Table III. Characterization of seven BLD patients, who developed HCC.

Case no.	1	2	3	4	5	6	7
Age	58	70	63	70	53	60	59
Gender	M	F	F	F	M	M	F
CH/LC	LC	CH	LC	LC	LC	LC	CH
HCV/NBNC	HCV	HCV	HCV	NBNC	HCV	HCV	HCV
AFP (ng/ml)	5.3	8.3	10.7	10.9	27.8	28.5	32.0
c-AFP-L3%	ND	ND	29.5	4.9	15.9	12.2	3.4
hs-AFP-L3%	6.0	7.0	32.6	8.4	12.2	9.6	3.7
ALT (IU/l)	31	48	23	39	41	65	116
Months until HCC detection	13	31	5	13	18	8	31

F, female; M, male; ND, not detectable.

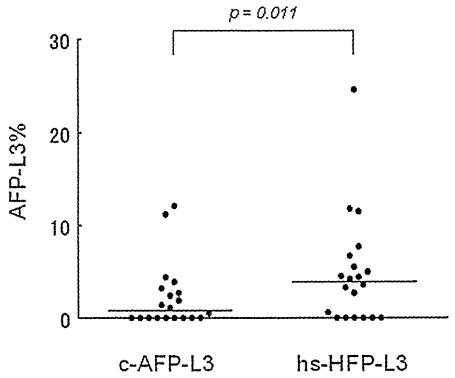

Figure 5. Patients with well-differentiated HCC showed an increase in hs-AFP-L3%. hs-AFP-L3% (HS) was significantly higher than c-AFP-L3% in patients with well-differentiated HCC; this was confirmed by histological examination.

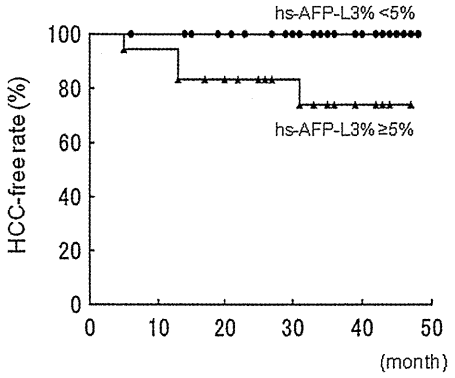

Figure 6. No patients with both serum AFP <20 ng/ml and hs-AFP-L3% <5% developed HCC. Patients with BLD (n=74) were periodically followed by US, CT, or MRI during the follow-up period (median, 35 months; range, 5-48 months). In cases of BLD with AFP <20 ng/ml (n=59), HCC was newly detected in 4 patients with hs-AFP-L3% ≥5%. The HCC-free rate in patients with hs-AFP-L3% ≥5% (▲) was significantly higher than in patients with hs-AFP-L3% <5% (●) (log-rank test and Wilcoxon test; p=0.0012 and p=0.0017, respectively). Importantly, no patients with hs-AFP-L3% <5% developed HCC.

at a cut-off level of 5% were 44.6 and 71.2%, whereas those of c-AFP-L3% were 12.5 and 98.3%, respectively. These results suggest that hs-AFP-L3% is useful for early detection of HCC, even when serum AFP is <20 ng/ml.

Serum hs-AFP-L3% increases in patients with well-differentiated HCC. Most HCC, initially present as well-differentiated HCC, develops in patients with chronic liver disease. Therefore, we evaluated c-AFP-L3% and hs-AFP-L3% in 20 patients with well-differentiated HCC, which was confirmed by histological examination. Fifteen patients (75.0%) exhibited small HCCs (<20 mm), and 9 (45.0%) suffered from liver cirrhosis. Serum AFP was 14.2±12.4 ng/ml (1.4-54.1), and 18 patients (90%) exhibited serum AFP levels <20 ng/ml. hs-AFP-L3% was measurable in 14 patients (70%), while 11 patients (55%) exhibited detectable levels of c-AFP-L3% (Fig. 5). Consequently, hs-AFP-L3% was significantly higher than c-AFP-L3% [4.81±5.91 (0.6-24.6) vs. 2.24±3.53% (0.5-12.1), p=0.011]. These results support the possible utility of hs-AFP-L3% for detection of early-stage HCC.

hs-AFP-L3% increases prior to detection of HCC in patients with BLD. Seven of 74 patients with BLD developed HCC during the follow-up period (median, 35 months; range, 5-48) (Table III). Five patients suffered from liver cirrhosis, and 6 exhibited hepatitis C virus infection. Two of the patients with chronic hepatitis required a longer period (31 months) for appearance of HCC than did the 5 patients with cirrhosis (5-18 months). Five patients exhibited measurable c-AFP-L3%, and an increase in c-AFP-L3% (≥5%) was observed in 3 patients. In contrast, hs-AFP-L3% was measurable in all 7 patients prior to detection of HCC, and 6 patients (85.7%) exhibited hs-AFP-L3% ≥5%. In 59 BLD patients with serum AFP <20 ng/ml, 4 patients developed HCC (Table III). An increase in c-AFP-L3% (≥5%) was observed only in 1 patient, who developed HCC during the follow-up period, whereas the other three patients exhibited undetectable levels or <5% of c-AFP-L3%. Conversely, all 4 patients with serum AFP <20 ng/ml exhibited an increase in hs-AFP-L3% (≥5%) prior to detection of HCC.

Next, we analyzed the HCC-free rate in BLD patients with serum AFP <20 ng/ml during the follow-up period (Fig. 6). The HCC-free rate in patients with hs-AFP-L3% ≥5% was

significantly higher than those with hs-AFP-L3% <5%. Of importance, HCC was not detected in BLD patients with both serum AFP <20 ng/ml and hs-AFP-L3% <5%, whereas 3 out of 58 patients with both serum AFP <20 ng/ml and <5% of c-AFP-L3% developed HCC. These results suggest that an increased hs-AFP-L3% allows prediction of HCC development; measurement of hs-AFP-L3% is useful for selecting BLD patients with higher risk of HCC.

Discussion

Most HCC occurs in patients with chronic liver diseases, especially cirrhosis. Therefore, periodical measurement of tumor markers for HCC, such as AFP and DCP, is recommended in patients who are at high risk for HCC. However, recent advances in diagnostic imaging techniques, including US, CT and MRI, facilitate the detection of small and early-stage HCC (19-21), resulting in an increase in the number of HCC patients diagnosed without an observed increase in serum AFP. Indeed, the 18th survey and follow-up study of primary liver cancer in Japan has reported that most patients with HCC exhibited low levels of serum AFP, <15 ng/ml. Additionally, although AFP-L3% status is known to be a specific marker for HCC, measurement of c-AFP-L3% has not always been reliable in patients with AFP <20 ng/ml.

In this study, we investigated the clinical utility of hs-AFP-L3%, which was measured by a newly developed and highly sensitive method, μ-TAS, in patients with BLD and HCC. Here, we showed that although most HCC patients with stage I cancer did not exhibit an increase in serum AFP levels (≥20 ng/ml), hs-AFP-L3% was measurable in ~70% of the patients, and was significantly increased in comparison with c-AFP-L3% (Fig. 2). Since hs-AFP-L3% is reliable even when serum AFP is <20 ng/ml, it is possible to set the cut-off value for hs-AFP-L3% at 5-7% (18,22,23). We show here that at a cut-off level of 5%, the sensitivity and specificity of hs-AFP-L3% were 44.6 and 71.2%, respectively, in HCC patients with serum AFP <20 ng/ml (Fig. 4). Recent investigations have shown that diagnostic sensitivity of hs-AFP-L3% at a cut-off level of 5 or 7% was 41.5 or 41.1%, respectively, in HCC patients with serum AFP <20 ng/ml (18,22). Therefore, our findings in this study support the specificity of hs-AFP-L3% in patients with serum AFP <20 ng/ml, as previously reported.

