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Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study

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Abstract

Background We investigated whether the administration of maintenance doses of interferon prevented hepatocellular carcinoma (HCC) in patients with chronic hepatitis C. *Methods* Study 1: A multicenter, retrospective, cooperative study was carried out to determine whether long-term administration of low-dose peginterferon alpha-2a (PegIFNα-2a) prevented HCC development in patients with chronic hepatitis C. In total, 594 chronic hepatitis C patients without a history of HCC were enrolled and treated with 90 μg PegIFNα-2a administered weekly or bi-weekly for at least 1 year. Study 2: HCC developed in 16 of 99 additional patients without PegIFNα-2a treatment during 3.8 years of observation. A propensity-matched control study was then carried out to compare the incidence of

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HCC between the 59 patients who received low-dose PegIFNα-2a (PegIFNα-2a group) and 59 patients who did not receive PegIFNα-2a treatment (control group), matched for sex, age, platelet count, and total bilirubin levels.

Results Study 1: HCC developed in 49 patients. The risk of HCC was lower in patients with undetectable hepatitis C virus RNA, ≤40 IU/L alanine aminotransferase (ALT), or ≤10 ng/L alpha-fetoprotein (AFP) 24 weeks after the start of therapy. Study 2: The incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group.

Conclusions Low-dose and long-term maintenance administration of PegIFNα-2a decreased the incidence of HCC in patients with normalized ALT and AFP levels at 24 weeks compared with patients without normal ALT and AFP levels.

Keywords Chronic hepatitis C · Hepatocellular carcinoma · Peginterferon

Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, often develops because of long-term hepatitis B or C virus infection [1, 2]. In particular, chronic hepatitis C and hepatic cirrhosis increase the risk of HCC; the annual incidence of tumor development in such patients may be as high as 2–4 % [3–5]. The incidence of HCC decreases in patients who achieve a sustained virological response (SVR) to interferon (IFN) treatment, although the incidence remains high in non-SVR patients [6–9]. A detailed analysis of HCC development revealed that chronic hepatitis C patients aged 65 years or more, especially those with advanced fibrosis of the liver, were at an increased risk of developing HCC [10]. For patients

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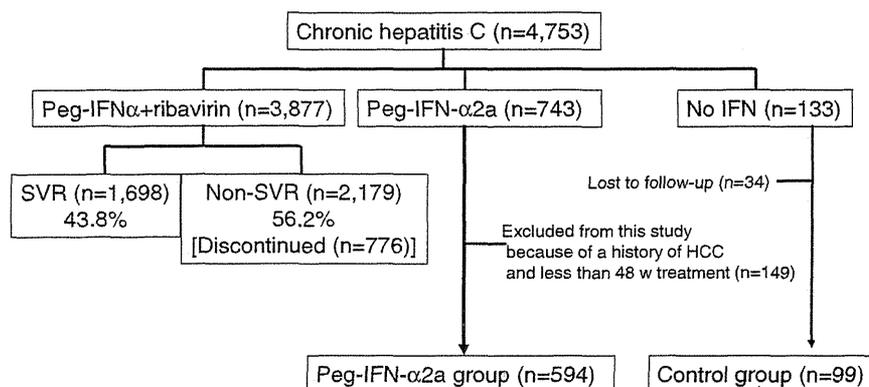Ehime University, Matsuyama, Japan

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65 years or older with advanced liver fibrosis, the dose of ribavirin is often reduced or the agent is discontinued, resulting in lower SVR rates in those with discontinuation of ribavirin. Establishing an effective treatment strategy for preventing the development of HCC is important for these high-risk patients.

Factors related to the development of HCC have been analyzed in patients who did not achieve an SVR even after IFN treatment; advanced fibrosis of the liver and high levels of serum alanine aminotransferase (ALT), and alpha-fetoprotein (AFP) are risk factors for HCC development [11, 12]. A randomized controlled trial was conducted in Western countries to determine whether combined peginterferon and ribavirin treatment with weekly administration of 90 μg peginterferon alpha-2a (PegIFNα-2a) could prevent HCC in non-responders. A 3.5-year follow up showed that administration of a maintenance dose of PegIFNα-2a did not reduce tumor incidence in these patients [13]. However, after 8.5 years of observation, the incidence of HCC was decreased among those in the PegIFNα-2a group with cirrhosis [14]. Meanwhile, Bruix et al. [15] reported that maintenance therapy with PegIFNα-2b did not prevent HCC in chronic hepatitis C patients with cirrhosis. In Japan, long-term low-dose administration of natural IFN has been reported to decrease the incidence of HCC [16]. In light of these conflicting results, investigations should be carried out in a large number of patients with chronic hepatitis C to resolve the question of whether IFN treatment prevents the development of HCC.

We carried out a multicenter retrospective cooperative study of patients with chronic hepatitis C to determine whether those treated with 90 μg PegIFNα-2a without ribavirin had a reduced incidence of HCC compared with those not treated with IFN.

