

Original Article

Impaired brain activity in cirrhotic patients with minimal hepatic encephalopathy: Evaluation by near-infrared spectroscopy

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Aim: Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the regional cerebral oxygenated hemoglobin (oxy-Hb) concentration with high time resolution. The aim of the present study is to reveal the time-dependent regional cerebral oxy-Hb concentration change coupled with brain activity during task performance in patients with minimal hepatic encephalopathy (MHE).

Methods: Cerebral oxy-Hb concentration was measured by using NIRS in 29 cirrhotic patients without overt hepatic encephalopathy (HE). Of those, 16 patients who had abnormal electroencephalography findings were defined as having MHE. Responsive increase in oxy-Hb during a word-fluency task was compared between MHE and non-MHE patients.

Results: There was no difference in the maximum value of oxy-Hb increase between patients with and without MHE (0.26 ± 0.12 vs 0.32 ± 0.22 mM·mm, $P = 0.37$). However, the

pattern of the time course changes of oxy-Hb was different between the two groups. The MHE group was characterized by a gradual increase of oxy-Hb throughout the task compared to steep and repetitive increase in the non-MHE group. Increase in oxy-Hb concentration at 5 s after starting the task was significantly small in the MHE group compared to the non-MHE (0.03 ± 0.05 vs 0.11 ± 0.09 mM·mm, $P = 0.006$).

Conclusion: The cerebral oxygen concentration is poorly reactive in response to tasks among cirrhotic patients without overt HE but having abnormal electroencephalography findings. These impaired responses in regional cerebral oxy-Hb concentration may be related to the latent impairment of brain activity seen in MHE.

Key words: hepatic encephalopathy, near-infrared spectroscopy

INTRODUCTION

HEPATIC ENCEPHALOPATHY (HE) is a major complication of liver cirrhosis. Apart from

clinically overt HE (OHE), minimal HE (MHE) is troublesome because it is associated with reduced quality of life (QOL), reduced cognitive function, lowered work efficiency, higher risk of progression to OHE and may be a cause of traffic accidents.^{1–3} MHE treatment can improve QOL, driving capability and progression of OHE.^{4–6} Adequate diagnosis of MHE and early therapeutic intervention are precluded by the lack of reliable diagnostic standards, and HE is usually diagnosed only after the presentation of overt symptoms. For the diagnosis of MHE, neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, Wechsler adult intelligence scale (WAIS) or electro-psychological tests

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including electroencephalography (EEG), cerebral evoked potential, p300 event-related potential, psychometric hepatic encephalopathy score (PHES) and critical flicker test⁷⁻¹⁵ have been employed. Diagnostic specificity can be improved by combining these tests, but complexity becomes a major disadvantage.

Recent advances in diagnostic imaging, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), made it possible to map brain function in tomographic images with high space and time resolutions. Recent study using PET¹⁶ revealed that the primary event in the pathogenesis of OHE is inhibition of cerebral energy metabolism evidenced by reduced cerebral oxygen consumption and reduced cerebral blood flow. Whether the same mechanism could be applied to MHE is not known. Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the cerebral blood volume as an oxygenated hemoglobin (oxy-Hb) concentration. The space and time resolution of NIRS is equivalent or higher than that of PET and fMRI. Moreover, NIRS is highly portable, does not have any restriction in the posture and flexible in setting tasks. Therefore it is possible to perform tests in a natural environment and to evaluate brain function as reflected by the dynamic changes in regional cerebral oxy-Hb concentration in response to a given task. The latter may be especially important to disclose a latent abnormality of brain function.

Recent study suggested that astrocytes regulate the cerebral blood flow and provide the oxy-Hb to the activation site of the brain.¹⁷⁻¹⁹ In hepatic encephalopathy patients, function of astrocyte is impaired which may lead to cerebral oxygen consumption and blood flow.^{16,20-22} We hypothesized that clinically latent abnormality of brain function in MHE also may be linked to

the impairment of adequate increase in cerebral energy metabolism in response to the stimulation for activating the brain due to impaired function of astrocytes. In the present study, we used NIRS to evaluate the latent abnormality of brain function in patients with MHE, by measuring the increase of regional cerebral oxy-Hb concentration in response to task stimulation.

METHODS

Patients

A TOTAL OF 29 liver cirrhosis patients without OHE were enrolled. The underlying etiology of liver disease was hepatitis C virus infection in 19 patients, hepatitis B virus infection in two, alcoholic liver disease in five and other liver disease in three. All participants were examined by two psychiatrists to exclude mental disorders. No patient had any history of taking antidepressants or other psychotropic drugs. Subjects were examined by brain MRI or brain CT and they had no apparent brain structural disease including brain infarction. The study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of Musashino Red Cross Hospital and National Center of Neurology and Psychiatry. Informed consent was obtained from each subject. MHE was defined as those who had abnormal EEG findings. According to this definition, 16 patients were assigned to the MHE group and 13 were assigned to the non-MHE group. Table 1 shows the clinical characteristics of patients. The age and sex ratio did not differ between groups.

NIRS measurements

Concentration of oxy-Hb was measured by a 52-channel NIRS machine (Hitachi ETG4000; Hitachi Medical,

Table 1 Patient characteristics

	MHE (n = 16)	Non-MHE (n = 13)	P-value
Age	67.9 ± 8.9	70.1 ± 10.2	0.53
Sex (M/F)	7/9	7/6	0.72
Albumin (g/dL)	2.68 ± 0.39	3.63 ± 0.47	<0.0001
T-Bil (mg/dL)	1.83 ± 1.22	0.88 ± 0.34	0.011
PT%	64.5 ± 10.8	85.2 ± 12.7	<0.0001
Child-Pugh (A/B/C)	0/9/7	11/2/0	<0.0001
Etiology (HC/HB/Alc/Others)	8/2/4/2	11/0/1/1	0.28
NH3 (mmol/L)	90.1 ± 64.3	40.1 ± 18.3	0.012

Alc, alcoholic liver disease; HB, hepatitis B; HC, hepatitis C; MHE, minimal hepatic encephalopathy; PT%, prothrombin time percentage; T-Bil, total bilirubin.

Tokyo, Japan). NIRS detects changes in brain activity by capturing increases in regional cerebral blood flow caused by neural activity. For each channel, an optic fiber device is connected to an application probe that is placed on the subject's scalp. The 52 channels cover the frontal lobe, upper temporal lobe and anterior parietal lobe of the brain (Fig. 1). The near-infrared light penetrates the scalp and skull, passes through the brain tissue, and is partially absorbed by oxy-Hb. The reflected light is detected by a probe positioned 30 mm away from the application probe. The changes in concentration of oxy-Hb can be calculated by measuring reflected light.²³ In this study, the results measured by the seven channels which were previously reported to be diagnostic for mental disorders; (channels 36–38 and 46–49)^{24–26} were selected for the analysis. The time-dependent changes in oxy-Hb concentration in each of these seven channels were compared between MHE and non-MHE patients. The sum of increase in oxy-Hb concentration in these seven channels was calculated and compared between MHE and non-MHE patients. For this analysis, increase of oxy-Hb at 5 s and maximum increase were used.

Activation task

A word-fluency task was used to stimulate frontal lobe activity. Subjects were instructed to generate as many words as possible with a given letter. For example, with

a task involving “naming words starting with the letter ‘T’”, subjects were given 20 s to say as many words as they could starting with the letter “T”, such as “tomato”, “tail” and “tea”. Three tasks were presented for a total of 60 s. During the word-fluency test, the real-time changes in the oxy-Hb concentration were measured at each channel. Data are expressed as a wave form as well as in the form of topographic images.