The sensitivity of c-AFP-L3% is relatively low (22.2-38.6%) in early-stage HCCs <20 mm in diameter (24,25). In this study, although the sensitivity of c-AFP-L3% was <20% in patients with HCC at stage I, hs-AFP-L3% was significantly higher than c-AFP-L3% in patients with solitary or small (<20 mm) HCC or with stage I HCC (Figs. 2 and 3); consequently, ~50% of HCC patients at stage I exhibited hs-AFP-L3% ≥5%. Additionally, in patients with well-differentiated HCC, hs-AFP-L3% was also significantly higher than c-AFP-L3%. Conversely, patients with stage III or IV HCC (multiple or larger (≥20 mm) tumors) exhibited an increase in both hs- and c-AFP-L3%, with no statistical difference. HCC initially develops as well-differentiated HCC, and then progresses to moderately- to poorly-differentiated HCC via a process of dedifferentiation. Thus, an increase in hs-AFP-L3% in patients with well-differentiated HCC and early-stage HCC supports the conclusion that measurement of hs-AFP-L3% is useful for early detection of HCC.

HCC often develops in patients with chronic infection of hepatitis B or C virus; especially in patients with chronic HCV infection, the annual incidence of HCC increases as a function of the stage of liver fibrosis, from 0.5% at stages F0 to F1 to 7.9% at stage F4 (cirrhosis) (26). Recently, Tateyama *et al* demonstrated that elevated AFP levels are a risk factor for the development of HCC in patients with HCV infection; the 10-year cumulative incidence rates of HCC in the patients with AFP levels of <6, 6-20 and ≥20 ng/ml at entry were 6.0, 24.6 and 47.3%, respectively, and that AFP levels may be used as a non-invasive and predictive marker in place of stage of fibrosis (27). In this study, all 7 BLD patients who developed HCC during the follow-up period exhibited measurable hs-AFP-L3% prior to detection of HCC, and 6 patients exhibited hs-AFP-L3% ≥5%. Of particular note, even when serum AFP levels increased to up to 20 ng/ml, HCC was not detected in patients with hs-AFP-L3% <5% (Fig. 6).

Although prolonged observation will be required in order to clarify whether hs-AFP-L3% is useful for prediction of HCC, the findings presented here indicated that hs-AFP-L3% is useful for early detection of HCC in BLD patients even with serum AFP <20 ng/ml, and also that an increase in hs-AFP-L3% prior to detection of HCC by various advanced imaging modalities may contribute to more precisely identifying BLD patients with a higher risk of HCC.

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REVIEW

Changing etiologies and outcomes of acute liver failure: A perspective from Japan

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Key words
acute liver failure, fulminant hepatitis, Japan, liver transplantation, viral hepatitis.

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Abstract

Acute liver failure in Japan usually consists of fulminant hepatitis (FH) due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury. The annual incidence of FH was estimated at 429 cases in 2004. FH is classified into acute or subacute type, and the prognosis of the latter is poor. Hepatitis B virus (HBV) is the most frequently identifiable agent that causes FH in Japan. Transient HBV infection is more prevalent in the acute than subacute type, whereas the frequency of HBV carriers is greater in the subacute type. FH due to HBV reactivation from resolved hepatitis B has been increasingly observed in patients with malignant lymphoma treated with rituximab and corticosteroid combination therapy. The prognosis is poor in HBV carriers with acute exacerbation, especially in patients with HBV reactivation from resolved hepatitis B. Despite careful investigation, the etiology is still unknown in 16% and 39% of the acute and subacute type of FH, respectively. Autoimmune hepatitis and drug-allergy-induced liver injury are found in 7% and 10%, respectively, and are more frequently observed in the subacute type of FH. Living donor liver transplantation is now the standard care for individuals with poor prognosis. Artificial liver support with plasmapheresis and hemodiafiltration plays a central role while waiting for a donor liver or for the native liver to regenerate. Further research is necessary to identify the causes of unknown origin. In addition, to improve the prognosis of FH, it is necessary to establish treatment modalities that are effective for liver regeneration.

Introduction

Acute liver failure is a clinical syndrome that is marked by the sudden loss of hepatic function in a person without chronic liver disease. The causes of acute hepatic failure are varied and differ geographically. In Japan, fulminant hepatitis (FH) is defined as having hepatitis, when grade II or worse hepatic encephalopathy develops within 8 weeks of the onset of the disease symptoms, with a prothrombin time of ≤40%. FH due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury is the main cause of acute liver failure in Japan. In contrast, other causes, including paracetamol overdose, other drug toxicity, metabolic liver disease, and acute fatty liver of pregnancy, are infrequent.

The Intractable Hepato-biliary Diseases Study Group of Japan annually performs a nationwide survey of patients with FH and late-onset hepatic failure (LOHF). This paper summarizes the results of the survey and addresses the characteristics and trends of acute liver failure in Japan.

Definition and methods

In 1969, Trey and Davidson defined acute liver failure as the occurrence of encephalopathy within 8 weeks of the onset of acute hepatic illness, and in the absence of pre-existing liver disease.[1] Thereafter, patients with hepatic encephalopathy that develops between 8 and 24 weeks after disease onset are defined as having LOHF.[2] Other definitions based on the duration of illness have subsequently been used to classify patients:[2–4] hyperacute, <7 days; acute, 7–28 days; and subacute, 28 days to 6 months. In Japan, patients with FH are classified into acute or subacute type, in which the encephalopathy occurs within 10 days, or later than 11 days, respectively, of the onset of disease symptoms.[5,6] Based on the previous survey, patients with FH who present within 10 days of symptom onset have significantly higher survival rates than similar patients who present with encephalopathy at 10 days after symptom onset.[7,8]

The survey was performed in hospital with active members of the Japan Society of Hepatology and the Japanese Society of Gastroenterology. Patients who meet the diagnostic criteria for FH and LOHF were entered into the survey (Table 1). Besides the diagnostic criteria, patients under 1 year of age and those with alcoholic hepatitis were excluded from the analysis.

The etiology of acute liver failure is classified into five categories: viral infection, autoimmune hepatitis, drug-allergy-induced liver injury, unknown, and indeterminate (Table 2). Patients with viral infection consist of those with hepatitis A virus (HAV),

Table 1 Diagnostic criteria for fulminant hepatitis in Japan according to the Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labour (2003)

Fulminant hepatitis (FH) is defined as hepatitis in which hepatic encephalopathy of coma grade greater than II develops in the patients within 8 weeks after the onset of disease symptoms with highly deranged liver functions showing prothrombin time less than 40% of the standardized values.

FH is classified into two subtypes: the acute typeand subacute type in which the encephalopothy occurs within 10 days and later than 11 days, respecitvely.

Note 1: Patients with chronic liver diseases are excluded from FH, but asymptomatic HBV carriers who develop acute exacerbation are diagnosed with FH.

Note 2: Acute liver failure accompanying no liver inflammation, such as drug or chemical intoxication, microcirculatory disturbance, acute fatty liver of pregnancy, and Reye's syndrome are excluded from FH.

Note 3: The grading of hepatic encephalopathy is based on the criteria from the Inuyama Symposium in 1972.

Note 4: The etiology of FH is based on the criteria from the Intractable Liver Diseases Study Group of Japan in 2002 (Table 2).