Patients and methods

Study 1: analysis of risk factors for HCC in patients treated with long-term low-dose-PegIFNα-2a

In total, at 21 hepatitis centers throughout Japan, 743 patients with hepatitis C who had received 90 μg of PegIFNα-2a therapy weekly or bi-weekly for 1 year or more without having received the full dose (180 μg) since December 2003 were examined retrospectively for the development of HCC. The end of enrollment in this study was the end of December 2008 and the end of follow up was the end of December 2010. Patients with a history of HCC before the start of therapy and those with a therapy period of less than 48 weeks were excluded, leaving 594 patients who had undergone long-term administration of PegIFNα-2a for analysis. At the 21 centers involved in this

Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

study, 4,753 patients with chronic hepatitis C had been treated; Peg-IFN and ribavirin combination treatment had been administered to 3,877 patients, 743 patients had received Peg-IFN alone, and 133 patients had not agreed to receive IFN (a flow diagram of the enrollment of patients in this study is shown in Fig. 1). In the patients with Peg-IFN and ribavirin combination treatment, the SVR rate was 43.8 %; SVR was not achieved in 2,179 patients, and in 776 of these patients, the combination therapy was discontinued owing to adverse events or the patient's choice. Patients who failed to achieve an SVR were not included in this study, because the incidence of HCC is known to be reduced even in non-responders to IFN [17].

The backgrounds of the 594 patients studied are shown in Table 1. Findings from the liver biopsies of the patients were classified according to international standards [18]. Long-term PegIFNα-2a treatment is approved by the Japanese Medical Insurance system. Written informed consent was obtained from all patients prior to participation in this study. The study design was approved by the regional ethics committees of the 21 centers involved in this study, including the Musashino Red Cross Hospital, in accordance with the Helsinki Declaration. The 743 patients treated with PegIFNα-2a alone were not indicated for Peg-IFNα and ribavirin combination therapy because of anemia or heart disease. The 133 patients who did not agree to receive IFN served as the control group (see Fig. 1). A large proportion of the 594 study patients had advanced fibrosis of the liver and active inflammation. A dose of 90 μg PegIFNα-2a was administered to 512 and 82 patients weekly and biweekly, respectively, according to the patients' wishes. There were no significant differences between the weekly and biweekly groups in the patients' background data (data not shown).

The median duration of follow up in the PegIFNα-2a group was 1,273 days (range 228–2,768 days) and HCC was observed in 49 of the 594 patients (Table 1). Pretreatment and on-treatment factors associated with the development of HCC were analyzed by Student's *t*-test, the

Table 1 Background data of patients treated with PegIFNα-2a (*n* = 594)

	n = 594
Age (years)	61.7 ± 11.7
Sex (male/female)	258/336
BMI	23.2 ± 3.3
Genotype (1/2)	443/151
Diagnosis (ASC/CH/LC)	4/460/130
History of excess alcohol consumption (≥60 g/day; yes/no)	118/376
Fibrosis (F0, 1, 2/F3, 4)	443/151
Inflammatory activity (A0, 1/A2, 3)	469/125
Diabetes mellitus (no/yes)	499/95
LDL cholesterol (mg/dL)	94.2 ± 31.1
Fasting blood sugar (mg/dL)	106.3 ± 28.5
White blood cell count (/mm^3)	4,360 ± 1,470
Red blood cell count (×10^6/μL)	423.8 ± 56.4
Hemoglobin (g/dL)	13.3 ± 1.8
Platelet count (×10^3/μL)	137 ± 56
Albumin (g/dL)	4.0 ± 0.5
Total bilirubin (mg/dL)	0.8 ± 0.6
AST (IU/L)	65.8 ± 47.8
ALT (IU/L)	72.1 ± 68.0
Gamma-GTP (IU/L)	55.2 ± 51.3
Esophageal varices (no/yes)	344/31
Alpha fetoprotein (ng/L)	6.9 (4.2–13.8)
Once weekly or biweekly PegIFNα-2a	512:82
Baseline HCV RNA (KIU/mL)	1,024 (73–2,130)
Development of HCC (no/yes)	545/49

PegIFN pegylated interferon, *BMI* body mass index, *ASC* asymtomatic carrier, *CH* chronic hepatitis, *LC* liver cirrhosis, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GTP* guanosine triphosphate, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

Values are means ± SD, with ranges in parentheses

Mann–Whitney *U*-test, and the χ^2 test (Table 2). Independent factors for the development of HCC were assessed by multivariate analysis using logistic regression. The

incidence of HCC was analyzed according to the ALT, AFP, and hepatitis C virus (HCV) RNA levels 24 weeks after the start of PegIFNα-2a administration by using the Kaplan–Meier method. The risk of HCC was analyzed, using the Kaplan–Meier method, only in the non-responders with detectable HCV RNA during PegIFNα-2a administration by dividing them according to the ALT and AFP levels 24 weeks after the start of therapy. The incidence of HCC was compared between the patients with ALT levels of <41 IU/L and those with levels of ≥41 IU/L, and between patients with serum AFP levels of <10 ng/L and those with levels of ≥10 ng/mL at 24 weeks after starting treatment, because at most of the centers participating in the this study, the upper normal range of serum ALT is set at 40 IU/L, and the most significant difference in the incidence of HCC was observed between the PegIFNα-2a and control group with the cut-off serum ALT set at 41 IU/L and cutoff serum AFP set at 10 ng/mL, 24 weeks after starting treatment. The HCV RNA level was measured using the Amplicor Monitor method with a lower detection limit of 50 IU/L (Roche Diagnostics, Tokyo, Japan). A history of excess alcohol consumption was determined as >60 g alcohol per day in order to exclude alcoholic liver disease.

An asymptomatic carrier was defined as a patient with a serum ALT level within the normal range and minimal inflammation or fibrosis in the biopsied tissues of the liver. Chronic hepatitis was defined as mild-to-severe fibrosis of the liver according to liver biopsy [18]. The diagnosis of liver cirrhosis was based on the results of histological examination of the biopsied liver tissues.