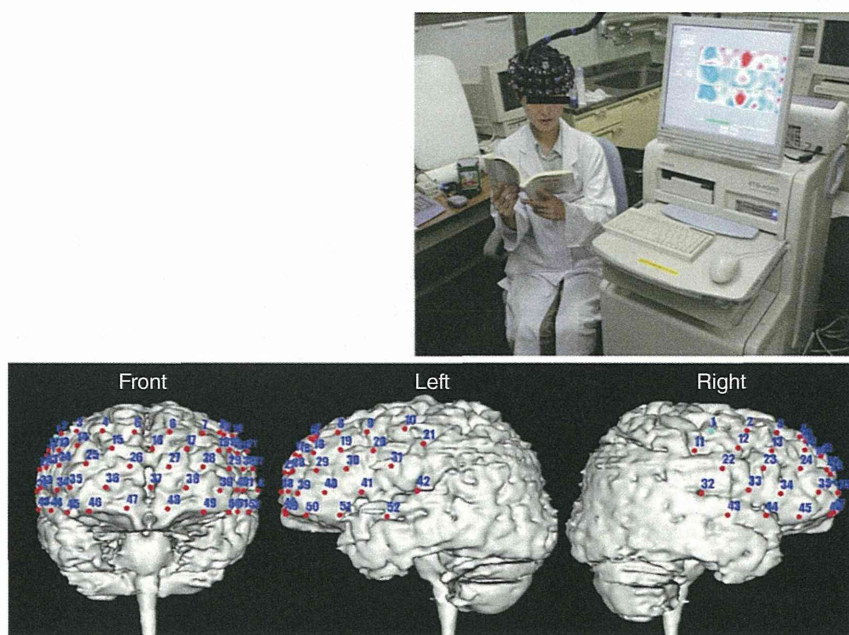
Statistical analysis

The SPSS software package ver. 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

THE NUMBER OF words generated by the word-fluency task did not differ significantly between the MHE and non-MHE groups (10.8 ± 3.4 vs 10.7 ± 2.5 words, $P = 0.93$). Figure 2 shows the time-dependent changes in the oxy-Hb concentration during the task in the representative seven channels. The average value of the seven channels (36–38 and 46–49) is shown in Figure 2. These changes reflected frontal lobe activation by the word-fluency test and correspondingly elevated cerebral blood flow in the frontal lobe. In the non-MHE

Figure 1 Near-infrared spectroscopy. An optic fiber device connected to a probe is placed on the subject's scalp covering the frontal to temporal regions. The relative concentration of oxygenated hemoglobin (oxy-Hb) was measured every 0.1 s during word-fluency testing.



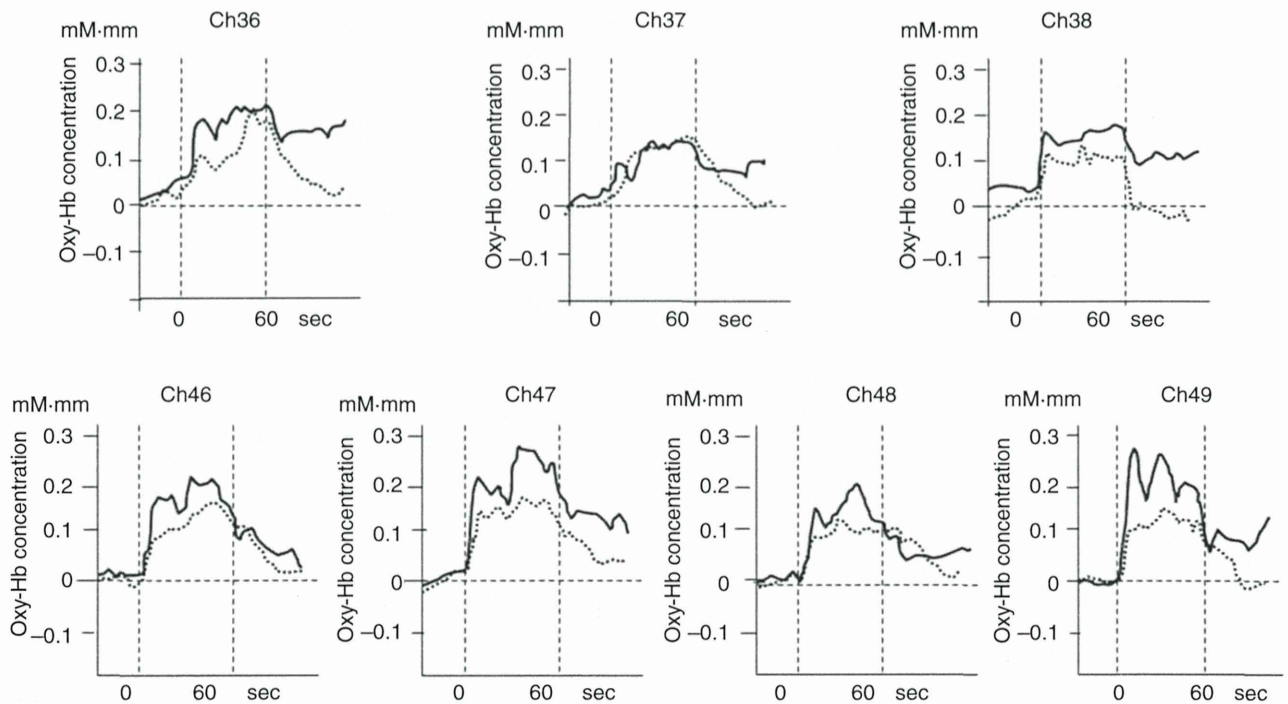


Figure 2 Time-dependent changes in oxygenated hemoglobin (oxy-Hb) concentration in response to tasks. The average waveforms of time-dependent changes in oxy-Hb concentration in representative channels (Ch) are shown. The solid and broken line represents non-minimal hepatic encephalopathy (MHE) and MHE groups, respectively. The area between the two vertical lines corresponds to the 60 s of the word-fluency test.

group, the oxy-Hb concentration increased immediately after the start of the task, remained high with repetitive steep peaks during the task, and decreased after the end of the task. In contrast, the time course of oxy-Hb changes was somewhat different in the MHE group, characterized by a slow increase of oxy-Hb throughout the task, gradually reaching a plateau at the end of the task (Fig. 2). These differences in the degree of oxy-Hb changes also could be visualized by the topographic presentation. In the topographic image, increase of oxy-Hb concentration is expressed as a deepening of the red shading. Figure 3 shows a topographic image showing the increase in oxy-Hb concentration in response to a task. The image in Figure 3 is the average value (arithmetic mean topographic image) of all patients. The concentration of oxy-Hb is small in the MHE group, as reflected by blue or green color, compared to the non-MHE group, as reflected by orange or red color.

When the average value of the seven channels were calculated, the maximum value of oxy-Hb increase was smaller in MHE compared to non-MHE patients but it did not reach statistical significance (0.26 ± 0.12

vs 0.32 ± 0.22 mM·mm, $P = 0.37$) (Fig. 4). On the other hand, increase in oxy-Hb concentration at 5 s after starting the task was significantly small in MHE compared to non-MHE patients (0.03 ± 0.05 vs

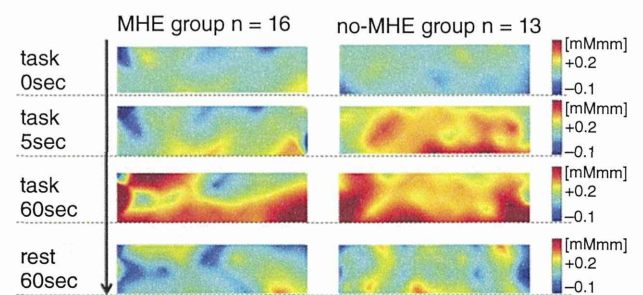


Figure 3 Topographic image showing cumulative increase in oxygenated hemoglobin (oxy-Hb) concentration. Increase in oxy-Hb concentration is shown by deepening of the red shading. The concentration of oxy-Hb is small in the minimal hepatic encephalopathy (MHE) group, as reflected by the blue or green color compared to the non-MHE group as reflected by orange or red color.

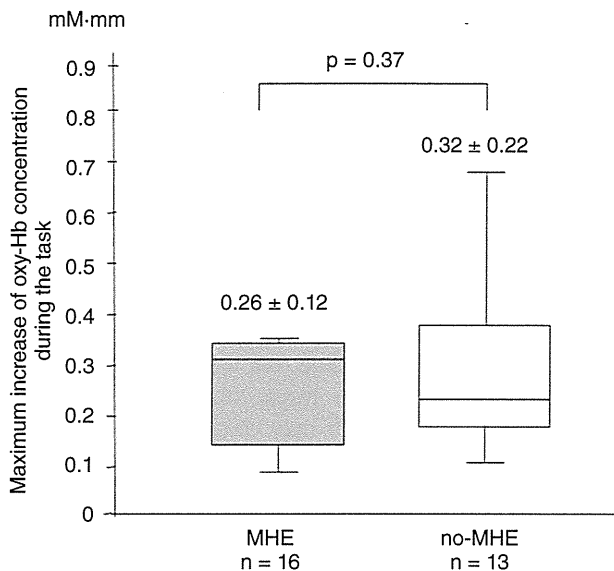


Figure 4 Comparison of maximum increase in oxygenated hemoglobin (oxy-Hb) concentration between patients with and without minimal hepatic encephalopathy (MHE). The average value of maximum increase in oxy-Hb did not differ significantly between the MHE and non-MHE groups.