Note 5: Patients with no hepatic encephalopathy or encephalopathy of coma grade I, even showing prothrombin time <40% of the standardized values, are diagnosed with severe acute hepatitis. Patients in whom encephalopathy develops between 8 and 24 weeks after disease onset, with prothrombin time <40% of the standardized values, are diagnosed with late onset hepatic failure (LOHF). Both are related to FH, but are regarded differently from FH.

hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV) and other viruses. Patients with HBV infection are further classified into transient infection and acute exacerbation of HBV carrier status. In 2002, the criteria were modified to define FH due to autoimmune hepatitis and HEV, and the etiology of patients between 1998 and 2001 was re-assessed according to these new criteria.

Demographic features

From 1998 to 2006, 934 patients were enrolled in the surveillance.[9] Among these patients, 856 (432, acute type and 424, subacute type) were classified as having FH and 78 as having LOHF (Table 3). Based on the nationwide epidemiology surveillance, the annual incidence of FH was estimated at 3700 cases in 1972, 1050 cases in 1995, and 429 cases in 2004.[10] About 30% of patients with severe acute hepatitis were presumed to develop hepatic encephalopathy of coma grade II or more.[11]

The male : female ratio was higher for the acute type than subacute type and LOHF. The age of the patients was significantly higher for the subacute type and LOHF than for the acute type. The frequency of HBV carriers was highest for the subacute type and lowest for LOHF. There were many patients with complications, such as metabolic syndrome, malignancy and psychiatric disorders, which preceded the onset of acute liver failure, and most of these patients had received daily medication. This tendency was more obvious in patients with the subacute type and LOHF.

The survival rates of non-liver-transplanted patients were 54% for acute and 24% for subacute type FH, and 15% for LOHF. The prognosis of patients with subacute type FH and LOHF was evidently poor. These annual rates have not improved between 1998 and 2006. When compared to a previous survey,[12] prognosis of FH in acute type patients improved until 1998, although the prognosis remained poor in the subacute type with no liver transplantation during that period (Fig. 1). This improvement was probably achieved by progress in artificial liver support.

Table 2 Criteria for etiology of fulminant hepatitis and late onset hepatic failure

I. Viral infection
  1. HAV: positive for serum IgM anti-HAV
  2. HBV: positive for either serum HBsAg, IgM anti-HBc or HBV DNA
    A. Transient infection: fulfilling either (a) or (b):
      (a) Negative for serum HBsAg before onset of acute liver injury.
      (b) Positive for serum IgM anti-HBc and negative for anti-HBc in serum diluted to 1:200.
    B. Acute exacerbation of carrier status: fulfilling either (a) or (b):
      (a) Positive for serum HBsAg before onset of acute liver injury
      (b) Negative for serum IgM anti-HBc and positive for anti-HBc in the serum diluted to 1:200.
    C. Undetermined: neither (a) nor (b)
  3. HCV: fulfilling either (a) or (b):
    (a) Negative for serum anti-HCV or HCV RNA before onset of acute liver injury.
    (b) Positive for serum HCV RNA and low titer positive for serum anti-HCV core protein.
  4. HEV: positive for serum HEV-RNA
  5. Other virus: e.g. EBV.

II. Autoimmune hepatitis: fulfilling either (a) (b) or (c):
  (a) Diagnosed as definite or probable according to the International Scoring System for autoimmune hepatitis.
  (b) Attenuation of liver injury after glucocorticosteroid administration and/or aggravation of liver injury following withdrawal of glucocorticoid.
  (c) Positive for serum antinuclear antigen and/or serum IgG levels >2 g/dL.

III. Drug-allergy-induced: drugs responsible for liver injury are determined by clinical course of liver injury and/or d-LST.

IV. Unknown: etiology is unknown despite sufficient examinations available.

V. Undetermined: etiology is undetermined because of insufficient examinations.

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; EBV, Epstein-Barr virus; d-LST, drug-induced lymphocyte stimulation test.

Causes of FH

Viral hepatitis

In Japan, the cause of FH has been identified as HAV, HBV or other viruses in about 50% of patients (Table 4). The causes of acute liver failure differed depending on the disease type. The frequencies of viral infection were 69% and 31% for patients with the acute and subacute types of FH, respectively, and 17% for LOHF patients.

Journal of Gastroenterology and Hepatology **26** (2011) Suppl. 1; 65–71

Table 3 Demographic features of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Men/women	431/423	228/203	197/226	33/45
Age (years; mean ± SD)	48 ± 17	46 ± 16	49 ± 17**	53 ± 15**
HBV carrier rate (%)	14	12	16*	7***
Complications (%)	39	35	44*	49*
History of medication (%)	46	41	51**	54*
Survival rate (no LT) (%)	40	54	24**	15**
Survival rate (LT) (%)	77	73	79	81

*$P < 0.05$; **$P < 0.01$ versus acute type; ***$P < 0.05$ versus subacute type.
HBV, hepatitis B virus; LT, liver transplantation.

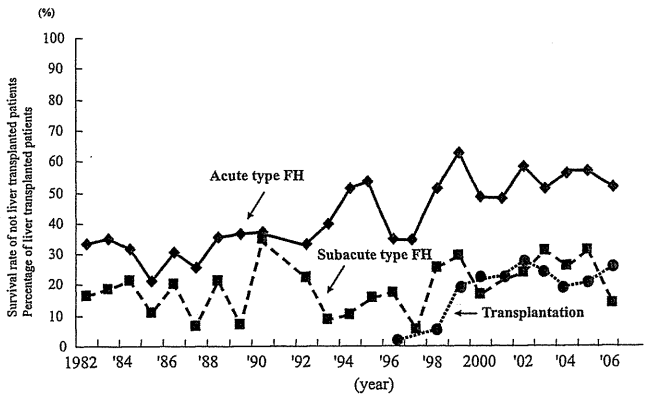

Figure 1 Survival rate of not liver transplanted patients with fulminant hepatitis(FH) and percentage of liver transplanted patients.

Table 4 Percentage etiology of fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Viral infection	51	69	31	17
HAV	6	11	1	1
HBV	42	56	27	13
(Transient infection)	(25)	(40)	(9)	(5)
(Carrier)	(13)	(11)	(16)	(4)
(Undetermined)	(4)	(6)	(2)	(4)
HCV	1	1	1	1
HEV	1	1	1	0
Other virus	1	1	1	1
Autoimmune hepatitis	7	2	12	18
Drug-allergy-induced	10	8	13	15
Unknown	30	18	42	47
Indeterminate	3	3	3	3

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Infection with HAV was found in 6% of patients with FH and frequently observed in the acute type. As annual incidence of acute hepatitis A has declined over the past decade,[13] so too has the incidence of FH. However, as the overall immunity of the Japanese population to hepatitis A is only 12%[14] and is decreasing gradually as in other non-endemic areas, the increasing risk of future outbreaks of acute hepatitis A is probable. With regard to the severity of hepatitis A, age, sex, and drug toxicity have been identified as potential contributing factors.[15] HAV susceptibility and the risk of severity have likely increased recently.

In most of the patients, viral infections were due to HBV. HBV infection was found in 42% of patients with FH and 13% of those with LOHF. Among these, transient HBV infection was more frequent than acute exacerbation of HBV carrier status. Transient HBV infection was more frequent in the acute type (40%) than subacute type (9%) of FH, whereas the frequency of HBV carrier status was greater in the subacute type (16%) than in the acute type (11%). Annual incidence of FH due to HBV infection, both in transient HBV infection and acute exacerbation of HBV carrier status, has declined over the past decade. The routes of transmission of HBV indicate that, at present, sexual transmission from HBV carriers is a major route for FH. The preventive administration of HBV hyperimmune globulin and vaccination against HBV of neonates born to HBV-carrier mothers has been practiced nationwide since 1985 in Japan.[16] Therefore, the HBV carrier rate in the population has significantly decreased, and as a result, a marked decrease in the incidence of FH caused by HBV is expected.