Study 2: incidence of HCC in the PegIFNα-2a therapy and non-administration (control) groups in comparison with propensity-matched controls

Ninety-nine of the 133 chronic hepatitis C patients who had not received IFN were examined as controls; patients in this group received liver-protective agents such as glycyrrhizin or were untreated, and the group was observed for more than 1 year. None of the individuals in the control groups had received IFN alone or PegIFNα and ribavirin combination treatment. They were treated for a median of 1,395 days (range 75–6,556 days). Fifty-nine of these patients underwent liver biopsy before the treatment and were considered the control group for the propensity-matched study. For the propensity-matched study, 59 patients were selected from the PegIFNα-2a group according to their age, sex, platelet count, and total bilirubin levels, which had been identified as independent pretreatment risk factors for the development of HCC in Study 1. The rates of HCC were analyzed using the Kaplan–Meier method, and the risk of HCC was analyzed particularly in patients with advanced fibrosis of the liver (F3 and F4).

Table 2 Comparison of HCC and non-HCC patients with long-term PegIFNα-2a administration (n = 594)

	Patients with or without development of HCC		p value
	With HCC (n = 49)	Without HCC (n = 545)	
Pretreatment parameters			
Age (years)	63.8 ± 1.7	61.3 ± 0.5	<0.05
Sex (male/female)	32/17	226/319	<0.01
BMI	24.0 ± 0.5	23.1 ± 0.2	n.s.
Genotype (1/2)	47/6	397/148	n.s.
History of excess alcohol consumption (≥60 g/day; yes/no)	11/38	107/338	n.s.
Fibrosis (F0, 1, 2/F3, 4)	25/24	418/127	<0.001
Inflammatory activity (A0, 1/A2, 3)	7/42	462/83	<0.001
Diabetes mellitus (no/yes)	38/11	461/84	n.s.
LDL cholesterol (mg/dL)	88.2 ± 9.0	94.7 ± 2.6	n.s.
White blood cell count (/mm^3)	4,355 ± 210	4,360 ± 64	n.s.
Red blood cell count (×10^6/μL)	420.8 ± 8.1	424.1 ± 2.6	n.s.
Hemoglobin (g/dL)	13.6 ± 0.3	13.3 ± 0.1	n.s.
Platelet count (×10^3/μL)	106 ± 8	140 ± 2	<0.001
Albumin (g/dL)	3.8 ± 0.1	4.0 ± 0.1	<0.001
Total bilirubin (mg/dL)	1.2 ± 0.1	0.8 ± 0.1	<0.001
AST (IU/L)	78.1 ± 6.8	64.6 ± 2.1	n.s.
ALT (IU/L)	72.8 ± 9.7	72.0 ± 2.9	n.s.
Gamma-GTP (IU/L)	68.7 ± 7.5	53.9 ± 2.3	n.s.
Alpha fetoprotein (ng/L)	17.1 (4.4–36.8)	16.7 (4.1–23.1)	n.s.
Esophageal varices	29.0 % (9/31)	6.4 % (22/344)	<0.01
On-treatment parameters			
ALT (IU/L)	59.4 ± 5.7	44.6 ± 1.8	<0.05
Alpha fetoprotein (ng/L)	9.8 (4.6–17.4)	5.5 (3.7–11.1)	<0.01
HCV RNA level (KIU/mL)	236 (<0.5–2,210)	21 (<0.5–1,780)	<0.05

n.s. not significant

Statistical analysis

Categorical data were compared using the χ^2 test or Fisher's exact test. The distributions of continuous variables were analyzed using Student's t-test and the Mann–Whitney U-test for two groups. Multivariate analysis was

conducted using logistic regression. The cumulative incidence curve was determined using the Kaplan–Meier method and differences between groups were assessed by the log-rank test. For all methods, the level of significance was set at $p < 0.05$. Multivariate analysis of the risk of HCC was carried out using the Cox proportional hazard model. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 11.0 (SPSS, Chicago, IL, USA). In Study 1, age, sex, platelet count, and total bilirubin levels were identified as independent factors for the development of HCC; therefore, these factors were selected for the propensity-matched control study (Study 2) in which 59 patients from the PegIFNα-2a group were included.

Results

Study 1

We analyzed the factors involved in the development of HCC in patients who received 90 μg PegIFNα-2a weekly or biweekly for more than a year. The incidence of HCC did not differ significantly between the groups treated with PegIFNα-2a weekly and biweekly (34 of 512 vs. 15 of 82, respectively). As shown in Table 2, univariate analysis revealed statistically significant differences in the pretreatment parameters including age, sex, fibrosis of the liver, platelet count, albumin level, and total bilirubin, between patients who developed HCC and those who did not. Endoscopy was carried out in 375 patients, and esophageal varices were noted in 31 of them. The incidence of HCC was higher in patients with esophageal varices than in those without varices [29.0 % (9 of 31) vs. 6.4 % (22 of 344)]. Assessment of on-treatment factors by univariate analysis revealed statistically significant differences in serum ALT, AFP, and HCV RNA levels 24 weeks after the start of PegIFNα-2a maintenance treatment (Table 2).

Multivariate analysis including pretreatment parameters revealed that age, sex, fibrosis of the liver, platelet count, and total bilirubin were independent risk factors for HCC development (Table 3). Multivariate analysis including on-treatment parameters identified ALT levels of ≥41 IU/L and AFP levels of ≥10 ng/L 24 weeks after the start of the PegIFNα-2a therapy as independent risk factors for HCC development (Table 3).

