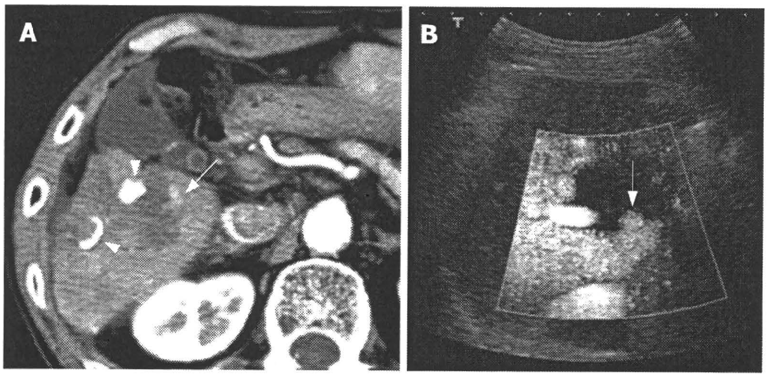

Figure 3 Assessment of therapeutic response after PEI for HCC. **A**: Contrast-enhanced CT with dynamic study; **B**: Contrast-enhanced US (Advanced Dynamic Flow with Levovist). Contrast-enhanced CT showed enhancement appearance which needed additional treatment within the treated area (arrow), and contrast-enhanced US could demonstrate a similar finding. Arrow heads: Lipiodol.

ADVANCED TECHNOLOGY

Recent US systems have provided three-dimensional visualization of the combined tissue structures and color blood-flow appearance under easy handling[24,25]. Additional anatomical information of the tumor with tumor-associated vessels is available at any plane from multiple directions[113-116]. With the remarkable progress in microelectronic technology, the US transducer has achieved full digital specification (Matrix transducer, iu22, Philips) with 3000 elements[117,118]. Including built-in micro-beamforming composed of a 150-computer board, it can visualize "Live 3D", which presents real-time three-dimensional anatomical views visible from any angle with volume rendering for pyramidal volume (90*70 angles). Contrast-enhanced 3D or 4D ultrasonographies using microbubble contrast agents might become a standard method for the characterization and/or evaluation of the therapeutic effect on liver tumors (Figure 4)[119].

HIFU is a novel technology that enables transcutaneous ablation effect without needle puncture[120,121]. While controlling the energy and focusing of US, successful HIFU results in necrosis of the tumor in the focal area with less damage of surrounding tissues. A number of clinical studies have been carried out using HIFU for the treatment of liver tumors as well as breast cancer and myoma uteri. In regard to liver tumors, it was reported that the anti-tumor effect and survival time by HIFU combined with TACE were superior to those by TACE alone in 50 patients with advanced HCC[122]. Although some of the subjects seemed to have a complete ablation effect, the precise effect for complete tumor necrosis by HIFU was not clear in this study. Furthermore, as the background of the HCCs showing sufficient ablation effect was not fully analyzed, it remains to be solved whether HIFU is valuable as a reliable method for curative treatment of small HCC. Nonetheless, this non-invasive method is really expected to be used for HCC treatment, as an alternative to PEI or RFA, because needle puncture is an invasive procedure for cirrhotic patients.

Normal ventilation is one of the serious problems in the completion of HIFU treatment for liver tumor, as movement of the liver may cause ablation failure that results on non-tumor tissue damage and/or incomplete therapeutic effect for the tumor. Wu *et al* reported that three-dimensional US images were used as a monitor to localize the tumor during HIFU treatment, and changes in echogenicity of the tumor just after the treatment were evaluated by US[122]. Advances in imaging technology for real-time 3D sonography would help the improvement of the therapeutic ability of HIFU.

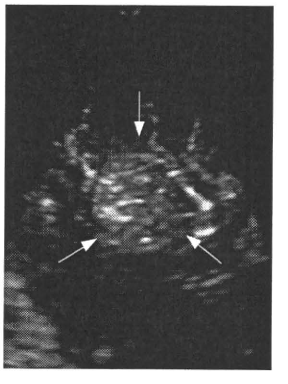

Figure 4 Real-time three-dimensional imaging of HCC (contrast-enhanced LIVE 3D with Sonazoid, iu22, Philips). Abundant tumor vessels were dramatically demonstrated in the HCC nodule. (Arrows: HCC nodule).