Reactivation of HBV is a well-recognized complication in patients with chronic HBV infection who are undergoing cytotoxic chemotherapy or immunosuppressive therapy. HBV reactivation can be clinically severe and result in death from acute liver failure. Among acute exacerbation of HBV carrier status in the survey, HBV reactivation has been increasingly observed in patients with hematological malignancies. Furthermore, among the 12 patients with HBV reactivation, six with serological evidence of resolved hepatitis B [without hepatitis B surface antigen (HBsAg), but with antibody to hepatitis B core antigen (anti-HBc) and/or antibody to HBsAg (anti-HBs) in serum] developed reactivation with reappearance of HBsAg in serum. Most of these patients had received rituximab and coriticosteroid. Recently, combination therapy with rituximab and corticosteroid has been identified as a risk factor for HBV reactivation in HBsAg-negative patients with malignant lymphoma.[17,18] A study in Japan has revealed that 22% of *de novo* hepatitis B and that caused by HBV reactivation from resolved

hepatitis developed into fulminant hepatic failure, and mortality was 100%.[19] This problem deserves careful attention, because HBsAg-negative, anti-HBc-and/or anti-HBs-positive patients, which account for 20–25% of hospitalized patients in Japan, represent a high-risk group.[20]

HCV infection is rare in the etiology of patients with FH and LOHF. HCV infection was found in 1% of patients with FH, independent of the disease type. Reactivation of HCV as a cause of acute liver failure following chemotherapy has been reported.[21] However, none of these patients were found in the survey.

HEV infection was found in 1% of FH patients. HEV is a common cause of acute hepatitis in endemic areas, such as South Asia, Africa and South America.[22] The virus is now also known to exist indigenously in Japan, and can contribute to acute liver disease.[23,24] In Japan, the zoonotic transmission from pigs, wild boar and deer, either food-borne or otherwise, is the cause of HEV infection in non-endemic areas.[24,25] As for the geographical distribution of clinical HEV infection in Japan, it has been reported that there was wide variation with a higher prevalence in the northern part of Japan (Hokkaido Island and the northern part of mainland Honshu).[26] In the survey, two-thirds of the patients were from this area. Moreover, most of the patients were elderly men and there were no pregnant women, who have the highest attack rate of the virus in endemic areas.

In the survey, Epstein–Barr virus, cytomegalovirus, herpes simplex virus, human herpesvirus type-6 and parvovirus were infrequent causes of other forms of viral hepatitis.

Autoimmune hepatitis

Although autoimmune hepatitis is a chronic disease, an acute presentation occurs in approximately 22% of patients, and an even smaller number present with acute liver failure.[27] In the survey, autoimmune hepatitis was found in 7% of patients with FH and 18% of those with LOHF, respectively. In 2001, FH due to autoimmune hepatitis was recognized in Japan, because there were patients with non-HAV/HBV FH in which IgG levels were >2 g/dL, with positive antinuclear antigen in the serum. Although the diagnosis generally relies on the presence of serum autoantibodies, higher IgG levels (>2 g/dL), liver histology (if available), and response to corticosteroid therapy, the diagnosis of acute-onset autoimmune hepatitis is often difficult. The serum gammaglobulin or IgG concentrations are often lower than those in patients with chronic hepatitis.[28]

Drug-allergy-induced liver injury

Formation of toxic reactive metabolites has been suggested as a potential mechanism for causing idiosyncratic drug-induced liver injury.[29] Drug-allergy-induced liver injury was seen in 13% of patients with subacute type FH and in 15% of those with LOHF. The diagnosis relied mostly on the clinical course or drug-induced lymphocyte stimulation test (D-LST). Numerous types and classes of drugs have been implicated. Anti-tuberculosis agents (isoniazid, rifampicin, ethambutol and pyrazinamide), nonsteroidal anti-inflammatory drugs (loxoprofen, lornoxicam and acetaminophen), anti-cancer agents (tegafur, UFT and flutamide), drugs for metabolic syndrome (allopurinol and acarbose), and various herbal and natural remedies were the probable causative agents in the survey.

Table 5 Survival rates and etiology of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 678$)	Acute type ($n = 369$)	Subacute type ($n = 309$)	($n = 62$)
Viral infection	45	55	23*	36*
HAV	74	77	40	100
HBV	39	50	18*	38
(Transient infection)	(51)	(56)	(32*)	(33)
(Carrier)	(22)	(35)	(13*)	(67)
(Undetermined)	(23)	(33)	(0)	(0)
HCV	67	75	60	0
HEV	60	100	33	—
Other virus	60	50	67	0
Autoimmune hepatitis	21	25	21	18
Drug allergy-induced	42	58	29*	0*
Unknown	36	54	26*	10*
Indeterminate	28	36	14	0

*$P < 0.05$ versus acute type.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Unknown etiology

The etiology was unknown in 42% and 47% of patients with subacute type FH and LOHF, respectively. Although the roles of GB virus C (GBV-C)/hepatitis G virus (HGV) and transfusion transmitted virus (TTV) have been discussed, in this survey, neither GBV-C/HGV or TTV appeared to be a major cause of FH. It is possible that the patients with drug-allergy-induced liver injury were contaminated with those of unknown etiology, because the ratio of medication history was high in these patients. The relationship between daily dose of oral medication or medication with significant hepatic metabolism and idiosyncratic drug-induced liver injury has been reported.[30,31] The higher numbers of patients with complications and daily medication in the survey support this evidence. Furthermore, HEV infection needs further investigation, because serum HEV RNA and IgM antibody to HEV were measured less in the survey.

Prognosis

The prognosis of patients with FH and LOHF differed depending on the etiology (Table 5). It was excellent in patients with HAV infection: the survival rate was 77% and 40% in patients with acute and subacute types of FH, respectively, and 100% in those with LOHF. In contrast, the prognosis was especially poor in HBV carriers who showed acute exacerbation. The survival rates of acute and subacute types of FH were 35% and 13%, respectively. It is noteworthy that none of the patients with HBV reactivation from resolved hepatitis B after rituximab and corticosteroid combination therapy survived. In contrast, the survival rate was 56% in acute type FH and 32% in subacute type in patients with transient HBV infection. The prognosis was poor in autoimmune hepatitis independent of disease type. Prognosis was also poor in patients

Journal of Gastroenterology and Hepatology **26** (2011) Suppl. 1; 65–71

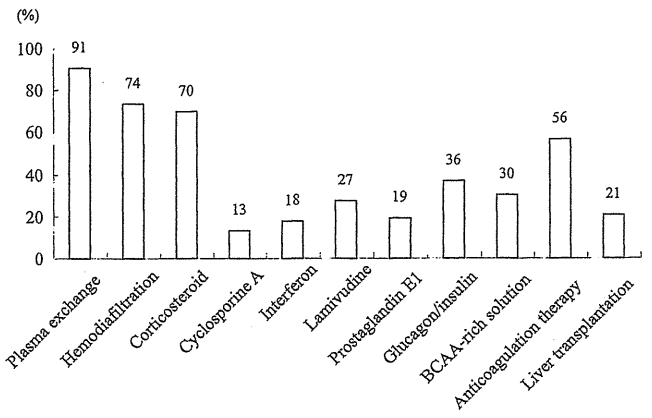

Figure 2 Percentage incidence of therapies performed for fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006). BCAA, branched-chain amino acid.

with subacute type FH and LOHF caused by drug-allergy-induced liver injury, and in those of the unknown etiology.