The incidence of HCC was significantly lower in patients with ALT levels of ≤40 IU/L than in those with ALT levels of ≥41 IU/L 24 weeks after the start of observation (Fig. 2). The incidence of HCC was also significantly lower in patients with AFP concentrations of <10 ng/mL at 24 weeks after the start of observation than in those with AFP concentrations of

≥10 ng/mL (Fig. 3). The dose of PegIFNα-2a was reduced to 45 μg in 16 patients because of neutropenia and thrombocytopenia. In addition, PegIFNα-2a was discontinued in 18 patients because of adverse events, including depression (7 patients), interstitial pneumonitis (3 patients), thrombocytopenia (3 patients), neutropenia (1 patient), itching (1 patient), and ascites (3 patients). No statistically significant differences were found between the patients with reduced dosage or treatment interruption and those without treatment modifications with respect to overall survival, HCC incidence, ascites formation, variceal bleeding, hepatic encephalopathy, and 2-point increases in the Child-Pugh score. No patients underwent liver transplantation.

Table 3 Independent risk factors for HCC development in patients treated with 90 μg PegIFNα-2a weekly or bi-weekly, evaluated by multivariate analysis (logistic regression analysis)

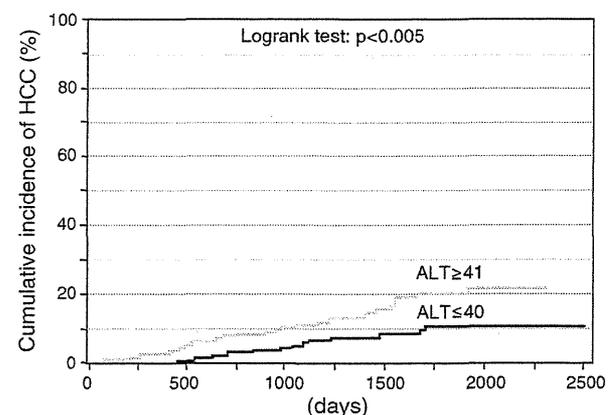|	Multivariate analysis		
	Odds ratio	95 % Confidence interval (CI)	p
Age (years) (every 5 years)	2.24	1.76–9.33	<0.005
Sex (male/female)	3.16	1.56–10.7	<0.005
Fibrosis (F3, 4/F0, 1, 2)	1.69	1.18–5.2	<0.01
Platelet count (<120 × 10³/μL vs. ≥120 × 10³/μL)	3.24	1.44–27.6	<0.01
Total bilirubin (mg/dL)	1.59	1.09–2.58	<0.05
ALT (at 24 weeks) (≥41 vs. <40 IU/L)	2.49	1.51–8.28	<0.05
AFP (at 24 weeks) (≥10 vs. <10 ng/L)	3.78	1.92–11.8	<0.01

Fig. 2 Comparison of HCC rates in patients administered with PegIFNα-2a ($n = 594$) with respect to alanine aminotransferase (ALT) levels 24 weeks after the start of therapy. Black line patients with ALT ≥41 IU/L in the first 24 weeks, gray line patients with ALT ≤40 IU/L in the first 24 weeks
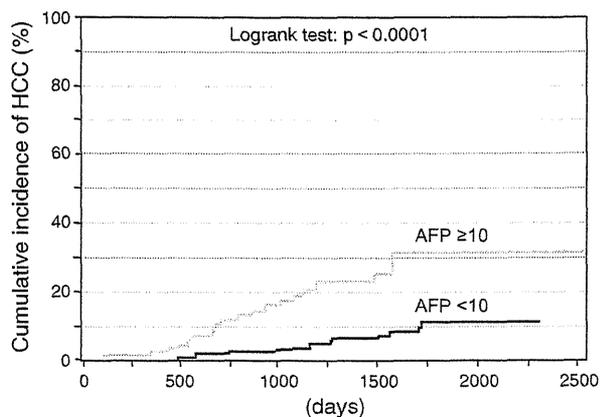
Fig. 3 Comparison of HCC rates in patients administered PegIFNα-2a ($n = 594$) with respect to alpha-fetoprotein (*AFP*) levels in the first 24 weeks after the start of therapy. *Black line* patients with AFP ≥10 ng/mL at 24 weeks, *gray line* patients with AFP <10 ng/mL at 24 weeks

Study 2

We compared the incidence of HCC between 59 patients in the control group and the same number of patients in the PegIFNα-2a group using the matched-pair test. The backgrounds of the patients are shown in Table 4. The PegIFNα-2a group had higher rates of advanced fibrosis (F3 and F4) and active inflammation (A2 and A3). No other differences were found between the two groups, except for the white blood cell count (Table 4).

Development of HCC was observed in 2 patients in the PegIFNα-2a group and 8 in the control group. The incidence of HCC was compared between the two groups, using the Kaplan–Meier method. The incidence of HCC in the PegIFNα-2a group was significantly lower than that in the control group (log-rank test, $p = 0.0187$; Fig. 4). Among the patients with advanced fibrosis of the liver (F3 and F4), those in the PegIFNα-2a group had a lower incidence of HCC than those in the control group. The independent risk factors for the development of HCC were analyzed using the stepwise Cox proportional hazard model. Only PegIFNα-2a administration and age were identified as independent risk factors for the development of HCC (Table 5).