0.11 ± 0.09 mM·mm, $P = 0.006$) (Fig. 5). For the diagnosis of MHE, the receiver-operator curve analysis identified an optimal cut-off of 0.05 mM·mm for the oxy-Hb concentration at 5 s after starting the task. The area under the curve was 0.774 ($P = 0.012$; 95% confidence interval, 0.60–0.95), sensitivity and specificity of NIRS for the diagnosis of MHE was 69% and 77%, respectively. The positive predictive value was 79% and negative predictive value was 67%.

DISCUSSION

USING NIRS, WHICH can detect changes in regional cerebral oxy-Hb concentration with an extremely high level of sensitivity, we found that increase in cerebral oxy-Hb concentration in response to tasks was slow and small among cirrhotic patients without OHE but having abnormal electroencephalography findings. The impairment of response was most significant at an early time point after the start of the task. These findings indicated that cerebral oxygen metabolism is poorly reactive in response to tasks among patients with MHE and that this impaired cerebral oxygen metabolism may be related to the pathogenesis of latent impairment of brain activity seen in

MHE. To the best of our knowledge, our study appears to be the first evaluating MHE with NIRS. The non-invasiveness and high time resolution of NIRS give it potential as a valuable research tool for the examination of brain function in HE, as well as a clinically useful tool for the diagnosis of MHE.

Hepatic encephalopathy in its early stage, such as latent or minimal HE, can reduce cognitive function, lower work efficiency, reduce QOL^{27,28} or impair driving skill.^{1,2,29,30} Although there are several practical requirements for the diagnosis of MHE, adequate diagnosis of MHE is difficult due to the lack of reliable diagnostic standards.^{31,32} Several diagnostic methods such as neuropsychological function tests, number connection test, light/sound reaction time, inhibitory control test, WAIS or electro-psychological tests including EEG, spectral EEG, and cerebral evoked potential, PHES, critical flicker test and computer-aided quantitative neuropsychological function test system (NP-test)^{7–15} have been proposed,^{32–36} but there is no ideal test for MHE as yet. Because these tests are developed for the screening of MHE, these are not diagnostic. Establishment of a reliable diagnostic method for MHE is imperative. We

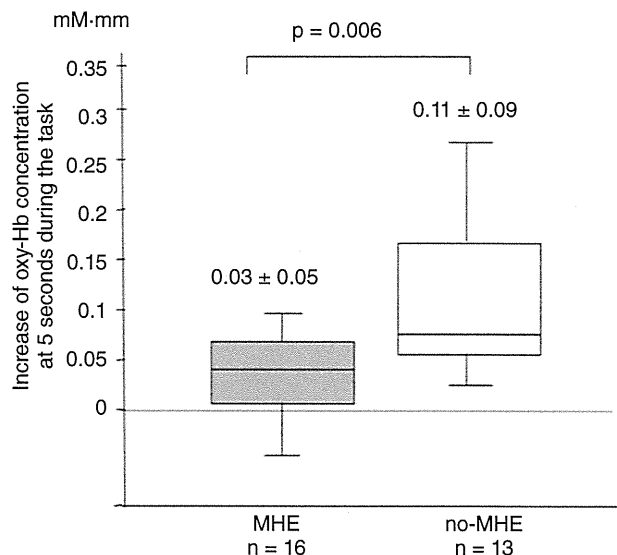


Figure 5 Comparison of increase in oxygenated hemoglobin (oxy-Hb) concentration at 5 s after the start of task between patients with and without minimal hepatic encephalopathy (MHE). The average value of increase in oxy-Hb was compared between the MHE and non-MHE groups at 5 s after starting the word-fluency task. The increase in the oxy-Hb concentration was significantly lower in patients with MHE compared to non-MHE ($P = 0.006$).

have some cases in which NIRS results improved with lactulose and branched-chain amino acid. A prospective study is ongoing to evaluate the effect of treatment by NIRS. The major advantage of NIRS over “paper and pencil tests” is the absence of learning effect which is generally seen in other neuropsychological function tests³⁷ and NIRS could also discriminate other mental disorders.^{24,25}

Neuroimaging using MRI, magnetic resonance spectroscopy and PET has made it possible to non-invasively assess hepatic encephalopathy.^{38–47} However, these tests require extensive equipment and are therefore costly. NIRS is a new methodology for brain research and brain function testing, and has applications in various areas of medicine, being used not only in research, but also in clinical medicine.^{23–25,48} NIRS has been approved for identifying the language-dominant hemisphere before brain surgery and measuring epileptic foci.⁴⁹ In human studies comparing NIRS and fMRI,^{50–52} a correlation was seen between blood-oxygen-level-dependent signal and oxy-Hb concentration as measured by NIRS. In brain function analysis, the detection sensitivity of NIRS is comparable to that of fMRI, but the time resolution of NIRS is greater. Furthermore, the advantages of NIRS are convenience, bedside analysis, non-invasiveness, free task setting and low cost.

Here, we used multichannel NIRS to measure the changes in oxy-Hb concentration during task performance from the frontal to temporal regions of the cortex in MHE patients and compared the results with those of liver cirrhosis without MHE. In all subjects, oxy-Hb increased during task performance and gradually decreased after the completion of task performance. However, the time-dependent changes in the degree of increase in oxy-Hb concentration differed between patients with and without MHE. The degree of increase in oxy-Hb concentration during task performance was smaller and more gradual in MHE compared to non-MHE patients. The increase of the oxy-Hb concentration reflects the increase of cerebral blood volume in the area of the brain activated by the task. Iversen *et al.* found that the cerebral oxygen consumption and blood flow were both reduced in cirrhotic patients with an acute episode of OHE¹⁶ and that the oxygen delivery was approximately twice the oxygen consumption, indicating that oxygen delivery or blood flow was not a limiting factor for the oxygen consumption. Consequently, cerebral blood flow seems to be reduced as a result of diminished cerebral oxygen requirement during HE, and not vice versa.¹⁶ It is reported that neuron-to-astrocyte signaling is a key mechanism in functional

hyperemia,^{17–19,53,54} and that function of astrocytes is impaired in hepatic encephalopathy patients.^{20–22} Therefore, impaired astrocyte-mediated control of cerebral microcirculation can result in slow increase of cerebral blood flow during task performance in MHE patients. Thus, the sluggish increase in cerebral blood flow seen in MHE in the present study may reflect the impaired brain activity and dysfunction of astrocytes and impaired cerebral oxygen metabolism in these patients.

There are several limitations in the present study. The number of patients was not enough to make a comparison stratified by Child grade. We would like to analyze this important point in a future study. It may be possible that cerebral oxy-Hb may change due to aging or by the arteriosclerotic changes. In the present study, age was not related to NIRS results. All patients were examined by brain MRI or brain CT and they had no apparent brain structural disease including brain infarction. However, it was not possible to evaluate the arteriosclerotic changes. This may be another limitation of this study. Many neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, WAIS or electro-psychological tests including EEG, cerebral evoked potential, p300 event-related potential, PHES and critical flicker test have been employed for the diagnosis of MHE. In Japan, Kato and colleagues established the computer-aided quantitative neuropsychological function test system called NP-test.⁷ However, these tests were not simultaneously measured in the present study. Because we recognize the importance of comparing NIRS with other tests, we would like to solve this issue in future study.

In conclusion, NIRS, with its high degree of time resolution, enabled us to identify the characteristic time course of oxy-Hb concentration changes during tasks in MHE. The observations imply that cerebral oxygen supply and metabolism is poorly reactive in MHE, which may be related to the pathogenesis of latent impairment of brain activity.

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Original Article

Hepatocellular carcinoma risk assessment using gadoxetic acid-enhanced hepatocyte phase magnetic resonance imaging

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Aim: To investigate whether the patients with hypovascular liver nodules determined on the arterial phase and hypointensity on the hepatocyte phase gadoxetic acid-enhanced magnetic resonance imaging (hypovascular hypointense nodules) are at increased risk of hepatocarcinogenesis, we assessed subsequent typical hepatocellular carcinoma (HCC) development at any sites of the liver with and without such nodules.

Methods: One hundred and twenty-seven patients with chronic hepatitis B or C and without a history of HCC, including 68 with liver cirrhosis, were divided into those with (non-clean liver group, $n = 18$) and without (clean liver group, $n = 109$) hypovascular hypointense nodules. All the patients were followed up for 3 years, and HCC development rates and risk factors were analyzed with the Kaplan–Meier method and the Cox proportional hazard model, respectively.