Complications

Complications that occurred during the course of acute liver failure also seemed to affect patient prognosis. Disseminated intravascular coagulation, renal failure and bacterial infection were found as complications in >30% of patients. Brain edema, gastrointestinal bleeding and congestive heart failure were seen in about 30%, 20% and 10%, respectively. Any of these complications significantly decreased survival rate. Furthermore, the number of these complications influenced prognosis.

Management

Specific therapies

The frequency of antiviral therapy with lamivudine has increased since 1998. As antiviral agents, lamivudine and interferon have been used in 27% and 18% of patients with FH and LOHF, respectively, between 1998 and 2006 (Fig. 2). Lamivudine has been used in 67% of patients with HBV-related FH or LOHF. Lamivudine has been reported to be efficacious for acute liver failure.[31,32] Recently, another guanosine nucleoside analog, entecavir, has been administered more frequently.[33] A preliminary study of entecavir for acute liver failure has revealed that the agent beneficially affects disease course. Lamivudine therapy is more efficacious when started early in acute liver failure. However, in the case of HBV reactivation from HBsAg-negative patients, it is difficult to prevent development of liver failure, even when lamivudine is administered after the onset of hepatitis. Two study groups in Japan have proposed guidelines for prevention of immunosuppressive-therapy- or chemotherapy-induced HBV reactivation. These guidelines recommend that patients with resolved infection should be routinely monitored for liver function and HBV DNA levels during and after chemotherapy, and antiviral therapy should be administered immediately when HBV DNA increases above the detection levels.

Corticosteroids were administered in 70% of patients with FH and LOHF. Steroid pulse therapy, methylprednisolone at a daily dose of 1 g injected intravenously, was administered to attenuate liver necrosis by suppressing excessive immune response. The efficacy of corticosteroids for improving the prognosis of acute liver failure is still obscure. Some randomized controlled trials have shown that corticosteroids provide no benefit overall in acute liver failure.[34] However, FH due to autoimmune hepatitis might be a candidate for therapy.[35] Anticoagulant therapy was performed in 56% of patients with FH and LOHF. Antithrombin III concentrate and protease inhibitor compounds such as gabexate mesylate and nafamostat mesylate were used as anticoagulants. They were effective for inhibition of disseminated intravascular coagulation and microcirculatory disturbance due to sinusoidal fibrin deposition. Glucagon/insulin, branched-chain amino acid-rich solution, cyclosporine A and prostaglandin E1 therapy was administered less frequently, and the frequency decreased compared to that in patients in the previous survey between 1995 to 1997.

Methods of liver support

In Japan, powerful artificial liver support with plasmapheresis and hemodiafiltration plays a central role in the treatment of acute liver failure. Plasmapheresis and hemodiafiltration were performed in 91% and 74% of patients with FH and LOHF, respectively (Fig. 2). In the late 1990s, hemodiafiltration therapy was developed and plasma exchange combined with hemodiafiltration therapy became popular. The increased frequency of this combination therapy in the 1990s could be implicated in the tendency for the survival rate to increase for acute type FH (Fig. 1). The effect of plasmapheresis on survival from acute liver failure has been difficult to determine. However, these support systems are efficacious for helping patients to remain in good condition until sufficient regeneration of the liver can be obtained, or liver transplantation can be performed. Recently, more powerful hemodiafiltration using large buffer volumes[36] or on-line hemodiafiltration[37] has been developed and has shown greater efficacy for improving hepatic coma.

Liver transplantation

Despite significant advances in critical care and an improved understanding of the pathophysiology of acute liver failure, the mortality rate remains high. Liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. In Japan, living donors have been used because of the insufficiency of organ donation since 1988. Living donor liver transplantation was performed in 17% of patients with FH and LOHF between 1998 and 2006, and the frequency in those patients was significantly greater in the subacute type (21%) than in the acute type (13%). Recently, these frequency ratios have been almost steady (Fig. 1). The survival rates were 77% and 81% in patients with FH and LOHF, respectively, and there was no difference in the rates among the disease types. Patient and graft survival rates were 94% and 87% at 1 year, and 91% and 81% at 5 years, respectively. There was no significant difference in patient and graft survival according to etiology.[38]

Appropriate judgment to move forward to liver transplantation is the most important step. The indications for liver transplantation

in cases of FH are determined according to the 1996 Guidelines of the Acute Liver Failure Study Group of Japan. Re-evaluation of the guidelines has revealed that the accuracy in patients not receiving liver transplantation was 68% and 78% in acute and subacute types of FH, respectively, and 84% among those with LOHF.[39] The sensitivity and specificity of the assessment in patients with acute and subacute types were very low. To improve this situation, new guidelines for using a scoring system have been proposed by the Intractable Hepato-biliary Disease Study Group of Japan.[40] By using these guidelines, the accuracy in patients not receiving liver transplantation was increased to 75% and 87% in acute and subacute types of FH, respectively.

Experimental methods of liver support

To improve the prognosis of acute liver failure, advances in the treatment for liver regeneration are urgently needed. Hepatocyte growth factor (HGF) acts as a stimulator of liver regeneration, as well as an anti-apoptotic factor. We have started a clinical trial to examine the effects of recombinant human HGF (rhHGF) in patients with FHor LOHF, and in the four patients with FH or LOHF enrolled in this study; repeated doses of rh-HGF did not produce any severe side effects. Although two patients were rescued in this study, evaluation of this therapeutic agent is still under investigation.[41]

Several clinical trials of bone marrow cell infusion in patients with liver cirrhosis have shown clinical improvement. A clinical trial of autologous bone marrow infusion for patients with advanced liver cirrhosis due to chronic HBV infection has shown clinical improvement with no serious adverse events.[42] The recent discovery of pluripotent stem cells has yielded a new cell type for potential application in regenerative medicine. Strategies to achieve high levels of hepatocyte survival and the development of methods to engineer a functional liver system *in vivo* are expected in the future.

Conclusion

In Japan, the incidence of FH has decreased gradually and the clinical characteristics of patients and the therapeutic approach have changed in the past decade. The prognosis differs in patients with FH and LOHF depending on the disease type and etiology. HBV is the major cause of FH in Japan. Recently, careful attention has been necessary because of an increase in HBV reactivation from resolved hepatitis B. Despite careful investigation, a significant group with FH of unknown origin remains and needs further investigation. Living donor liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. Artificial liver support systems are efficacious while waiting until the native liver regenerates or a donor is found. New therapeutic modalities are required to regenerate the liver, in particular, for the subacute type of FH.

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Original Article

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Nucleic Acid Substitutions and Response to Interferon in HBeAg-positive Chronic Hepatitis B Patients with Genotype C HBV Infection

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Abstract

To elucidate the effect of nucleic/amino acid substitutions on the response to interferon treatment, we retrospectively analyzed 27 HBeAg-positive patients infected with genotype C hepatitis B virus (HBV), who were treated with interferon (IFN). Nucleic/amino acid sequences of the core upstream regulatory sequences (CURS), basic core promoter (BCP), and precore-core regions (nucleotide (nt.) 1643-2452) HBV DNA were determined. Sustained virological response (SVR) was defined as normalization of serum alanine aminotransferase (ALT) level, HBeAg loss, and decrease in HBV DNA level to less than 5 log copies/ml at 24 weeks after the end of treatment. The other patients were defined as having no response (NR). Of the 27 patients, 6 achieved SVR and 21 showed NR. There were no significant differences in clinical characteristics between both groups. The total number of substituted nucleic acids in the CURS and BCP is higher in the patients who achieved SVR (SVR: 4.33, NR: 3.05, $p=0.018$). Amino acid mutations in the precore-core region were not related to the response to IFN treatment. Our study suggests that nucleic acid sequences in the CURS and BCP may be related to the response to IFN.