Discussion

The number of HCC cases resulting from HCV infection continues to increase worldwide [19]. To date, IFN therapy is the most effective preventive measure against HCC in patients with chronic hepatitis C; furthermore, the

Table 4 Backgrounds of the patients in the propensity-matched control study (PegIFNα-2a group, $n = 59$; control group, $n = 59$)

	PegIFNα-2a group ($n = 59$)	Control group ($n = 59$)	p value
Age (years)	60.5 ± 13.0	63.3 ± 10.5	n.s.
Gender (male/female)	24/35	25/34	n.s.
BMI	22.9 ± 3.6	22.9 ± 3.4	n.s.
Genotype (1/2)	49/10	46/13	n.s.
History of excess alcohol consumption (60 g/day; yes/no)	10/49	4/55	n.s.
Fibrosis (F0, 1, 2/F3, 4)	37/22	43/16	<0.05
Development of HCC (F0–2/F3, 4)	1/1	1/7	n.s.
Inflammatory activity (A0,1/A2, 3)	19/40	30/29	<0.05
Diabetes mellitus (no/yes)	57/2	56/3	n.s.
LDL cholesterol (mg/dL)	95.3 ± 23.8	117.0 ± 4.2	n.s.
White blood cell count (/mm^3)	4,260 ± 1,239	5,193 ± 2,078	<0.05
Red blood cell count (×10^{-4}/μL)	430 ± 57.8	441 ± 44.9	n.s.
Hemoglobin (g/dL)	13.6 ± 1.5	13.6 ± 1.9	n.s.
Platelet count (×10^{-3}/μL)	14.5 ± 5.7	15.8 ± 5.7	n.s.
Albumin (g/dL)	4.1 ± 0.5	4.1 ± 0.4	n.s.
Total bilirubin (mg/dL)	0.7 ± 0.5	0.9 ± 0.7	n.s.
AST (IU/L)	58.3 ± 47.7	49.7 ± 26.6	n.s.
ALT (IU/L)	63.6 ± 68.7	58.0 ± 39.2	n.s.
Gamma-GTP (IU/L)	78.3 ± 81.3	55.3 ± 75.1	n.s.
Baseline alpha-fetoprotein (AFP) (ng/L)	7.2 (4.3–14.2)	7.7 (3.9–13.8)	n.s.
Baseline HCV RNA level (KIU/mL)	1,230 (24–3,870)	1,024 (38–3,110)	n.s.

incidence of HCC is reduced in patients who achieve an SVR to IFN [6–9] Therefore, achieving an SVR is the most effective approach for reducing the risk of developing HCC. In Japan, the incidence of HCC is elevated in older patients with hepatitis C. Corroborating this finding, the results of a Japanese study show a higher risk of HCC in patients aged 65 years and more [10]. Therefore, prevention of HCC in aged patients is an important challenge.

In the present multicenter, cooperative, retrospective study conducted in Japan, the incidence of HCC was reduced in patients who received 90 μg PegIFNα-2a weekly or biweekly and had AFP values of <10 ng/mL and ALT values of ≤40 IU/L 24 weeks after the start of the treatment. The results of the matched case–control study of the PegIFNα-2a group and the non-IFN control group show that the incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group, especially in patients with advanced fibrosis of the liver (F3 and F4). However, there could have been a selection bias between
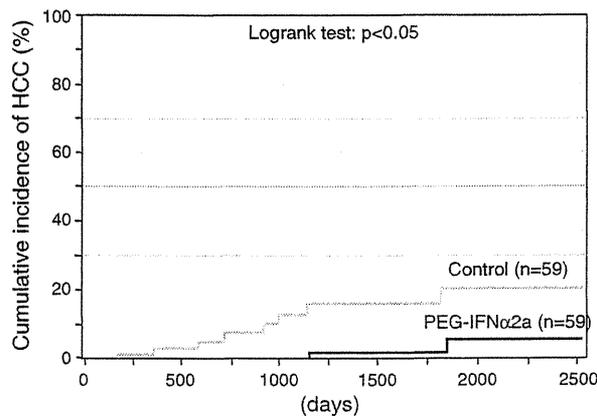
Fig. 4 Comparison of HCC rates between the long-term PegIFNα-2a administration group ($n = 59$) and non-administration group ($n = 59$) in the propensity-matched control study (Kaplan–Meier log-rank test, $p = 0.019$)

Table 5 Risk factors for HCC in the propensity-matched control study (Cox proportional hazard model)

Variables	Risk ratio	95 % CI	p value
PegIFN versus control	0.17	0.03–0.75	<0.05
Age (every 1 year)	1.12	1.02–1.25	<0.05
Fibrosis (F3, 4 vs. F0, 1, 2)	1.70	0.75–4.16	n.s.
Platelet count (every $10 \times 10^3/\mu L$)	0.89	0.73–1.09	n.s.
Albumin (every 1.0 g/dL)	0.80	0.10–6.68	n.s.
On-treatment AFP (<10 vs. ≥10 ng/L)	4.07	0.59–40.12	n.s.

the PegIFNα-2a group and the control group (patients who did not agree to receive IFN treatment), because this was a retrospective and non-randomized study. However, concordant with the findings of the HALT-C study [14], the present results show that PegIFNα-2a inhibits the development of HCC in patients with advanced fibrosis of the liver.

Recent studies show that polymorphisms in the host *IL28B* gene are important factors in the response to PegIFNα and ribavirin combination therapy [20, 21]. However, the mechanism of IL28B involvement in the response to PegIFNα and ribavirin has not been elucidated completely. A recent report has shown that IL28B is a significant factor in the development of HCC as well as in the response to IFN therapy [22]. Further studies are warranted to analyze the relationship between IL28B and inhibition of the development of HCC by PegIFNα in chronic hepatitis C.

