.001), platelet count (*p*=0.03), plasma TG level (*p*=0.009), IR (*p*=0.03), and HCV viral load (*p*=0.008) were associated with visceral obesity. Multivariate analysis revealed that the presence of metabolic syndrome (*p*=0.007), platelet count (*p*=0.020), serum HDL level (*p*=0.048), and HCV viral load (*p*=0.027) were independently associated with visceral obesity.

Factors associated with HCV viral load

Because visceral obesity was associated with HCV viral load in elderly patients, further examination was performed to confirm this association by stepwise linear regression analysis (Table 3). In the case of elderly patients, univariate analysis showed that serum viral load was significantly correlated only with visceral obesity (*p*=0.004), while multivariate analysis revealed that HDL (*p*=0.038) and visceral obesity (*p*=0.004) were independently associated with the serum viral load. In the case of non-elderly patients, both univariate and multivariate analyses showed that only plasma TG level was a significant factor associated with the serum viral load (*p*=0.009). No significant correlation was observed between HCV viral load and visceral obesity in the case of

Table 1. Patient Characteristics

	All patients (N = 180)	Non-elderly (N = 101)	Elderly (N = 79)	p value
Age, years	60 (20–85)	56 (20–64)	70 (65–85)	
Male gender, N (%)	91(49.4)	59 (58.4)	32 (40.5)	0.017 ‡
BMI, kg/m ²	23.2 (14.1–36.8)	23.7 (18.1–36.8)	22.5 (14.1–35.3)	0.10 †
Visceral fat area, cm ²	66.9 (2.4–298.6)	69.3 (8.5–298.6)	62.6 (2.36–279.9)	0.27 †
Metabolic syndrome, N (%)	31 (16.8)	18 (17.5)	12 (15.2)	0.68 ‡
Arterial hypertension, N (%)	94 (51.4)	42 (40.8)	50 (64.1)	0.002 ‡
Type 2 diabetes, N (%)	24 (13.3)	10 (9.8)	14 (18.2)	0.10 ‡
Past history of interferon therapy, N (%)	14 (7.8)	8 (7.9)	6 (7.6)	0.94 ‡
Viral load, log IU/mL	6.4 (3.9–7.8)	6.4 (3.9–7.8)	6.4 (4.6–7.7)	0.65 †
ALT, IU/L	58 (14–470)	53 (16–470)	60 (14–235)	0.56 †
Platelet count, ×10 ³ /μL	16.1 (4.0–85.9)	17.8 (4.0–85.9)	14.7 (6.8–33.8)	0.003 †
AFP, ng/mL	7 (1–307)	6 (1–142)	8 (2–307)	0.003 †
Total cholesterol, mg/dL	170 (110–251)	171 (110–251)	167 (113–246)	0.37 †
HDL, mg/dL	55 (27–123)	54 (27–123)	58 (31–104)	0.12 †
TG, mg/dL	89 (30–379)	96.5 (30–379)	88 (32–278)	0.10 †
LDL, mg/dL	92 (33–172)	92.5 (33–172)	89 (36–169)	0.37 †
Blood glucose, mg/dL	101 (69–235)	100 (70–235)	103 (69–226)	0.33 †
HbA1c, %	5.1 (3.8–8.3)	5.0 (3.8–8.3)	5.1 (3.8–7.9)	0.11 †
IR, N (%)	55 (43.7)	35 (50)	20 (35.7)	0.11 †

Data are shown as median (range)

p values are for comparison between non-elderly patients and elderly patients.

BMI: body mass index, ALT: alanine transaminase, AFP: alpha-fetoprotein, HDL: high-density lipoprotein, TG: triglyceride,

LDL: low-density lipoprotein, HbA1c: hemoglobin A1c, IR: insulin resistance

† Mann-Whitney *U* test.