Key words

hepatitis B virus, core, precore, genotype, interferon

Introduction

Chronic hepatitis B (CHB) is an intractable disease that sometimes leads to progressive liver disease with advanced fibrosis and hepatocellular carcinoma. Interferon (IFN) and nucleoside analogues (NAs) are the current approved treatments for CHB in most countries. IFN is superior to NAs in that IFN can remit hepatitis even after the withdrawal of treatment[1]. Furthermore, IFN, which mainly act as a immunomodulator, does not induce drug-resistant mutants as seen in the case of NAs[1-3]. Therefore, the role of IFN treatment is still considerable even after the use of NAs.

Although long-term virological and biochemical responses may be expected in IFN treatment, the proportion of patients who exhibit long-term response is not large[3-5]. Furthermore, IFN treatment is accompanied by several adverse effects in most patients[1]. Therefore, prediction of outcome

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before the start of IFN treatment is important.

Previous studies have shown some factors that may affect the treatment efficacy of IFN in CHB treatment. The major pretreatment factors that correlated with long-term response were high ALT levels, low HBV DNA, female sex, greater degrees of activity, and fibrosis on liver biopsy[3)6)].

HBe antigen (HBeAg) has a major role in determining the response to IFN. HBeAg modulates the immune response to HBV[7)] and affects the therapeutic efficacy of IFN. Patients who are positive for serum HBe antigen (HBeAg) respond better to IFN treatment than those who are negative for HBeAg[3-5)].

HBV genotype is another factor that affects treatment efficacy. Studies from Europe show that a higher rate of HBeAg seroconversion following IFN treatment was found in those infected with genotype A than in those with genotype D[8-10)].

The rate of HBeAg loss was also significantly higher in patients with genotype B than in those with genotype C in Taiwanese studies[11) 12)].

In addition to HBeAg and HBV genotype, variations in nucleotide sequences have been shown to affect the response to IFN. Some reports have shown that patients with nucleic acid mutations at nt. 1762/1764 in the basic core promoter (BCP) region respond better to IFN treatment[9)]. Another report has shown that patients with nucleic acid substitution at nt. 1896 in the precore (preC) region, which terminate HBeAg translation, have a poorer response to IFN than those without the substitution[13)]. However, most previous studies have focused on nt. 1762/1764/1896. The effect of other regions on the efficacy of interferon treatment has not been determined.

Therefore, we examined the nucleic/amino acid sequences of core upstream regulatory sequences (CURS), nt. 1643–1742, BCP, preC, and core regions of HBV DNA in the serum of HBeAg positive chronic hepatitis B patients who were infected with genotype C and were treated with IFN-α. The relationship between sequence polymorphisms and the response to treatment was also studied.

Materials and Methods

Patients

Twenty-seven consecutive patients with chronic hepatitis B with HBeAg who received IFN-α treatment from 1996 to 2007 for chronic active hepatitis with abnormal ALT were retrospectively analyzed. Six megaunits of interferon alpha was administered daily in the first 2 weeks, followed by 3 times per week for the next 22 weeks.

Patients whose serum alanine aminotransferase (ALT) level was normalized and whose serum hepatitis B virus (HBV) DNA level decreased to less than 5 log copies/ml 24 weeks after the end of treatment were categorized as achieving sustained virological response (SVR). This is because DNA above this level usually accompanies ALT elevation and HBV DNA over 5-log copies/ml was indicated for antiviral therapy in HBeAg-positive patients by The clinical study group for the treatment of viral liver disease in Japan. The other patients were categorized as showing no response (NR).

Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines stipulated in the 1975 Declaration of Helsinki and was approved by the Ethics Committees of St. Marianna University School of Medicine (No. 1163) and Seizankai Kiyokawa Hospital.

HBV DNA and genotype

HBV DNA was measured using transcription mediated amplification TMA-HPA (Chugai Diagnostics Science, Tokyo, Japan) assay or PCR (Amplicor HBV monitor. Roche Diagnostics, Tokyo, Japan).

The HBV genotypes were determined using a commercial enzyme-linked immunosorbent assay kit (SMITEST HBV genotype detection kit, Genome Science Laboratories, Fukushima, Japan).

Nucleic/amino acid sequences of HBV DNA

DNA was extracted from the serum using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The serum samples, collected just before treatment and 24 weeks after treatment, were preserved at −80°C until use. DNA was dissolved in 200μl of distilled water.

For amplification and sequencing, the CURS, BCP, and preC-core regions were divided into three fragments, namely, those spanning nt. 1590–1974, nt. 1632–2389, and nt. 2111–2680, and amplified by PCR using KOD-Plus-Ver. 2 (Toyobo Co., Ltd., Osaka, Japan). The primers used in this study, which were selected using "primer 3" on Website (http://frodo.wi.mit.edu/primer3/), are shown in **Table 1**. The cycle profile for the first round of PCR was 94°C for 2 min, followed by 98°C for 10 s for

Talbe 1. Primers in This Study

Primer	Sequence
HBVCURSIS	5'-TTCACCTCTGCACGTCGCAT-3' (nt.1590-1609)
HBVCURSIAS	5'-GGAAAGAAGTCAGAAGGCAAA-3' (nt.1974-1954)
HBVR4OS	5'-CAAGTCTTGCCCAAGGTCTTA-3' (nt.1632-1652)
HBVR4OAS	5'-AGGCGAGGGAGTTCTTCTTCT-3' (nt.2389-2369)
HBVR5OS	5'-TGGGTGGGAAGTAATTTGGA-3' (nt.2111-2130)
HBVR5OAS	5'-AAGGGCAAATATTTGGTAAGG-3' (nt.2680-2660)

Talbe 2. Pretreatment Background of the Patients

	SVR	NR
Mean Age (median)	38.5 (34)	37.4 (39)
Sex (Male:Female)	5: 1	19: 2
Mean ALT (IU/L)	206.2±206.4	254.2±277.2
HBV DNA (logcopy/ml)	7.65±1.00	7.34±1.15

The pretreatment background is not statistically different between SVR and NR patients.

denaturation, 52°C for 30 s for annealing, and 68°C for 30 s for polymerization for 40 cycles. Standard precautions to avoid contamination were taken during PCR, with a negative control included in each run. The PCR products were analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized by ultraviolet transillumination.

Sequencing of PCR products

The amplification products were purified using a QIAquick@Spin purification system (QIAGEN, Hilden, Germany) and sequenced in forward and reverse directions using the PCR primers.

Statistical analysis

The data were analyzed by chi-square test, Student's t-test, and Mann-Whitney U-test. *P* values less than 0.05 were regarded as statistically significant.

Results

Clinical features of patients

Of the 27 enrolled patients, 6 patients achieved SVR and the other 21 patients showed NR. The clinical background of the patients is shown in **Table 2**. Age, sex, levels of ALT, and HBV DNA were not different between SVR patients and NR patients.

Nucleic acid sequences of nt. 1762/1764 in the BCP and nt. 1896 in the preC regions and response to IFN treatment

Nucleic acid sequences of nt. 1762/1764 in the BCP regions, which were reported to be frequently substituted in responders to the IFN treatment, were studied. The BCP sequences were determined in 6 SVR and 18 NR patients. The preC sequence was determined in 6 SVR and 20 NR patients.