Results: A total of 17 patients (10 in the non-clean liver group and seven in the clean liver group) developed typical

HCC. Cumulative 3-year rates of HCC development were 55.5% in the non-clean liver group and 6.4% in the clean liver group ($P < 0.001$), and those at the different sites from the initial nodules was also higher in the non-clean liver group (22.2%) than the clean liver group (6.4%) ($P = 0.003$). Multivariate analysis identified older age ($P = 0.024$), low platelet counts ($P = 0.017$) and a non-clean liver ($P < 0.001$) as independent risk factors for subsequent HCC development.

Conclusion: Patients with hypovascular hypointense liver nodules are at a higher risk for HCC development at any sites of the liver than those without such nodules.

Key words: gadoxetic acid, hepatocellular carcinoma, hepatocyte phase, magnetic resonance imaging, risk assessment

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INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide and is a major cause of death in patients with chronic viral liver disease. Despite many advances in multidisciplinary treatment, complete curative treatment of early stage HCC remains the only possible therapeutic choice for long-term survival. Therefore, surveillance programs for patients at a high risk for HCC that include imaging-based evaluations are crucial for the detection and treatment of early stage HCC.

The newly introduced magnetic resonance imaging (MRI) contrast agent, gadolinium ethoxybenzyl

diethylenetriamine pentaacetic acid (gadoteric acid), has enabled concurrent assessment of tumor vascularity and unique hepatocyte-specific contrast (hepatocyte phase).^{1–3} This has led to the frequent identification of hypovascular nodules determined on the arterial phase with hypointensity on the hepatocyte phase (hypovascular hypointense nodules),^{4–8} while many of these nodules are difficult to be detected by ultrasonography (US) or computed tomography (CT). Recently, the natural history of hypovascular hypointense nodules themselves were reported in several studies,^{9–12} revealing the high risk of subsequent progress to typical HCC from these nodules. However, it is not well known whether patients with such nodules have a higher risk of developing typical HCC at any sites of the liver, including at the different sites from initial nodules, compared to those without such nodules.

If patients with these nodules may have a high risk of developing typical HCC not only at the same sites but also at the different sites from initial nodules, a significant proportion of these nodules are precancerous lesions or early stage HCC as reported,^{13–15} and more importantly, the liver with these nodules may reflect a higher potential for hepatocarcinogenesis or the presence of undetectable precursor lesions in other sites of the liver. Conversely, the absence of these nodules potentially identifies the patients at a low risk for subsequent typical HCC development at any sites. The purpose of this study was to assess the risk of subsequent typical HCC development at any sites of the liver with and without hypovascular hypointense nodules on gadoteric acid-enhanced MRI.

METHODS

Ethical review

THE PROTOCOL OF this retrospective study was approved by the ethics committee of Yamanashi University Hospital, which waived the requirement for written informed consent because the study was a retrospective data analysis, with appropriate consideration given to patient risk, privacy, welfare and rights.

Patients

We recruited 559 consecutive outpatients with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection who underwent gadoteric acid-enhanced MRI at Yamanashi University Hospital between January 2008 and December 2010. The exclusion criteria were as follows: (i) presence or history of typical HCC

($n = 420$), because intrahepatic metastasis does not always develop through the usual multistep hepatocarcinogenesis process, skipping the early pathological stage with hypovascularity to an advanced pathological stage even when the size is small;^{16,17} (ii) Child–Pugh class C disease ($n = 9$), because the hepatocyte phase findings are not reliable in patients with this condition because of reduced gadoteric acid uptake in the liver;¹⁸ and (iii) patients who dropped out during the 3-year follow-up period ($n = 3$).

After excluding 432 patients, 127 patients were included in this retrospective cohort study. They were divided into groups with hypovascular nodules determined on the arterial phase and hypointensity on the hepatocyte phase (non-clean liver group; $n = 18$ patients) and without such nodules (clean liver group; $n = 109$ patients) as shown in Figure 1. In this study, we divided cases into two groups according to the presence or absence of these nodules at the baseline, even when such nodules were initially detected during the follow-up period; we assigned these patients to the clean liver group.

Follow up and diagnosis of HCC

All 127 patients were followed up at the liver disease outpatient clinic of our institution with blood tests, including those for tumor markers and diagnostic imaging modality (US, CT or MRI). The development of typical HCC that required treatment as proposed by the American Association for the Study of Liver Diseases (AASLD) guidelines¹⁹ and that was diagnosed according to imaging criteria, showing arterial hypervascularity and venous phase washout, or based on histological examination of liver biopsies from hypovascular nodules that grew to more than 10 mm during follow up. Biopsies were obtained using a 21-G core needle. Two patients each had a liver nodule of more than 10 mm in diameter on initial MRI (12 mm and 13 mm), which were diagnosed on the basis of the biopsy as dysplastic nodules.

The end-point of this study was the development of typical HCC not only from the hypovascular hypointense nodules observed initially but also from other areas without these nodules (“de novo HCC”). Dynamic CT and/or MRI were also performed in cases with hepatic nodules detected by US, liver cirrhosis, a tendency of tumor marker elevation and difficult evaluation of the liver parenchyma by US. All 127 patients were followed up for 3 years after the initial gadoteric acid-enhanced MRI examination. When imaging

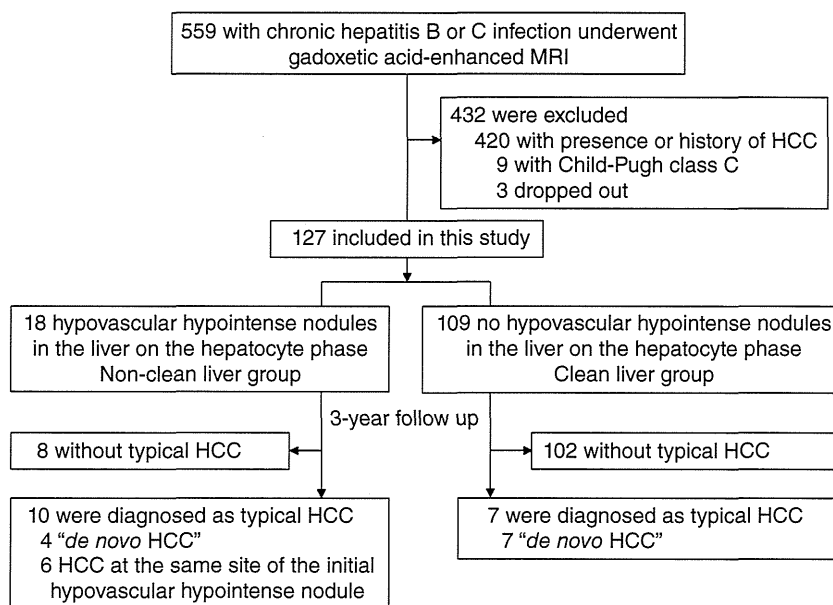


Figure 1 Patient inclusion criteria. “De novo HCC” is a typical hepatocellular carcinoma that developed at sites in which no nodules had been seen on the initial gadoxetic acid-enhanced magnetic resonance imaging (MRI).

modalities led to diagnosis of HCC, recognizing hypervascularization by more than one experienced radiologist and other imaging modalities was regarded as the time of diagnosis of HCC. When needle biopsy was performed to investigate nodules, the time of diagnosis of HCC was when the pathologists and physicians examined pathological tissue and diagnosed as HCC.

MRI

Magnetic resonance imaging was performed using a superconducting magnet that operated at 1.5 Tesla (Sigma EXCITE HD; GE Medical Systems, Milwaukee, WI, USA) and an 8-channel phased-array coil. First, we obtained fast spoiled gradient-echo T₁-weighted images (T1WI) with dual echo acquisition and respiratory-triggered fat-saturated fast spin-echo T₂-weighted images (T2WI). Dynamic fat-suppressed gradient-echo T1WI were obtained using a 3-D acquisition sequence before (precontrast) and 20–30 s, 60 s, 2 min, 5 min, 10 min and 20 min after the administration of gadoxetic acid (Primovist; Bayer Schering Pharma, Berlin, Germany). This contrast agent (0.025 mM/kg bodyweight) was administered i.v. as a bolus at a rate of 1 mL/s through an i.v. cubital line (20–22 G) that was flushed with 20 mL saline from a power injector. The delay time for the arterial phase scan was adjusted according to a fluoroscopic triggering method.²⁰ All images were acquired in the transverse plane. Sagittal plane T1WI were also

obtained during the hepatocyte phase at 20 min after the injection of the contrast agent.