‡ Chi-square test

Table 2. Univariate and Multivariate Analyses of Factors Associated with Visceral Obesity

Variables	Non-elderly				Elderly			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Male gender	0.46 (0.17–1.29)	0.14			0.62 (0.22–1.72)	0.36		
BMI, kg/m ²	1.39 (1.19–1.62)	<0.001	1.30 (1.06–1.60)	0.011	1.22 (1.02–1.46)	0.03		
Type 2 diabetes	0.90 (0.18–4.58)	0.90			1.28 (0.35–1.28)	0.71		
Arterial hypertension	1.82 (0.71–4.63)	0.21			1.97(0.63–6.17)	0.24		
Metabolic syndrome	10.6 (3.38–33.05)	<0.001	9.69 (2.12–44.38)	0.003	10.18 (2.61–39.80)	0.001	11.53 (1.96–68.05)	0.007
History of IFN therapy	4.17(0.07–0.52)	0.06			3.56(0.66–19.38)	0.14		
ALT, IU/mL	1.00 (1.00–1.01)	0.21			1.00 (0.99–1.01)	0.63		
Platelet ×10 ³ /μL	1.01 (0.96–1.06)	0.71			1.11(1.01–1.22)	0.03	1.19 (1.02–1.38)	0.020
AFP, ng/mL	0.96 (0.90–1.04)	0.31			1.01 (0.99–1.02)	0.32		
TG, mg/dL	1.01 (1.00–1.02)	0.018			1.02 (1.01–1.03)	0.009		
HDL, mg/dL	0.98 (0.95–1.02)	0.30			0.98 (0.94–1.01)	0.15	0.95 (0.91–1.00)	0.048
HbA1c, %	1.28 (0.73–2.25)	0.40			1.12 (0.59–2.14)	0.73		
IR	3.11 (1.02–9.45)	0.046			3.43 (1.12–10.50)	0.03		
Log ₁₀ HCV RNA	0.92 (0.50–1.72)	0.80			6.05 (1.51–22.70)	0.008	8.28 (1.28–53.65)	0.027

Logistic regression analysis was used in the univariate and multivariate analyses.

BMI: body mass index, ALT: alanine transaminase, AFP: alpha-fetoprotein, HDL: high-density lipoprotein, TG: triglyceride, HbA1c: hemoglobin A1c, IR: insulin resistance

non-elderly patients.

Figure 1 shows the distribution of HCV viral load in terms of visceral obesity. In the non-elderly group, 22 patients (22%) had visceral obesity, while 79 patients (78%) did not, and their median HCV viral load were 6.4 logU/mL

(range, 3.9–7.7 logU/mL) and 6.5 logU/mL (range, 4.2–7.8 logU/mL), respectively. The difference between the 2 sub-groups of patients was not statistically significant. In the elderly group, 19 patients (24%) had visceral obesity, while 60 patients (76%) did not, and the median HCV viral loads

Table 3. Univariate and Multivariate Analyses of Factors Associated with HCV Viral Load

Variables	Non-elderly				Elderly			
	Univariate		Multivariate		Univariate		Multivariate	
	coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p value
Male gender	-0.154	0.12			0.147	0.20		
History of IFN therapy	0.032	0.76			0.086	0.46		
ALT, IU/L	0.022	0.82			-0.020	0.86		
Platelet, $\times 10^3/\mu\text{L}$	-0.143	0.15			0.177	0.12		
AFP, ng/mL	-0.047	0.64			0.055	0.64		
TG, mg/dL	0.241	0.015	0.291	0.009	0.107	0.36		
HDL, mg/dL	-0.187	0.07			0.182	0.13	0.256	0.038
HbA1c, %	0.119	0.25			0.192	0.10		
IR	0.129	0.24			0.158	0.60		
Visceral obesity	-0.024	0.81			0.328	0.004	0.363	0.004

Stepwise linear regression analysis was used for the univariate and multivariate analyses.

IFN: interferon, ALT: alanine transaminase, AFP: alpha-fetoprotein, TG: triglyceride, HDL: high-density lipoprotein, HbA1c: hemoglobin A1c, IR: insulin resistance

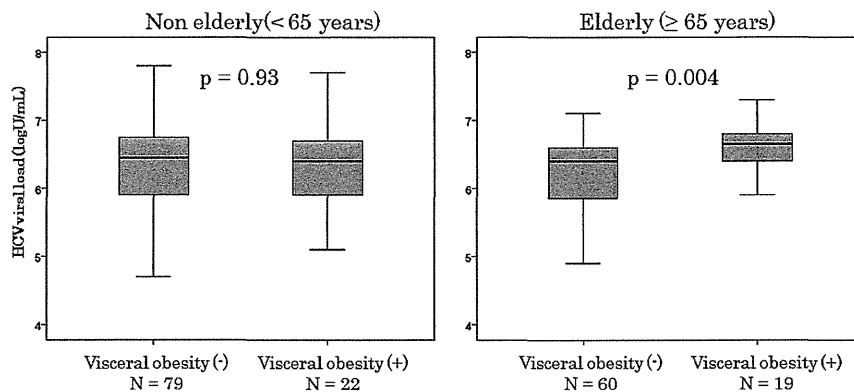


Figure 1. Comparison of serum viral load between patients without visceral obesity and those with visceral obesity stratified by age. Mann-Whitney *U* test was used for statistical analysis.

were significantly different, at 6.7 logU/mL (range, 5.4-7.7 logU/mL) and 6.4 logU/mL (range, 4.6-7.1 logU/mL), respectively ($p=0.004$).