As shown in **Table 3**, the nucleic acids in the BCP region (both nt. 1762 and 1764) were substituted in 5 of 6 SVR patients and 11 of 18 NR patients

Talbe 3. Nucleic Acid Substitutions in the BCP (nt. 1762/1764) and PreC Regions (nt. 1896)

	BCP1762	BCP1764	PC1896	Number of patients
SVR	W	W	W	1
	M	M	W	4
	M	M	M	1
NR	W	W	W	7
	M	M	W	9
	M	M	M	2
	NT	NT	NT/W	3

W: wild, M: mutant, NT: not tested

(p=0.32). The nucleic acid sequence at nt. 1896 in the preC region was substituted in 1 of 6 SVR patients and 2 of 20 NR patients (p=0.88). The nucleic acid sequences in the three positions (nt. 1762, 1764 and 1896) did not change after treatment except for 3 NR patients (NR3, NR10 and NR20).

Nucleic acid sequences in the CURS/BCP region and response to IFN treatment

The mean number of substituted nucleic acid sequences in the BCP region including nt. 1762/1764 was not different between SVR patients and NR patients (2.67 vs. 1.79, p=0.06). The mean number of substituted nucleic acids in the region ranging from CURS to BCP was larger in SVR patients than in NR patients (4.33±2.26 vs. 3.05±1.29, p= 0.018).

Table 4 shows the frequency of mutation in each position of nucleic acid. The nucleic acid at position 1688 in the CURS region was substituted from G to A in 5 of 19 NR patients and in none of the SVR patients (p=0.41). The nucleic acid at position 1719 in the CURS region was substituted from T to G in 7 of 19 NR patients and in 1 of the 6 SVR patients (p=0.67). The sequences at these positions after treatment did not change in most cases.

Amino acid sequences in the preC-core region and response to IFN treatment

The mean number of amino acid substitutions in the preC-core region before treatment is shown in **Table 5**. No significant difference was found between SVR and NR patients (3.00 vs. 2.71). The total number of mutated nucleic acids in CURS and BCP regions, which differed between SVR and NR patients, was not different. Two SVR patients and 5 NR patients showed no amino acid substitution in the region. There were no significant changes in the amino acids that are related to the response to IFN treatment (data not shown).

Discussion

IFN treatment for chronic hepatitis B was first reported by Greenberg *et al.* in 1976[14]. Eradication of HBV by IFN is somehow difficult because the effect of IFN was mainly on messenger RNA as the precursor of viral protein. HBV DNA including covalently closed circular DNA (cccDNA) is cleared or decreased by immunological response indirectly mediated by IFN. Therefore, elucidating the factors, including viral ones, related to the favorable response to IFN treatment is important, which prompted us to conduct this study.

Nucleic acid substitution at nt. 1896 from G to A creates a stop codon, which abolishes the translation of HBeAg at the transcriptional level[15-17]. Therefore, few patients in this study who were positive for HBeAg do not have G1896A. The mutation at nt. 1896, which may show that only minor clone produce 1896, may decrease the level of HBeAg and change both viral load and immune response viral protein. However, the nucleic acid mutation, found in one SVR and two NR patients, is not useful for predicting response to IFN treatment.

Nucleic acid substitutions at nt. 1762 from A to T and at nt. 1764 from G to A are known to upregulate transcription of pregenomic RNA and downregulate transcription of preC mRNA, which lead to lowered HBeAg production[18]. In this study,

Talbe 4. Changes in the Nucleic Acid Sequence in the CURS and BCP Regions after IFN Treatment

before

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
SVR1	W	W	W	W	3
SVR2	W	W	W	M	5
SVR3	W	W	W	W	6
SVR4	M	W	M	W	6
SVR5	M	W	W	W	3
SVR6	W	W	W	M	3
mean					4.33*

after

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
SVR1	W	W	W	W	3
SVR2	W	W	W	M	4
SVR3	W	W	W	W	---
SVR4	M	W	M	W	---
SVR5	M	W	W	W	---
SVR6	W	W	W	M	---
mean					3.50

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
NR1	W	W	W	W	3
NR2	NT	NT	NT	NT	---
NR3	W	W	M	W	5
NR4	NT	NT	NT	NT	0
NR5	W	W	M	W	4
NR6	W	M	W	W	3
NR7	W	W	M	W	3
NR8	W	M	W	W	3
NR9	W	M	W	W	1
NR10	W	W	W	W	4
NR11	W	W	M	W	3
NR12	W	W	M	W	3
NR13	W	W	W	W	3
NR14	W	W	W	W	3
NR15	W	W	W	W	4
NR16	W	W	W	W	2
NR17	W	M	W	M	4
NR18	W	W	W	W	3
NR19	W	M	W	W	2
NR20	W	W	M	W	2
NR21	W	W	M	W	3
mean					3.05*

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
NR1	W→M	W	W	W	6
NR2	NT	NT	NT	NT	---
NR3	W	W→M	M→W	W	3
NR4	NT	NT	NT	NT	0
NR5	W	W	M	W	5
NR6	W	M	W	W	3
NR7	W	W	M	W	3
NR8	W	M	W	W→M	4
NR9	W	M	W	W	1
NR10	W	W	W	W	3
NR11	W	W	M	W→M	5
NR12	W	W	M	W	3
NR13	W	W	W	W	2
NR14	W	W	W	W	---
NR15	W	W	W	W	3
NR16	W	W	W	W	4
NR17	W	M	W	M	4
NR18	W	W	W	W	2
NR19	W	M	W	W	4
NR20	W	W	M	W	1
NR21	W	W	M	W	---
mean					3.11

W: wild, M: mutant, NT: not tested
*: p=0.018

Talbe 5. Amino Acid Changes in the PreC and Core Regions after IFN Treatment

	Number of substituted amino acid in the precore and core regions	
	Pre	Post
SVR1	8	2
SVR2	4	5
SVR3	2	0
SVR4	0	Not done
SVR5	4	3
SVR6	0	Not done
Mean(SVR)	3	2.5
NR1	9	3
NR2	0	3
NR3	1	2
NR4	4	5
NR5	3	2
NR6	4	4
NR7	2	3
NR8	1	2
NR9	0	1
NR10	6	5
NR11	4	0
NR12	4	4
NR13	2	2
NR14	1	1
NR15	5	6
NR16	3	2
NR17	2	4
NR18	6	5
NR19	0	3
NR20	0	4
NR21	0	Not done
Mean(NR)	2.71	3.05

83% of SVR and 61% of NR patients have nucleic acid substitution at these positions. The results were inconsistent with a previous report showing that patients whose serum HBV DNA has nucleic substitutions at nt. 1753, 1766, and 1764 respond better to IFN treatment than those without the substitutions[8]. The reason for this inconsistency may be attributed to the HBV genotype. In this study, we investigated patients who are infected with genotype C HBV, many of whom were resistant to IFN treatment. Therefore, studies on a large number of patients may be necessary to show the effect of nucleic acid substitutions at nt. 1753/1766/1764 on IFN treatment.

Nucleic acid substitutions in the BCP region at nt. 1762/1764 are often seen in patients with genotype C HBV. These mutations predict delayed HBe clearance and development of advanced liver disease[19]. In our study, 16 patients had the substitutions. Among the 16 patients, 5 (31%) achieved SVR. In contrast, in 8 patients without the substitutions, only 1 (13%) patient achieved SVR (p=0.31). Therefore, we might expect that nucleic acid substitutions in the BCP region may be favorable for IFN treatment.

The mean total number of substituted nucleic acid sequences in the CURS and BCP regions is larger in SVR patients than in NR patients (4.33 vs. 3.05, p=0.018). The mechanism is unclear but it may be hypothesized that nucleic acid substitutions in the regions reduce HBe production and render tolerance to HBV, which may trigger clearance of HBV and remission of hepatitis[8].