Statistical analysis

All continuous values are expressed as median (range). Fisher’s exact probability test was used for comparisons between categorical variable and the non-parametric Mann-Whitney *U*-test was used to compare differences between continuous variables. Baseline clinical characteristics, including blood test results, were evaluated within 1 month of the initial MRI. We investigated whether or not HCC development was associated with age, sex, fibrosis, etiology (HBV or HCV), platelet count, serum alanine aminotransferase (ALT), γ -glutamyltransferase (γ -GT), α -fetoprotein (AFP), and the presence or absence of hypovascular hypointense nodules.

Cumulative HCC development was estimated according to the Kaplan–Meier method and differences in the curves were tested using the log-rank test. Risk factors for HCC development were determined according to the Cox proportional hazard model. Subgroup analyses with a Cox proportional hazard model were applied to estimation of the hazard ratio (HR) of the non-clean liver group versus clean liver group in the dichotomized subgroups. All statistical analyses were performed using JMP software, version 10 (SAS Institute Japan, Tokyo, Japan). A two-sided *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Characteristics of the patients and nodules

A TOTAL OF 127 patients were enrolled, of whom 26 had chronic HBV infections and 101 had HCV infections, and 68 had virus-associated cirrhosis. No statistically significant differences in the initial clinical characteristics were found between the non-clean liver and clean liver groups (Table 1). Thirty-five hypovascular hypointense nodules were found in 18 patients in the non-clean liver group (1–5 nodules per patient) at baseline (data not shown). Twenty-four of these 35 nodules were detectable only on the hepatocyte phase MRI and were undetectable by US, CT and non-hepatocyte phase MRI. None of the 35 nodules showed high intensity on T2WI. The median nodule diameter was 8 mm (range, 4–13 mm; 33 nodules with ≤ 10 mm, two nodules with 12 mm and 13 mm).

HCC incidence according to initial MRI findings

Hepatocellular carcinoma was diagnosed in 17 patients, 10 in the non-clean liver group and seven in the clean liver group; 14 of these patients had HCV infection. Thirteen patients were diagnosed according to the AASLD imaging criteria.¹⁹ Four patients were diagnosed pathologically by liver biopsies that were performed, based on enlargement of the nodules of more than 10 mm in diameter during the observation period.

The cumulative 1-, 2- and 3-year HCC incidence rates were 1.5%, 10.2% and 13.4%, respectively. As determined by the Kaplan–Meier method, these rates were 11.1% (95% confidence interval [CI], 0.0–25.6%), 38.8% (95% CI, 16.3–61.4%) and 55.5% (95% CI, 32.6–78.5%) in the non-clean liver group, and 0.0% (95% CI, 0.0–2.3%), 5.5% (95% CI, 0.0–9.8%) and

6.4% (95% CI, 1.8–11.0%) in the clean liver group; the former group showed significantly higher rates of development of typical HCC than the latter ($P < 0.001$) as shown in Figure 2. The median imaging intervals were 3 months (range, 3–6) in the non-clean liver group and 4 months (range, 2–12) in the clean liver group. The imaging interval of the non-clean liver group was shorter than the clean liver group (3 vs 4 months, $P = 0.015$). The median intervals between the initial MRI and HCC diagnosis was 16 months (range, 9–32) in the non-clean liver group and 21 months (range, 16–35) in the clean liver group.

In 11 of 17 patients with HCC development, HCC developed at sites in which no nodules had been seen on the initial gadoxetic acid-enhanced MRI, namely de novo HCC. These HCC were found in four of 18 patients in the non-clean liver group (3-year HCC incidence rates: 22.2%; 95% CI, 4.3–51.0%) and 7 in 109 patients in the clean liver group (3-year HCC incidence rates: 6.4%; 95% CI, 1.8–11.0%). The incidence rates of de novo HCC was significantly higher in the non-clean liver group than the clean liver group ($P = 0.003$, Fig. 3). In the remaining six patients, HCC developed at the same site of the initial nodules exclusively in 18 patients of a non-clean liver group by definition, and those HCC arose among the nodules of 8 mm or more in the initial MRI study.

Risk factors for HCC development

Univariate analyses showed that the significant risk factors for HCC development included older age ($P = 0.039$), cirrhosis ($P = 0.009$), a low platelet count ($P = 0.003$), a high AFP concentration ($P = 0.006$) and a non-clean liver ($P < 0.001$). Multivariate analysis with these variables revealed that older age (hazard ratio [HR], 1.08; 95% CI, 1.01–1.16; $P = 0.024$), a low plate-

Table 1 Baseline patient characteristics

Characteristics	Total ($n = 127$)	Non-clean liver ($n = 18$)	Clean liver ($n = 109$)	P
Age, years	65 (30–88)	68 (46–82)	64 (30–88)	0.15
Male/female	68/59	10/8	58/51	1.00
Non-cirrhosis/cirrhosis	59/68	6/12	53/56	0.31
HBV/HCV	26/101	5/13	21/88	0.53
Platelet count ($\times 10^9/L$)	122 (30–410)	102 (46–187)	125 (30–410)	0.07
ALT (IU/L)	32 (7–206)	32 (14–95)	32 (7–206)	0.97
γ -GT (IU/L)	31 (9–305)	31 (13–258)	31 (9–305)	0.68
AFP (ng/mL)	4 (1–582)	8 (2–181)	4 (1–582)	0.19

Continuous data are shown as medians (range).

γ -GT, γ -glutamyltransferase; AFP, α -fetoprotein; ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.

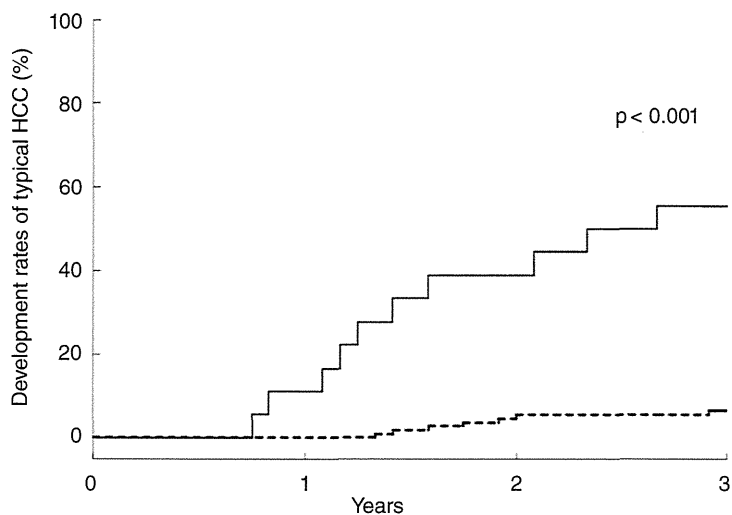


Figure 2 Cumulative incidence rates of typical hepatocellular carcinoma (HCC) development in the non-clean and clean liver groups. —, non-clean liver group ($n = 18$); ---, clean liver group ($n = 109$).

No. of patients at risk

Non-clean liver	18	16	11	8
Clean liver	109	109	103	102

let count (HR, 1.17; 95% CI, 1.03–1.35; $P = 0.017$) and a non-clean liver (HR, 9.41; 95% CI, 3.47–25.46; $P < 0.001$) were the only independent risk factors for HCC development (Table 2).

We further assessed the effect of a non-clean liver on the risk of HCC development in subgroups of these patients (Fig. 4). We found that belonging to the non-

clean liver group was a significant risk factor in patients without HBV. Notably, this designation was particularly valuable for patients who are generally regarded as low risk for HCC development: those without cirrhosis (HR, 37.23; 95% CI, 3.30–419.71; $P = 0.003$) and those with high platelet counts (HR, 33.42; 95% CI, 6.69–166.94; $P < 0.001$).