Visceral obesity and histological findings

We next investigated the relationship between visceral obesity and histological findings in patients with G1CHC (Table 4). Histological data were available for 160 of the 180 patients (89%). A total of 77 patients had mild hepatic fibrosis (F0-1), and 83 patients had severe hepatic fibrosis (F2-4). Severe necroinflammatory change (A2-A3) was observed in 120 of the 160 patients (75%). Median percentage of steatosis was 2.6% (range, 0-21%). A comparison of patients with or without visceral obesity revealed no significant difference between them in the prevalence of severe hepatic fibrosis and severe necroinflammatory change. On the other hand, the percentage of steatosis was significantly higher in patients with visceral obesity than in those without (3.8% vs. 2.2%, $p=0.036$). In addition, the percentage of steatosis was significantly correlated with HCV viral load ($r=0.214$, $p=0.003$).

Because the natural history of hepatic fibrosis progression

is age dependent (23), we further analyzed the correlation between patients' age and the fibrosis stage (Fig. 2). The analysis revealed a significant correlation between these factors in patients with visceral obesity ($r=0.355$, $p=0.021$), but not in those without visceral obesity. Furthermore, among patients with visceral obesity, severe fibrosis was observed in 8 of 22 non-elderly patients and 14 of 20 elderly patients. The prevalence of severe hepatic fibrosis was significantly higher in elderly patients than in non-elderly patients (70.0% vs. 36.4%, $p=0.029$), which is indicative of age-dependent progression of fibrosis. On the other hand, in patients without visceral obesity, the prevalence of severe fibrosis of elderly patients and non-elderly patients was 26 of 47 (55.3%) and 35 of 71 (49.3%), respectively, showing no significant difference between the 2 age groups.

Visceral obesity and adipokines

Adipose tissue releases a variety of proinflammatory and anti-inflammatory cytokines, including the adipokines: adiponectin, leptin, TNF- α , and IL-6, and excess accumulation of visceral adipose tissue is associated with adipokine dysregulation, which has adverse metabolic conse-

Table 4. Visceral Obesity and Histological Findings

	Visceral obesity (-)	Visceral obesity (+)	p value
Stage of fibrosis			
F 0 / 1 / 2 / 3 / 4	1 / 56 / 30 / 23 / 8	0 / 20 / 11 / 8 / 3	0.985 †
Grade of inflammation			
A 0 / 1 / 2 / 3	0 / 28 / 84 / 5	0 / 9 / 31 / 2	0.943 ††
Steatosis, (%)*	2.2 (0-21)	3.8 (0-18.4)	0.036 ‡

†Cramer's measure of association

††Chi-square test

‡Mann-Whitney *U* test.

*Data are shown as median (range)

quences (13, 14). In this point of view, we compared the serum levels of adiponectin, leptin, TNF- α , and IL-6 between G1CHC patients with and without visceral obesity (Fig. 3). Serum adipokine levels were evaluated in 159 of 180 patients. The median levels of adiponectin in patients without visceral obesity and those with visceral obesity were 13.7 ng/mL (range, 3.9-96.2 ng/mL) and 9.35 ng/mL (range, 1.9-31.6 ng/mL), respectively. Patients with visceral obesity showed significantly lower serum levels of adiponectin than those without visceral obesity ($p=0.012$). On the other hand, serum levels of leptin, TNF- α , and IL-6 did not show any significant difference between patients with and those without visceral obesity. A significant association was also noted between serum adiponectin level and HCV viral load ($r=-0.182$, $p=0.023$).