Nucleic acid substitutions from G to A at nt.

1688 and T to G at nt. 1719 in the CURS region tend to be found more often in NR than in SVR patients. Twelve out of 20 NR patients have nucleic acid substitutions at nt. 1688 and/or nt. 1719. In contrast, only 1 out of 6 SVR patients has the substitutions ($p=0.16$). As nt. 1719 is included in the HNF-3 binding region, the nucleic acid substitution at this position may lead to upregulation of the transcription of HBV and may reduce the response to IFN[8].

Nucleic and amino acid substitutions in the preC-core region were not related to response to IFN treatment in this study. It was reported that amino acid substitutions in the hypervariable region (AA 84–101) occur around the onset of hepatitis in HBeAg positive patients and may be related to breaking tolerance[20]. Our results, written above, did not show the relation between amino acid sequences in the core region and clinical outcome. The role of amino acid substitution in the core region in the response to IFN treatment seems low, which should be further studied.

The results of nucleotide/amino acid sequence was obtained using a set of primers. HBV clones that do not hybridize the primers may be overlooked. Using another set of primers and subcloning of PCR samples may show more accurate results.

We have shown that the nucleotide sequences of the regulatory regions upstream of the core region may be related to favorable response to IFN in HBeAg-positive patients. However, the results did not show that the sequences of these regions were related to the natural seroconversion to anti-HBe. However, the sequences might be related to the durability of anti-HBe, which should be further investigated.

In conclusion, nearly 20% of the patients who were infected with genotype C HBV and positive for HBeAg showed sustained virological response to IFN treatment. Nucleic acid substitutions in the regulatory regions upstream of the core region might be related to the response.

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Original Article

Occurrence of clinical depression during combination therapy with pegylated interferon alpha or natural human interferon beta plus ribavirin

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Aim: The onset of depression symptoms during pegylated interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to treatment discontinuation in some cases. In the present study, we conducted a questionnaire survey during treatment to determine whether natural human interferon β plus ribavirin (IFNβ/RBV) therapy is associated with a lower incidence of depression symptom onset compared with PEG-IFN/RBV therapy.

Methods: Seventy-seven patients with chronic hepatitis C received PEG-IFN/RBV (PR) or IFNβ/RBV (FR) therapy. A questionnaire survey was administered at the start of treatment, and at 4 and 12 weeks, using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).

Results: BDI-II scores in the PR group increased at 4 and 12 weeks, but remained unchanged in the FR group. At 12 weeks, the mean BDI-II score and incidence of abnormalities with a BDI-II score of ≥14 were significantly lower in the FR group than in the PR group. BDI-II scores during IFNβ/RBV therapy in 11 patients currently using antidepressants remained unchanged up to 12 weeks. None of these 11 patients required addition or dose increases of antidepressants, and there was no evidence of worsened depression symptoms. Nine PR patients had BDI-II scores of ≥14 and PSQI scores of ≥11 at 12 weeks.

Conclusions: IFNβ/RBV therapy was associated with a lower incidence of depression symptom onset during treatment. In patients already diagnosed with depression, there was no evidence that IFNβ/RBV therapy caused any worsening of symptoms, indicating that IFNβ/RBV therapy is safe for patients with depression.

Key words: Beck Depression Inventory II, chronic hepatitis C, depression, natural interferon β, pegylated interferon α, Pittsburgh Sleep Quality Index.

INTRODUCTION

INTRODUCTION OF PEGYLATED interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) in patients with chronic hepatitis C who are receiving interferon therapy.[1–6] An additional new treatment regimen has been introduced by adding Telaprevir to this PEG-IFN/RBV therapy.[7,8] However, adverse effects of PEG-IFN/RBV include the onset of symptoms of depression.[9–11] Thus, there are some difficulties in treating patients with depression or sleep disorders with PEG-IFN/RBV therapy.

In Japan, natural human interferon β (IFNβ), which has a low association with the onset of symptoms of depression, has been used in interferon therapy for chronic hepatitis C.[12,13] IFNβ plus ribavirin (IFNβ/RBV) combination therapy is now used.[14] However, there are no existing reports on the relationship between PEG-IFN/RBV or IFNβ/RBV therapy and the onset of depression symptoms. Therefore, in the present study, in order to determine if IFNβ/RBV therapy is associated with a lower incidence of the onset of symptoms of depression compared to PEG-IFN/RBV therapy, and to evaluate the safety of the IFNβ/RBV therapy in patients with depression, we conducted a questionnaire survey during PEG-IFN/RBV or IFNβ/RBV therapy to investigate the frequency, timing, and intensity of depression symptoms.

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METHODS

Study population

A TOTAL OF 77 Shinkokura Hospital patients with chronic hepatitis C who received IFN therapy for at least 12 weeks between January 2010 and April 2011 were included in the study. The study protocol was in compliance with both the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were assigned to one of the following three groups: (1) the PEG-IFN/RBV (PR) group, consisting of 41 patients who received PR therapy for a period of 24 to 48 weeks; (2) the IFNβ/RBV (FR) group, consisting of 25 patients who received the FR therapy for a period of 24 to 48 weeks; and (3) the FR-d group, consisting of 11 patients with depression who were on antidepressants and who received the FR therapy for a period of 24 to 48 weeks. Patients in the FR-d group received regular psychiatric consultation and experienced dose reduction, dose increase, or addition of antidepressants during treatment. Patients with depression, those with a previous history of depression, those who were on antidepressants, or those who were on sleep-inducing drugs were excluded from the PR and FR groups. Patients reporting some type of sleep disorder during treatment were given sleep-inducing drugs at the discretion of their primary physician. Treatment regimens of PR or FR therapy were determined by the physician. None of the patients required dose reduction of IFN due to neutropenia or thrombocytopenia prior to 12 weeks. This study is a prospective, non-randomized open trial.

Criteria for exclusion from the study were as follows: (i) clinical or biochemical evidence of hepatic decomposition and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFNβ/RBV: white blood cell count of less than 3000/mm^3 and platelet count of less than 50 000/mm^3, PEG-IFN/RBV: white blood cell count of less than 4000/mm^3 and platelet count of less than 80 000/mm^3; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within the 12 months prior to enrollment.

Interferon treatment

Patients in the PR group received the following treatment regimen. In brief, PEG-IFNα-2b (PEG-Intron; MSD Co., Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 lg/kg (range: 1.3–2.0 lg/kg) once a week. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Patients in the FR and FR-d groups received the following treatment regimen. Briefly, IFNβ (Feron; Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Hepatitis C virus (HCV) RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/mL. Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. Urinalysis and measurement of serum albumin levels were performed once every 4 weeks, from the start of treatment to Week 24.

Questionnaire

A questionnaire survey was conducted immediately before the start of treatment and at 4 weeks and 12 weeks using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).[15,16] The questionnaire survey was administered by one expert investigator, who remained blinded to the treatment regimens prescribed to patients, the timing of treatment, and other information. Patients with a BDI-II score of 14 or more were considered to have the onset of depression symptoms. Patients with a PSQI score of 11 or more were identified as having sleep disorder. All patients were given a questionnaire at 12 weeks, while a questionnaire was administered to 58 subjects at the baseline and at 4 weeks, including 28, 19, and 11 patients in the PR, FR, and FR-d groups, respectively.

Statistical analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups, as well as the BDI-II score and the PSQI score at 12 weeks. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the onset of symptoms of depression. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values less than 0.05, as determined by the two-tailed test, were considered significant. Variables were entered into