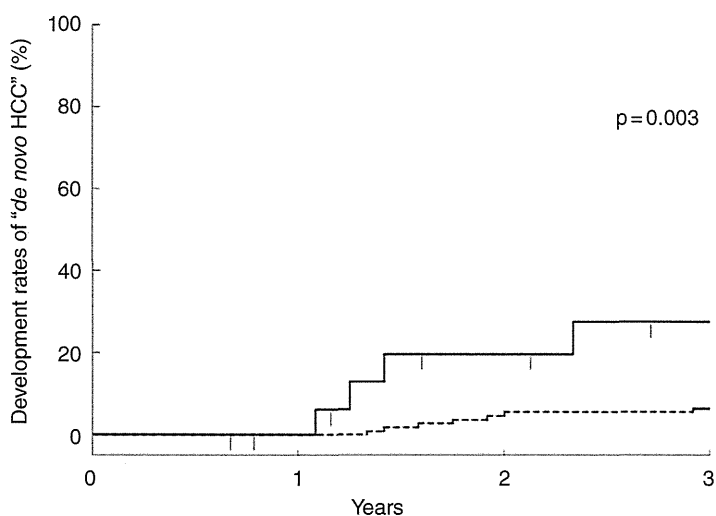


Figure 3 Cumulative incidence rates of typical hepatocellular carcinoma (HCC) at sites in which no nodules had been seen on the initial gadoteric acid-enhanced magnetic resonance imaging, namely, “de novo HCC”. —, non-clean liver group ($n = 18$); ---, clean liver group ($n = 109$).

No. of patients at risk

Non-clean liver	18	18	15	14
Clean liver	109	109	103	102

Table 2 Variables that predict HCC development: univariate and multivariate analyses

Variables	Univariate		Multivariate	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Male	0.56 (0.29–1.95)	0.755		
Age (per year)	1.06 (1.00–1.12)	0.039	1.08 (1.01–1.16)	0.024
Cirrhosis	14.37 (1.90–108.44)	0.009	3.54 (0.37–33.77)	0.231
HCV (vs HBV)	4.39 (0.58–33.17)	0.151		
Platelet count (per 10 ¹⁰ /L)	1.19 (1.06–1.33)	0.003	1.17 (1.03–1.35)	0.017
ALT (per IU/L)	1.00 (0.99–1.02)	0.423		
γ-GT (per IU/L)	1.00 (0.99–1.01)	0.688		
AFP >10 ng/mL	3.98 (1.47–10.77)	0.006	1.47 (0.49–4.33)	0.486
Non-clean liver	12.36 (4.68–32.61)	<0.001	9.41 (3.47–25.46)	<0.001

γ-GT, γ-glutamyltransferase; AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

DISCUSSION

THIS STUDY REVEALED presence of hypovascular hypointense liver nodules (non-clean liver) on gadoteric acid-enhanced MRI, is a significant risk factor for subsequent development of typical HCC not only at the same sites but also at the different sites from the initial nodules. The incidence of development of typical HCC in the non-clean liver patients was more than 50% during a 3-year follow-up period, indicating that these higher risk patients should be rigorously investigated for the early detection of HCC during follow up.

In the present study, six of the 18 patients in the non-clean liver group developed typical HCC at the

same site of the initial nodules during the subsequent 3 years (11.1%/year). Most of the hypovascular hypointense nodules on gadoteric acid-enhanced MRI are considered precursor lesions of typical HCC, such as early HCC or high-grade dysplastic nodules, on histological examination,^{13–15} while it has been reported that most hypovascular nodules exhibiting high-intensity to isointensity signals in the hepatocyte phase are benign hepatic nodules.^{14,15} Recent studies have suggested that a reduction of organic anion-transporting polypeptide 1B3 (OATP 8) transporter expression begins at the earliest stage of hepatocarcinogenesis,^{21,22} before changes in vascularity such as decreased portal flow or increased arterial flow. The progression rate of the small

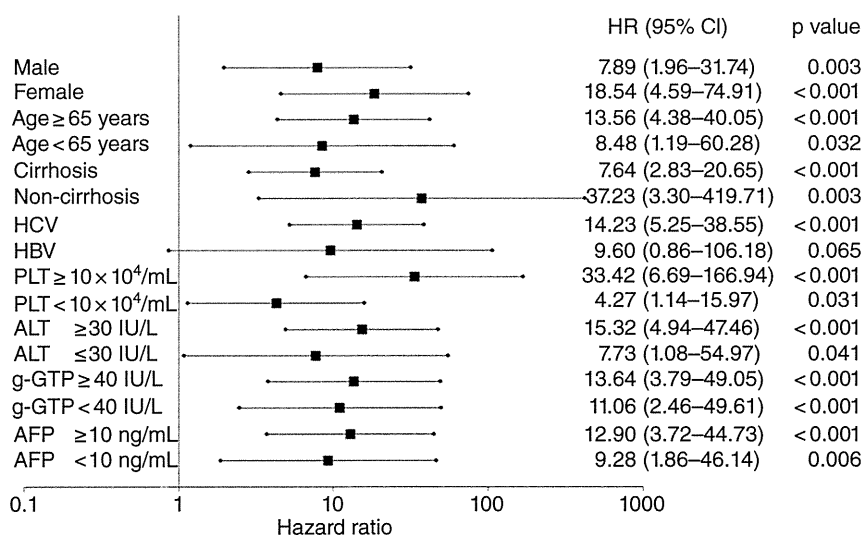


Figure 4 Stratified analyses of the non-clean liver as a risk factor for typical HCC development. AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; g-GTP, γ-glutamyltransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; PLT, platelets.

hypovascular hypointense nodules to typical HCC was reported as 10–17%/year,^{9,10} which is comparable to the present study. Typical HCC arose exclusively among the nodules of 8 mm or more, as in previous studies in which the larger hypovascular hypointense nodules were found to be the risk factor for progression to typical HCC in the initial MRI study.^{9,10}

Hyperintensity on T2WI¹² or diffusion-weighted images (DWI)¹¹ also was reported to be useful for prediction of typical HCC progress in hypovascular hypointense nodules. In our patients, none of the nodules in the non-clean liver group showed hyperintensity on T2WI, suggesting that the hepatocyte phase is more sensitive for detecting the early stage of hepatocarcinogenesis.¹⁵ DWI were not evaluated in this study because this usually detects pathologically advanced HCC of larger size or with hypervascularity.²³ Thus, it is reasonable that the hepatocyte phase can effectively recognize the earliest stage of HCC development without T2WI or DWI.

In 11 of 17 patients, typical HCC developed at sites other than the initially detected hypovascular hypointense nodules. As shown in Figure 3, the incidence rates of such HCC in the non-clean liver group was significantly higher than in the clean liver group ($P=0.003$), indicating that a non-clean liver itself is a risk factor for HCC development, apart from the detectable hypovascular hypointense nodules. In addition, in four patients with nodules even below 8 mm, two developed HCC at different sites from the initial nodules during follow up (data not shown). Taken together, a non-clean liver has the higher potential for hepatocarcinogenesis or for undetectable precursor lesions. The non-clean liver may reflect more advanced genetic or epigenetic changes in the background hepatocytes, however, the detailed biological mechanism is not clear in this study.

Non-clean liver was an independent risk factor for the development of typical HCC, apart from well-documented risk factors (Table 2), such as cirrhosis,²⁴ ALT,²⁵ γ -GT,²⁶ age and AFP.²⁷ A non-clean liver is a significant risk for HCC development also for those without cirrhosis or with high platelet counts (Fig. 4). This means patients at increased risk of HCC development can be discerned as having a non-clean liver even among low-risk subgroups.

Conversely, patients without such nodules (clean liver group) showed a significantly lower risk of developing typical HCC than those with non-clean livers (0.0% vs 11.1% at 1 year, 6.8% vs 55.5% at 3 years of follow up; $P<0.001$), suggesting that gadoteric acid-enhanced

MRI could detect precursor lesions sensitively enough to rule out immediate (within 1 year) development of typical HCC. Although seven patients in the clean liver group developed typical HCC only after 1 year, these patients had other risk factors for HCC development, including lower platelet counts, implying more advanced liver cirrhosis or high AFP (data not shown). Such HCC may arise from precursor lesions that cannot be visualized by current imaging techniques.

This study is a retrospective study and has some limitations. We included patients with HBV and HCV together, because gadoteric acid-enhanced MRI findings or HCC development do not differ between these two groups and HBV or HCV infection is not an independent risk factor for typical HCC development. However, the number of HBV patients was too small ($n=26$) to statistically confirm the current result when limited to HBV patients only. Prospective studies with larger numbers of patients who have uniform liver disease etiologies and imaging intervals are needed to verify our findings in different settings. Although the imaging interval of the non-clean liver group was shorter than the clean liver group (3 vs 4 months; $P=0.015$), the median intervals between the initial MRI and HCC diagnosis was 16 months in the non-clean liver group and 21 months in the clean liver group. They are short enough for cumulative detection of HCC development for 3 years and it is assumed that there was little influence on the conclusions.