Risk factors for the development of HCC have been discussed previously. Increased intrahepatic fat is involved in the development of HCC in chronic hepatitis C patients [23, 24]. In addition, diabetes-associated fat disorder [25,

26], hepatic iron overload [27], advanced fibrosis, older age, and fatty deposits in the liver are risk factors for HCC development [4]. Therefore, it is important to establish strategies to mitigate these risk factors to prevent the development of HCC and thus improve the outcomes of hepatitis C patients.

IFN therapy after HCC treatment is reported to inhibit the recurrence of tumors [28, 29], and a meta-analysis has revealed a trend toward inhibition of the recurrence of HCC [30, 31]. The prevention of HCC is an important issue that needs to be addressed to improve the survival of chronic hepatitis C patients. The findings of the present study and the HALT-C trial [14] indicate the effectiveness of long-term administration of maintenance IFN for preventing the development of HCC in chronic hepatitis C patients without an SVR. Improvement in ALT levels is also known to be an important predictor for the prevention of HCC [32]. A low AFP value during IFN administration is also recognized as a significant indicator of a lower risk of HCC [33, 34]. Recently, Osaki et al. [35] reported that a decrease of serum AFP during treatment with IFN was associated with a reduced incidence of HCC. Taking these findings and our own together, we conclude that maintenance administration of low-dose PegIFNα-2a weekly or biweekly to non-SVR patients with chronic hepatitis C decreases the incidence of HCC, especially in patients whose serum ALT and AFP levels are within the normal range 24 weeks after the start of treatment. The preventive effects of IFN against the development of HCC without elimination of the virus may be associated with its anti-carcinogenic effects [16, 35]; however, the precise mechanism should be investigated.

The limitations of the present study are that it is retrospective and multicentric; therefore, potentially there may have been a selection bias. However, the reduction of the rate of development of HCC by maintenance administration of PegIFNα-2a in the patients in whom serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment may be attributable to the anticarcinogenic effects of IFN without elimination of the virus.

Conclusion

The incidence of HCC was lower in non-SVR patients with chronic hepatitis C who were administered with maintenance low-dose PegIFNα-2a; especially in those whose serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment.

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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

including electroencephalography (EEG), cerebral evoked potential, p300 event-related potential, psychometric hepatic encephalopathy score (PHES) and critical flicker test[7-15] 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 PET[16] 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.
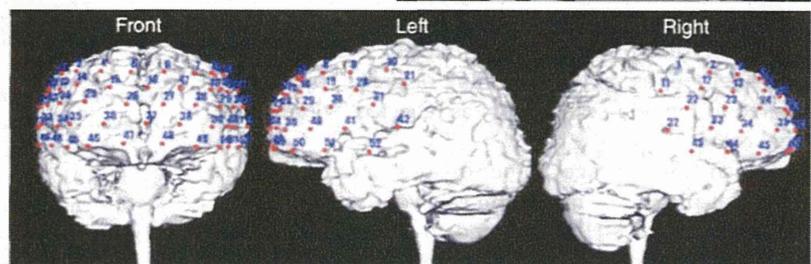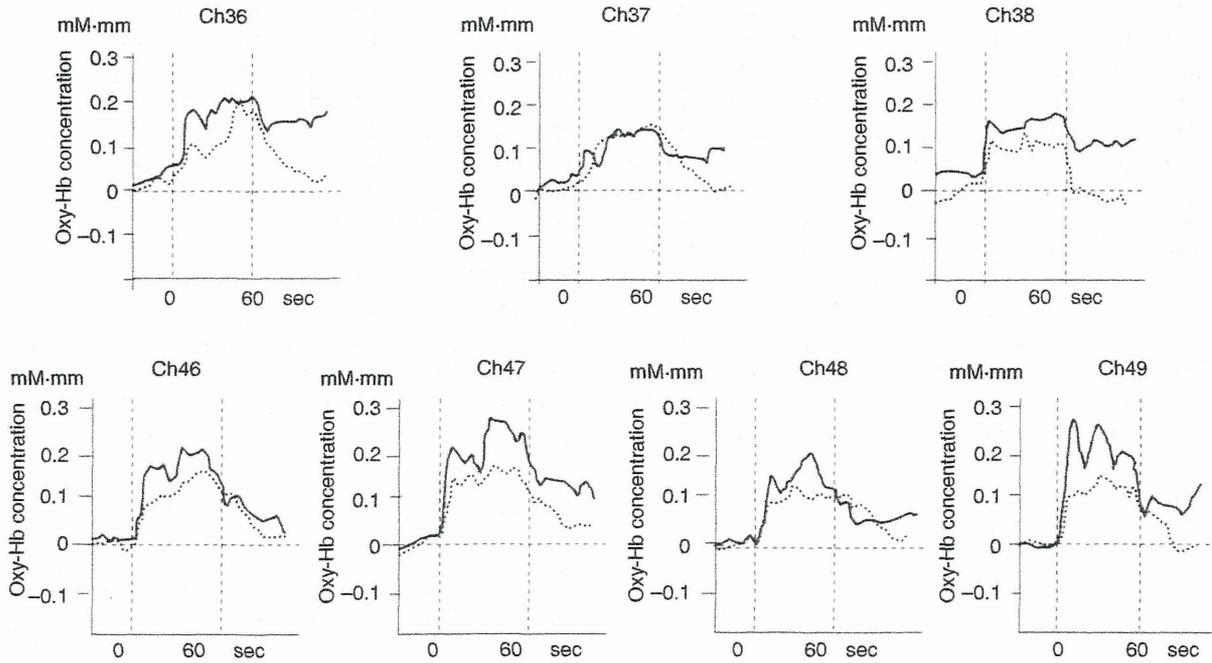
Figure 2 Time-dependent changes in oxygenated hemoglobin (oxy-Hb) concentration in response to tasks. The average waveforms of time-dependent changes in oxy-Hb concentration in representative channels (Ch) are shown. The solid and broken line represents non-minimal hepatic encephalopathy (MHE) and MHE 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
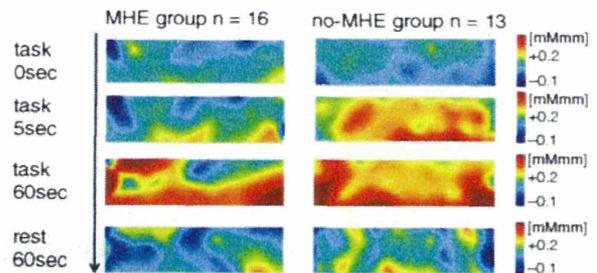