Discussion

Serum HCV viral load is clinically important because it has been identified as one of the most substantial factors that predict the outcome of interferon-based anti-virus treatment. For instance, among patients treated with 48 weeks of pegylated interferon and ribavirin, those with genotype 1 and a high viral load ($>2 \times 10^6$ copies/mL) showed a sustained virologic response rate of 46%, while those with genotype 1 and a low viral load ($<2 \times 10^6$ copies/mL) showed a rate was 61% (24). Interestingly, the serum viral load is persistently stable over prolonged periods in untreated patients with chronic hepatitis C (25, 26). However, differences in the serum viral load between individuals vary widely even among patients with identical genotypes. The interpersonal difference in serum viral load was noted to be greater than 6 logU/mL in our study (11). These observations suggest that interpersonal differences in the serum viral load might be affected by environmental predispositions, such as host demographic and biochemical characteristics. Several recent reports have confirmed the correlation between high serum viral load and hypertriglyceridemia or glucose intolerance in patients with G1CHC (11, 27, 28). However, the reasons for the correlation between these biochemical disturbances and HCV viral load have not yet been established.

Dyslipidemia and glucose intolerance are frequent find-

ings in patients with metabolic syndrome. It is generally accepted that excess accumulation of visceral adipose tissue plays an essential role in the development of this multiple risk factor syndrome (14). Therefore, the correlation between hypertriglyceridemia or glucose intolerance and HCV viral load suggests the involvement of visceral obesity in the interpersonal differences of serum viral load in patients with G1CHC. Thus far, many studies have shown the correlation between visceral obesity and glucose intolerance or between metabolic syndrome and insulin resistance or adiponectin level. However, only a few reports have indicated a correlation between visceral obesity and HCV viral load. Only one previous study showed the correlation between HCV viral load and visceral adiposity index, which is a surrogate marker of visceral adiposity (29). In the present study, by using CT to evaluate patients for the presence of visceral adiposity, we demonstrated the close correlation between visceral obesity and high serum viral load in elderly patients with G1CHC. It is unclear why the association between visceral obesity and viral load was observed only in elderly patients in our study. In general, the prevalence of metabolic syndrome increases in advancing age (15, 16). This finding suggests that elderly persons are more seriously affected by visceral obesity compared to non-elderly persons. In addition, recent large-scale Japanese cohort studies have revealed that the proportion of patients with substitution at amino acid 70 of the HCV core protein tend to increase with advancing age (30, 31). These observations suggest that some age-related viral factors are involved in the association between visceral obesity and HCV viral load only in the case of elderly patients.

Further, reports indicate that HCV infection per se is associated with host insulin resistance, independent of the visceral fat area (32). However, the correlation between HOMA-IR and HCV viral load was not found to be significant, both in this study and in a previous one (29). The reason for this discrepancy remains unclear. One of the differences among these studies was the eligible criteria of the patients. The former study excluded obese and diabetic patients, while the latter ones did not. In general, obesity itself causes insulin resistance, even in the absence of HCV infection. HOMA-IR can-not accurately reflect the degree of insulin resistance in individuals whose β -cells are unable to secrete sufficient insulin to overcome existing insulin resistance, for example, in patients with uncontrolled diabetes. Considering these findings together, we believe that the study cohort in the present investigation would be inadequate to assess the exact correlation between HCV viral load and insulin resistance itself.

Histological analysis in this study demonstrated that visceral obesity was associated with steatosis, as reported in a previous study (33, 34). In addition, age-dependent progression of liver fibrosis was significantly noted in patients with visceral obesity; however, no difference was noted in the prevalence of severe hepatic fibrosis between non-elderly and elderly patients without visceral obesity. These results

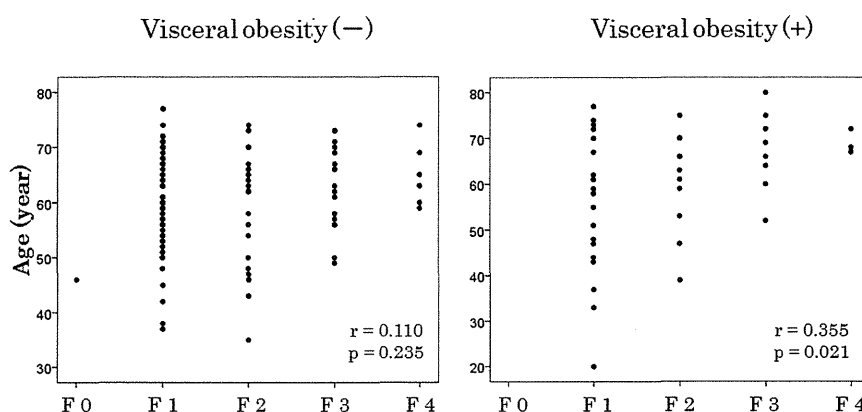


Figure 2. Comparison of age-dependent fibrosis progression between patients without visceral obesity (n=118) and those with visceral obesity (n=42). The Spearman's rank correlation coefficient was used for statistical analysis.