In conclusion, patients with chronic viral liver disease are at high risk for developing typical HCC at any sites of the liver if they have hypovascular hypointense nodules on gadoteric acid-enhanced MRI. These patients should be closely followed up for developing typical HCC not only at the same site but also at different sites from the initial nodule.

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Original Article

Prospective comparison of real-time tissue elastography and serum fibrosis markers for the estimation of liver fibrosis in chronic hepatitis C patients

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Aim: Real-time tissue elastography (RTE) is a non-invasive method for the measurement of tissue elasticity using ultrasonography. Liver fibrosis (LF) index is a quantitative method for evaluation of liver fibrosis calculated by RTE image features. This study aimed to investigate the significance of LF index for predicting liver fibrosis in chronic hepatitis C patients.

Methods: In this prospective study, 115 patients with chronic hepatitis C who underwent liver biopsy were included, and the diagnostic accuracy of LF index and serum fibrosis markers was evaluated.

Results: RTE imaging was successfully performed on all patients. Median LF index in patients with F0–1, F2, F3 and F4 were 2.61, 3.07, 3.54 and 4.25, respectively, demonstrating a stepwise increase with liver fibrosis progression ($P < 0.001$). LF index (odds ratio [OR] = 5.3, 95% confidence interval [CI] = 2.2–13.0) and platelet count (OR = 0.78, 95% CI = 0.68–

0.89) were independently associated with the presence of advanced fibrosis (F3–4). Further, LF index was independently associated with the presence of minimal fibrosis (F0–1) (OR = 0.25, 95% CI = 0.11–0.55). The area under the receiver–operator curve (AUROC) of LF index for predicting advanced fibrosis (0.84) was superior to platelets (0.82), FIB-4 index (0.80) and aspartate aminotransferase/platelet ratio index (APRI) (0.76). AUROC of LF index (0.81) was superior to platelets (0.73), FIB-4 index (0.79) and APRI (0.78) in predicting minimal fibrosis.

Conclusion: LF index calculated by RTE is useful for predicting liver fibrosis, and diagnostic accuracy of LF index is superior to serum fibrosis markers.

Key words: chronic hepatitis C, fibrosis, liver fibrosis index, real-time tissue elastography

INTRODUCTION

AN ADVANCED STAGE of liver fibrosis in chronic hepatitis C (CHC) is associated with hepatocellular carcinoma development and complications such as

esophageal variceal bleeding and liver failure.^{1,2} Therefore, accurate evaluation of the stage of liver fibrosis is most important in clinical practice. Liver biopsy is considered to be the golden standard for diagnosis of liver fibrosis.^{3–5} However, this method may be inaccurate because of sampling errors and interobserver variations.^{6,7}

Improvements in a variety of non-invasive methods for evaluating liver fibrosis have recently emerged as alternatives to liver biopsy. Liver fibrosis was reportedly predicted by measurement of liver stiffness using transient elastography^{8,9} and acoustic radiation force impulse (ARFI).^{10,11} As assessed by blood laboratory tests, the aspartate aminotransferase (AST)/alanine

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aminotransferase (ALT) ratio,¹² AST/platelet ratio index (APRI),^{13,14} and FIB-4 index^{15,16} have been reported to be useful for the prediction of liver fibrosis. We previously reported that the FIB-4 index is useful for the prediction of liver fibrosis progression.¹⁷

Real-time tissue elastography (RTE) is a non-invasive method for the measurement of tissue elasticity using ultrasonography.¹⁸ RTE calculates the relative hardness of tissue from the degree of tissue distortion and displays this information as a color image. RTE was recently reported to be useful for predicting liver fibrosis.^{19,20} To increase the objectivity of the evaluation, an image analysis method to evaluate the strain image features and a new algorithm to deliver an index were proposed. Liver fibrosis (LF) index is a quantitative method for evaluation of liver fibrosis that is calculated by nine RTE image features, and the significance of LF index for predicting liver fibrosis has been reported.^{21,22}

In the present study, we prospectively investigated the significance of LF index calculated by RTE for the prediction of liver fibrosis in CHC patients. Further, diagnostic accuracy for liver fibrosis was compared between LF index and serum fibrosis markers.

METHODS

Patients

A TOTAL OF 127 consecutive patients with CHC were prospectively investigated. All patients underwent liver biopsy at Musashino Red Cross Hospital between February 2011 and November 2012. Exclusion criteria comprised the following: (i) co-infection with hepatitis B virus ($n = 1$); (ii) co-infection with HIV ($n = 1$); (iii) history of autoimmune hepatitis or primary biliary cirrhosis ($n = 3$); (iv) alcohol abuse (intake of alcohol equivalent to pure alcohol ≥ 40 g/day) ($n = 0$); (v) portal tracts of biopsy sample of less than five ($n = 7$); and (vi) presence of serious heart disease ($n = 0$). After exclusion, 115 patients were enrolled in this study. Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees (application no. 24007).

Histological evaluation

Liver biopsy specimens were laparoscopically obtained using 13-G needles ($n = 93$). When laparoscopy was not conducted due to a history of upper abdominal surgery, percutaneous ultrasound-guided liver biopsy

was performed using 15-G needles ($n = 22$). Specimens were fixed, paraffin-embedded, and stained with hematoxylin–eosin and Masson-trichrome. A biopsy sample with minimum portal tracts of five was required for diagnosis. All liver biopsy samples were independently evaluated by two senior pathologists who were blinded to the clinical data. Fibrosis staging was categorized according to the METAVIR score:²³ F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Activity of necroinflammation was graded on a scale of 0–3: A0, no activity; A1, mild activity; A2, moderate activity; and A3, severe activity. Percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis and graded on a scale of 0–3: grade 0, no steatosis; grade 1, 1–33%; grade 2, 34–66%; and grade 3, 67% and over.

Clinical and biological data

The age and sex of the patients were recorded. Serum samples were collected within 1 day prior to liver biopsy and the following variables were obtained through serum sample analysis: AST, ALT and platelet count. FIB-4 index and APRI were calculated according to the published formula appropriate to each measure.^{13,15}

RTE and LF index

Real-time tissue elastography was performed using HI VISION Preirus (Hitachi Aloka Medical, Tokyo, Japan) and the EUP-L52 linear probe (3–7 MHz; Hitachi Aloka Medical) within 3 days of liver biopsy. RTE was performed on the right lobe of the liver through the intercostal space. An RTE image was induced by heartbeats. Five RTE images were collected for each patient and analyzed to calculate nine image features. RTE method and the equation that calculates LF index using nine image features has been previously detailed.²² Results are expressed as mean LF index of all measurements. Two hepatologists (N. T. and K. Tsuchiya, with 8 and 16 years of experience, respectively) performed RTE. In 32 patients with CHC, LF index was measured independently by two examiners. The correlation coefficient of LF index between two examiners was 0.85 ($P \leq 0.001$).

Statistical analysis

Correlations between LF index and histological fibrosis stage were analyzed using Spearman's rank correlation coefficients. Categorical variables were compared using Fisher's exact test, and continuous variables were compared using Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant. Logistic regression was

used for multivariate analysis. Receiver–operator curves (ROC) were constructed, and the area under the ROC (AUROC) was calculated. Optimal cut-off values were selected, to maximize sensitivity, specificity and diagnostic accuracy. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by using cut-offs obtained by ROC. SPSS software ver. 15.0 (SPSS, Chicago, IL, USA) was used for analyses.

RESULTS

Patient characteristics

THE CHARACTERISTICS OF all 115 patients are listed in Table 1. F0–1 was diagnosed in 52 cases (45%), F2 in 31 (27%), F3 in 20 (17%) and F4 in 12 (11%). Mean values of LF index of F0 (2.62) and F1 (2.60) were not significantly different ($P=0.9$), and only six patients with F0 were included in this study. Therefore, patients with F0 and F1 were integrated for the analysis. RTE imaging was successfully performed in all patients, and LF index was calculated.