Figure 3 Topographic image showing cumulative increase in oxygenated hemoglobin (oxy-Hb) concentration. Increase in oxy-Hb concentration is shown by deepening of the red shading. The concentration of oxy-Hb is small in the minimal hepatic encephalopathy (MHE) group, as reflected by the blue or green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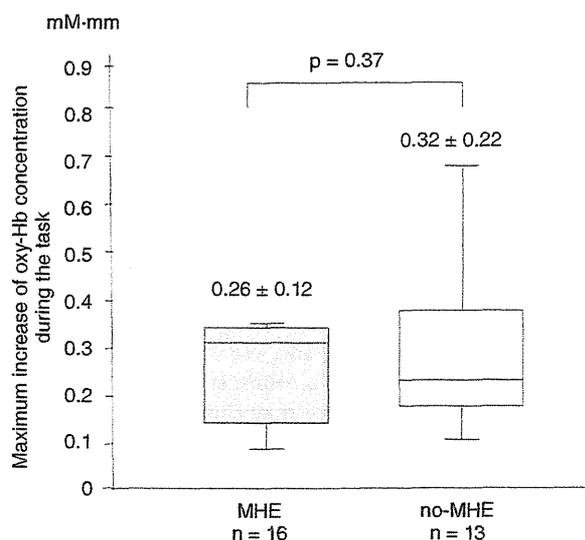
mM·mm

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.

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

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
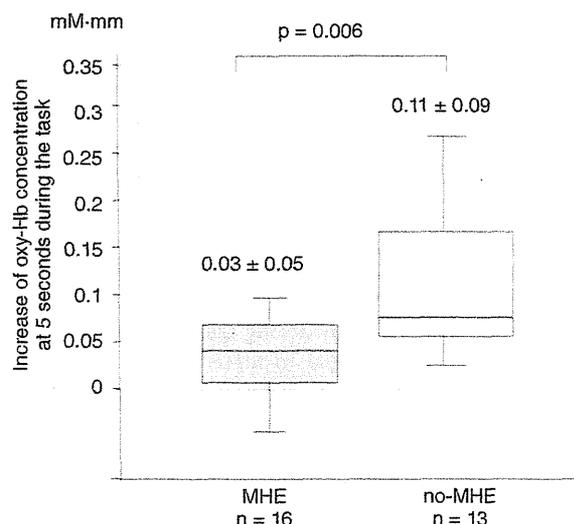
mM·mm

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 tests[37] 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 OHE[16]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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Noninvasive estimation of fibrosis progression overtime using the FIB-4 index in chronic hepatitis C

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SUMMARY. The FIB-4 index is a simple formula to predict liver fibrosis based on the standard biochemical values (AST, ALT and platelet count) and age. We here investigated the utility of the index for noninvasive prediction of progression in liver fibrosis. The time-course alteration in the liver fibrosis stage between paired liver biopsies and the FIB-4 index was examined in 314 patients with chronic hepatitis C. The average interval between liver biopsies was 4.9 years. The cases that showed a time-course improvement in the fibrosis stage exhibited a decrease in the FIB-4 index, and those that showed deterioration in the fibrosis stage exhibited an increase in the FIB-4 index with a significant correlation ($P < 0.001$). Increase in the ΔFIB-4 index per year was an independent predictive factor for the progression in

liver fibrosis with an odds ratio of 3.90 ($P = 0.03$). The area under the receiver operating characteristic curve of the ΔFIB-4 index/year for the prediction of advancement to cirrhosis was 0.910. Using a cut-off value of the ΔFIB-4 index/year <0.4 or ≥0.4, the cumulative incidence of fibrosis progression to cirrhosis at 5 and 10 years was 34% and 59%, respectively in patients with the ΔFIB-4 index/year ≥0.4, whereas it was 0% and 3% in those with the ΔFIB-4 index/year <0.4 ($P < 0.001$). In conclusion, measurement of the time-course changes in the FIB-4 index is useful for the noninvasive and real-time estimation of the progression in liver fibrosis.

Keywords: FIB-4, fibrosis, HCV, noninvasive.

INTRODUCTION

Advanced stage of liver fibrosis in chronic hepatitis C is associated with failure of interferon therapy or development of major concomitant disease such as variceal bleeding, liver failure and hepatocellular carcinoma [1–3]. Therefore, evaluation of the stage of liver fibrosis is essential in clinical practice. Liver biopsy is the gold standard for diagnosis of liver fibrosis [4,5], but inaccuracy in evaluation of fibrosis because of sampling errors [6–8] or by the inter-observer variation has been reported [9]. Real-time assessment of liver fibrosis may be clinically useful, but the invasiveness of liver biopsy precludes repeated examinations.