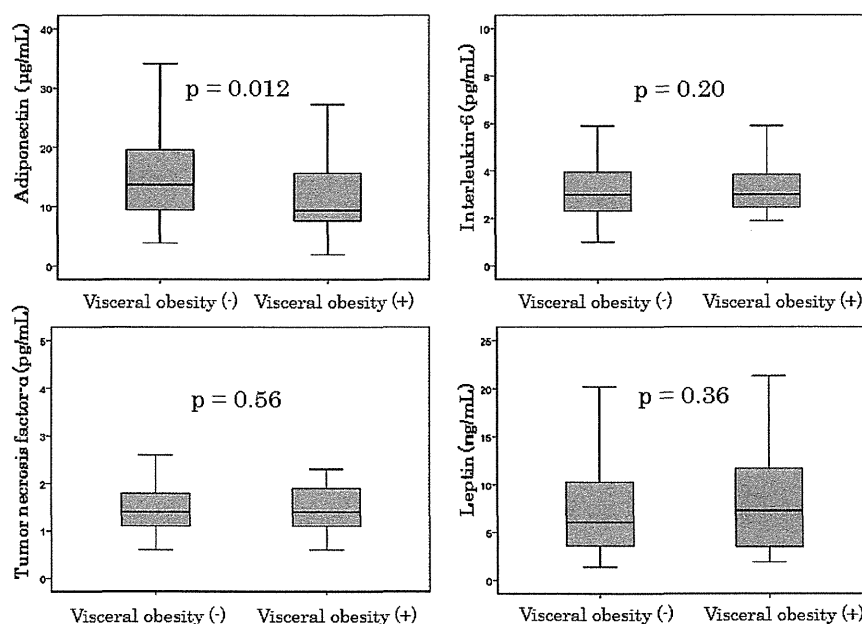


Figure 3. Comparison of serum levels of adiponectin, interleukin-6, tumor necrosis factor- α and leptin between without visceral obesity (n=121) and those with visceral obesity (n=38). Mann-Whitney *U* test was used for statistical analysis.

indicated that visceral obesity was possibly involved in age-dependent progression of liver fibrosis. Consistent with this finding, previous studies have shown that steatosis was a major determinant of the progression of fibrosis in HCV-infected patients (7, 8). Until now, many factors have been found to influence the progression of liver fibrosis in patients with CHC, such as age, duration of infection, alcohol consumption, male gender, hepatic steatosis, and anti-viral therapeutic response. Because these factors interact in a complex manner to influence the progression of fibrosis, it is difficult to determine which one is essential. However, our results suggested that visceral obesity could influence not only the therapeutic response but also the progression of hepatic fibrosis itself. Thus, we can infer that visceral obe-

sity is one of the important factors influencing hepatic fibrosis and that it is clinically important in the management of CHC patients.

Besides serving as a passive reservoir for energy storage, adipose tissue acts as an endocrine organ that secretes various bioactive peptides, known as adipokines (12). To identify the adipokines that might play a key role in the visceral obesity-associated elevation in the serum viral load in G1 CHC patients, we compared the serum levels of adiponectin, leptin, TNF- α , and IL-6 of patients with and those without visceral obesity. Among the 4 adipokines, only adiponectin showed a statistically significant difference between those with visceral adiposity and those without, i.e., the serum adiponectin level was significantly lower in the former. Fur-

thermore, a significant association was also observed between serum adiponectin level and HCV viral load. Importantly, a close association between the serum level of adiponectin and anti-HCV immune response was reported (35). Taken together, our findings indicate that visceral obesity might reduce the immune response to HCV via the down-regulation of adiponectin, resulting in the upregulation of HCV kinetics and elevation of serum viral load in patients with G1CHC.

In conclusion, visceral obesity was found to be associated with high viral load, steatosis, and age-dependent fibrosis progression in patients with G1CHC. These results are suggestive of the detrimental impact of visceral obesity both on the natural course of HCV infection and the effect of interferon-based anti-viral therapy. Strategies aiming at correct visceral obesity might have a beneficial effect on the management of patients with G1CHC.