Relationship between histological findings and LF index by RTE

The median value of LF index compared with the METAVIR fibrosis stage is shown in Figure 1. Median LF

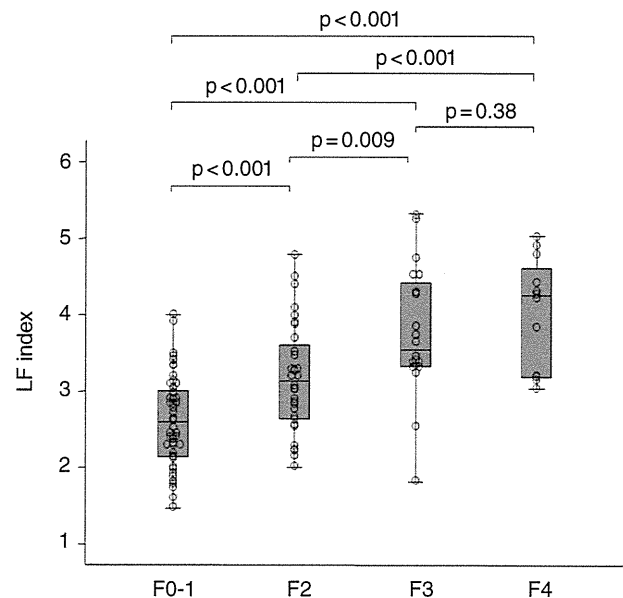


Figure 1 Correlation between liver fibrosis (LF) index calculated by real-time tissue elastography and fibrosis stage. Box plot of the LF index is shown according to each fibrosis stage. The bottom and top of each box represent the 25th and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and error bar indicates minimum and maximum non-extreme values.

Table 1 Patient characteristics

Characteristics	Patients ($n = 115$)
Female/male	68/47
Age (years)	57.9 \pm 10.9
AST (IU/L)	55.7 \pm 44.9
ALT (IU/L)	63.2 \pm 56.3
Platelet counts ($\times 10^9/L$)	162 \pm 53
Portal tracts of biopsy samples	12.6 \pm 5.0
Fibrosis stage	
F0–1 (%)	51 (44)
F2 (%)	32 (28)
F3 (%)	20 (17)
F4 (%)	12 (11)
Histological activity	
A0 (%)	0 (0)
A1 (%)	75 (65)
A2 (%)	34 (30)
A3 (%)	6 (5)
Steatosis grade	
Grade 0 (%)	65 (57)
Grade 1 (%)	47 (41)
Grade 2 (%)	3 (2)
Grade 3 (%)	0 (0)

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

index in patients with F0–1, F2, F3 and F4 were 2.61, 3.07, 3.54 and 4.25, respectively, demonstrating a step-wise increase with liver fibrosis progression ($P < 0.001$). LF index of each fibrosis stage significantly differed from each other (F0–1 vs F2, $P < 0.001$; F0–1 vs F3, $P < 0.001$; F0–1 vs F4, $P < 0.001$; F2 vs F3, $P = 0.009$; F2 vs F4, $P = 0.001$). On the other hand, mean values of LF index in patients with steatosis grade 0, 1 and 2 were 2.99, 3.29 and 2.60, respectively, demonstrating no significant correlation (Fig. 2a). LF index was compared with steatosis grade for each fibrosis stage. LF index was not significantly different between patients with steatosis and without steatosis (Fig. 2b).

Liver fibrosis index was compared with histological activity. A significant correlation existed between histological activity and fibrosis stage. Therefore, the relationship between LF index and histological activity was examined by each fibrosis stage. In patients with F0–1, the mean LF index of A1, A2 and A3 was 2.60, 2.58 and 2.40, respectively, demonstrating no significant correlation. Similarly, in patients with F2, F3 and F4, there was no significant correlation between LF index and histological activity (Fig. 3).

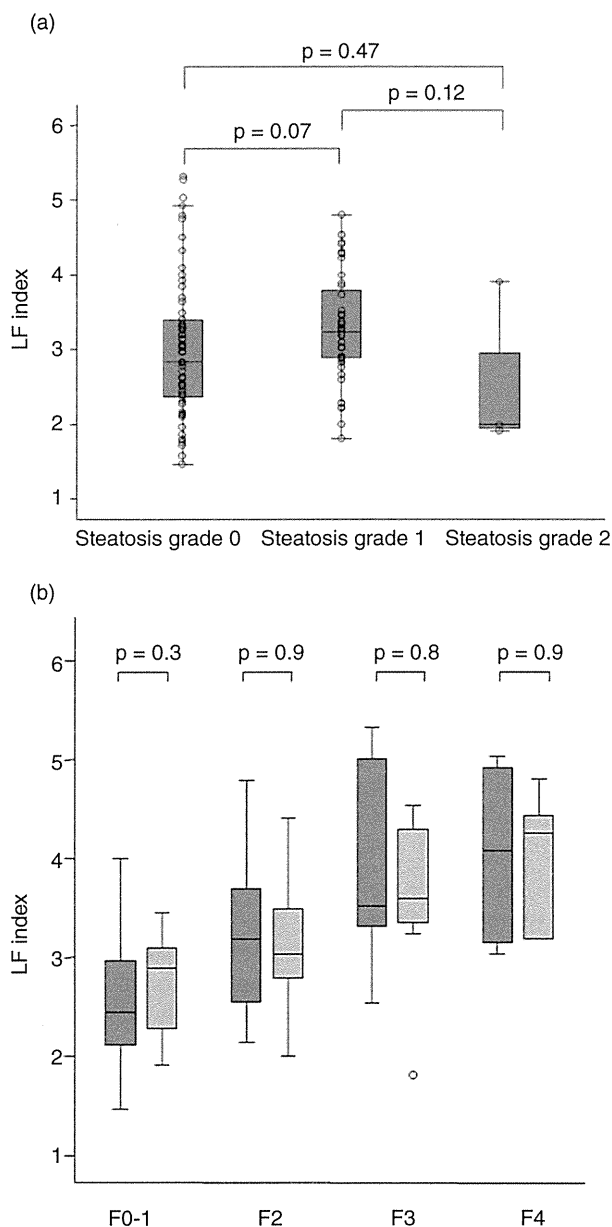


Figure 2 (a) Correlation between liver fibrosis (LF) index and steatosis grade. Box plot of the LF index is shown according to each steatosis grade. The bottom and top of each box represent the 25th and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and error bar indicates minimum and maximum non-extreme values. (b) Box plot of LF index for each fibrosis stage in relation to degree of steatosis grade. The bottom and top of each box represent the 25th and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and error bar indicates minimum and maximum non-extreme values. Dark grey bar chart indicates steatosis grade 0. Light grey bar chart indicates steatosis grade 1-2.

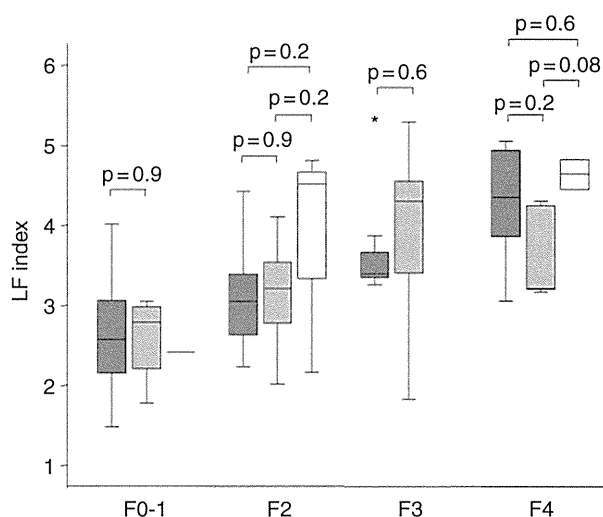


Figure 3 Box plot of liver fibrosis (LF) index for each fibrosis stage in relation to degree of necroinflammatory activity. The bottom and top of each box represent the 25th and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and error bar indicates minimum and maximum non-extreme values. Dark grey bar chart indicates activity grade 1. Light grey bar chart indicates activity grade 2. White bar chart indicates activity grade 3.

Comparison of variables associated with the presence of advanced fibrosis (F3-4) by univariate and multivariate analysis

Variables associated with the presence of advanced fibrosis (F3-4) were assessed by univariate and multivariate analysis (Table 2). The variables of age ($P = 0.03$) and LF index ($P < 0.001$) were significantly higher, and the variable of platelets ($P < 0.001$) was significantly lower in patients with advanced fibrosis than in patients with F0-2. Multivariate analysis showed that LF index (odds ratio [OR] = 5.3, 95% confidence interval [CI] = 2.2-13.0) and platelets (OR = 0.78, 95% CI = 0.68-0.89) were independently associated with the presence of advanced fibrosis.

Comparison of variables associated with the presence of minimal fibrosis (F0-1) by univariate and multivariate analysis

Variables associated with the presence of minimal fibrosis (F0-1) were assessed by univariate and multivariate analysis (Table 3). The variables of age ($P < 0.001$), AST ($P = 0.02$) and LF index ($P < 0.001$) were significantly lower, and the variable of platelets ($P < 0.001$) was significantly higher in F0-1 patients than F2-4 patients.