A variety of noninvasive methods to diagnose liver fibrosis have been proposed. Recently, transient elastography [10–13] and real-time tissue elastography [14] using ultrasonography

have been developed, but these modalities are not widely available. For blood tests, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio [15], the AST/platelet ratio index (APRI) [16,17] and the Fibrotest [18,19] have been reported to be useful. The FIB-4 index is another prediction value of liver fibrosis in chronic hepatitis C based on the standard biochemical values and age. The FIB-4 index has been reported to be markedly useful for the prediction of advanced liver fibrosis [20,21]. Given its noninvasiveness and simplicity, the FIB-4 index has the advantage of an easy follow-up of the time-course changes by repeated measurements.

In the present study, we investigated the utility of the real-time assessment of the FIB-4 index for the prediction of time-course progression in liver fibrosis.

PATIENTS AND METHODS

Patients

A total of 421 patients with chronic hepatitis C who had repeated liver biopsies between 1991 and 2010 at the Musashino Red Cross hospital were consecutively investigated. All patients received interferon therapy after the first biopsy and had nonsustained virological response. A second

biopsy was performed at least 6 months after the completion of interferon therapy. Exclusion criteria were as follows: (i) co-infection with HBV or HIV ($n = 1$), (ii) alcohol abuse (intake of alcohol equivalent to pure alcohol 40 g/day or more) ($n = 8$), (iii) the presence of nonalcoholic steatohepatitis ($n = 14$), (iv) the presence of hepatocellular carcinoma ($n = 15$), (v) interval between paired biopsies was <1.5 years ($n = 41$) and (vi) length of biopsy sample <15 mm ($n = 28$). The demographic characteristics of the 314 patients enrolled are shown in Table 1.

Assessment of liver fibrosis stage

Liver biopsy was carried out under laparoscopic or ultrasonographic guidance. A sample 15 mm or larger was collected and evaluated. The fibrosis stage was categorized according to the METAVIR score: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Two pathologists examined all samples and determined the fibrosis stage. When staging was inconsistent between the two pathologists, an appropriate stage was determined by discussion between the two.

Calculation of FIB-4 index

The FIB-4 index at the time of each liver biopsy was calculated based on the blood test results within 1 month before

Table 1 Clinical background of patients

	First biopsy	Second biopsy
Age (years)	53.7 ± 9.8	58.7 ± 9.4
Gender (male/female)	149/165	
AST (IU/L)	64.5 ± 36.7	58.5 ± 37.7
ALT (IU/L)	87.7 ± 58.9	69.9 ± 53.9
Platelet counts (× 10^9/L)	165 ± 48	159 ± 48
Histological findings		
Activity: 0/1/2/3	38/143/117/16	10/147/131/26
Fibrosis: 0–1/2/3/4	139/107/61/7	134/101/63/16
Interval of between biopsies (years)	4.9 ± 2.9	–

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

liver biopsy according to the following formula: The FIB-4 index = (age [years] × AST [IU/L])/(platelet count [10^9/L] × (ALT [IU/L])$^{1/2}$). Change in the FIB-4 index per year (ΔFIB-4 index/year) was calculated by the following formula: ΔFIB-4 index/year = (the FIB-4 index at the second liver biopsy − the FIB-4 index at the first liver biopsy)/ interval between paired biopsies (years). Change in AST, ALT, platelet counts per year (ΔAST/year, ΔALT/year, ΔPlatelet counts/year) and the degree of changes in the fibrosis stage per year were calculated similarly.

Statistical analysis

The SPSS software package 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Categorical data were analysed using Fisher's exact test. Continuous variables were compared with Student's t-test. Factors associated with the progression in liver fibrosis were analysed by multivariate logistic regression analysis. Association between progression in fibrosis stage and changes in the FIB-4 was analysed by Spearman's rank correlation test. Kaplan–Meier method and log-rank test were used to analyse time to occurrence of fibrosis progression to cirrhosis. A P-value of < 0.05 was considered statistically significant.

RESULTS

Changes in liver fibrosis stage overtime

The clinical backgrounds of patients at the first and second biopsies are shown in Table 1. The average interval was 4.9 years between the two liver biopsies. The fibrosis stage progressed over time in 23%, regressed in 17% and remained unchanged in 60%. Changes of fibrosis stage stratified by the fibrosis stage at the first liver biopsy are shown in Table 2.

Comparison of FIB-4 index and liver fibrosis stage

For the prediction of advanced liver fibrosis (F3–4), a FIB-4 index <1.45 had a negative predictive value of 97%, whereas a FIB-4 > 3.25 had a positive predictive value of 49% at first biopsy. Similarly, a FIB-4 < 1.45 had a negative predictive value of 98%, and a FIB-4 > 3.25 had a positive predictive value of 54% at second biopsy (Fig. 1).

Table 2 Changes of fibrosis stage over time

	Fibrosis stage at second biopsy				
Fibrosis stage at first biopsy	F0–1 (%)	F2 (%)	F3 (%)	F4 (%)	Total
F0–1	98 (71)	33 (24)	8 (5)	–	139
F2	33 (31)	50 (47)	21 (20)	3 (2)	107
F3	3 (5)	18 (29)	33 (55)	7 (11)	61
F4	–	–	1 (14)	6 (86)	7