The authors state that they have no Conflict of Interest (COI).

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HEPATOLOGY

Prediction of liver stiffness hepatocellular carcinoma in chronic hepatitis C patients on interferon-based anti-viral therapyYutaka Narita,* Takuya Genda,* Hironori Tsuzura,* Shunsuke Sato,* Yoshio Kanemitsu,* Sachiko Ishikawa,* Tetsu Kikuchi,* Katsuharu Hirano,* Katsuyori Iijima,* Ryo Wada[†] and Takafumi Ichida*Departments of *Gastroenterology and Hepatology and [†]Pathology, Juntendo University Shizuoka Hospital, Shizuoka, Japan**Key words**

chronic hepatitis C, hepatocellular carcinoma, liver stiffness, risk factor.

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Abstract**Background and Aim:** The purpose of this study was to evaluate the usefulness of liver stiffness measurement (LSM) for assessing the risk of hepatocellular carcinoma (HCC) in chronic hepatitis C (CHC) patients receiving interferon (IFN) therapy.**Methods:** One hundred fifty-one CHC patients who underwent LSM and received IFN therapy were included in the estimation cohort, and 56 were included in the validation study. The cumulative HCC incidences were evaluated using Kaplan–Meier plot analysis and the log-rank test. Multivariate Cox proportional hazard analyses were used to estimate the hazard ratios (HRs) of variables for HCC.**Results:** In the estimation cohort, 9 of 151 patients developed HCC during the median follow-up time of 722 days. Multivariate analysis identified three independent risk factors for HCC: LSM (≥ 14.0 kPa, HR 5.58, $P = 0.020$), platelet count ($< 14.1 \times 10^4/\mu\text{L}$, HR 5.59, $P = 0.034$), and non-sustained virological response (HR 8.28, $P = 0.049$). The cumulative incidence of HCC development at 3 years was 59.6%, 8.2%, and 0.0% in patients with all three risk factors, one to two risk factors, and none of these risk factors, respectively. The incidence of HCC was significantly different between these groups ($P < 0.001$). In the validation cohort, HCC incidence was also significantly different with respect to these risk factors ($P = 0.037$).**Conclusion:** LSM, platelet count, and IFN-therapeutic effect could be used to successfully stratify the risk of HCC in patients receiving IFN therapy and demonstrate the usefulness of LSM before IFN therapy for the management of CHC patients.**Introduction**

Persistent hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease leading to the development of HCC, the fifth most common cancer, and the third most common cause of cancer-related death worldwide.¹ HCV is responsible for 27–75% of the HCC cases in Europe and the United States and > 80% of the HCC cases in Japan.^{2,3} In fact, HCV-positive patients have a 20-fold higher risk of developing HCC than HCV-negative patients,⁴ indicating a significant carcinogenic role for persistent HCV infection. Because of this connection, many chronic hepatitis C (CHC) patients are treated with interferon (IFN)-based antiviral therapy because it not only eradicates HCV but also reduces the rate of HCC development. IFN therapy is most effective at decreasing the risk of developing HCC in patients that achieve a sustained virological response (SVR);^{5–7} however, the risk of HCC development persists after IFN therapy even in patients who do achieve SVR.⁸ HCC might develop immediately after IFN therapy in some cases, or during long-term IFN therapy in others.^{9,10}

Because assessing the risk of developing HCC is clinically important in the management of CHC patients, it is necessary to establish predictors for HCC development in patients who receive IFN therapy.

Some factors reported to predict the risk of HCC development after IFN therapy are older age, male gender, and severe fibrosis,^{11,12} with advanced fibrosis and cirrhosis significantly correlating with the risk of HCC development.¹³ To date, liver biopsy has been the gold standard for assessing the severity of liver fibrosis and cirrhosis,¹⁴ although sampling errors and intraobserver and interobserver variability can lead to understaging.^{15,16} In addition, it is difficult to perform liver biopsy for all patients because of its invasiveness and rare but potentially life-threatening complications.¹⁴ As a result, liver stiffness measurement (LSM), a type of transient elastography, has become a reliable alternative for assessing hepatic fibrosis and cirrhosis mainly in patients with CHC.^{17,18} LSM is non-invasive, reproducible, can be expressed numerically as continuous values, and has a wide dynamic range in the evaluation of hepatic fibrosis. These advantages over liver biopsy