In conclusion, US has made amazing strides in the last decades because of digital technology progress, and it will continue to grow. The advancement of imaging methods is expected to support the clinical management of patients with HCC.

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CLINICAL STUDIES

Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients

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Abstract

Background/Aims: Our aim was to evaluate the relationship between hepatitis C virus (HCV) infection and development of diabetes mellitus (DM) or insulin resistance (IR) in comparison with hepatitis B virus (HBV) infection and eradication of HCV infection by interferon treatment. *Methods:* This study consisted of 952 outpatients, including 544 HCV-infected (HCV+chronic), 286 HBV-infected (HBV+chronic) and 122 patients whose HCV was cleared by interferon treatment (HCV+cleared) (diabetes study). Among 849 without overt DM, IR was assessed in 423 patients, including 232 HCV-infected (HCV+chronic), 135 HBV-infected (HBV+chronic) and 56 HCV-eradicated patients (HCV+cleared) (IR substudy). *Results:* The prevalence of DM in the HBV+chronic, HCV+chronic and HCV+cleared groups was 6.3, 13.6 and 9.0%, respectively (HBV+chronic vs HCV+chronic, $P < 0.005$), in the diabetes study, and the prevalence of IR in the HCV+chronic group (54.3%) was also higher than that in the HBV+chronic (36.3%) ($P < 0.005$) and HCV+cleared groups (35.7%) ($P < 0.05$) in the IR substudy. However, HCV infection was not shown to be independently associated with DM development [odds ratio (OR) 1.669; $P = 0.0936$] and with IR (OR 1.531; $P = 0.2154$) by multivariate analysis in comparison with HBV infection as control. *Conclusions:* HCV-infected patients showed a higher prevalence of DM and IR than those with HBV infection. However, in Japan, other confounding factors appeared to be more important risk factors for the development of disturbance in glucose metabolism.

Approximately 1.5 million people in Japan and 170 million people worldwide have been infected with the hepatitis C virus (HCV), and chronic HCV infection causes chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (1, 2). In most western countries, HCV infection is becoming a major cause of HCC and liver transplantation (3).

It is estimated that seven million people are affected by diabetes mellitus (DM) in Japan (4). Type 2 DM is a lifestyle-associated disease and is increasing worldwide, including in Japan (5). The risk factors associated with type 2 DM include family history, age, gender, obesity, smoking and physical activity (6). A close association between DM and insulin resistance (IR) is also reported (7).

The liver is crucial to carbohydrate metabolism and glucose homoeostasis, and hepatic dysfunction causes glucose abnormalities leading to DM, which is prevalent in chronic liver disease, and especially in liver cirrhosis (8,9). Cirrhotic patients have glucose intolerance attributable to IR, which is caused by a post-receptor defect, decreased binding of insulin to target tissues and inadequate response of β-cells to secrete insulin appropriately to overcome the defect in insulin action (10). It has also been reported that DM increases the risk of developing chronic liver disease and HCC (11), and DM has been associated with non-alcoholic fatty liver disease including non-alcoholic steatohepatitis (12). Thus, there is a close association between DM and liver diseases.

Recently, there has been growing evidence to suggest an association between HCV infection and DM. A high prevalence of DM has been reported among patients chronically infected with HCV in comparison with controls or other liver diseases (13–18), in addition to a high prevalence of HCV infection among

Table 1. Clinical characteristics of HBV+chronic, HCV+chronic and HCV+cleared patients in diabetes study and insulin resistance substudy

				P value		
	HBV+ chronic	HCV+ chronic	HCV+ cleared	HBV+ chronic vs HCV+ chronic	HCV+ chronic vs HCV+ cleared	HBV+ chronic vs HCV+ cleared
Diabetes study						
Number	286	544	122			
Age	45.1 ± 13.6	58.4 ± 13.0	53.2 ± 13.0	< 0.0001	< 0.0001	< 0.0001
Gender male (%)	164 (57.3)	257 (47.2)	82 (67.2)	< 0.01	< 0.0001	NS
Clinical stage (asymptomatic carrier/chronic hepatitis/ cirrhosis)	100/161/25	78/353/113	−/−/−	< 0.0001		
Insulin resistance substudy						
Number	135	232	56			
Age	44.5 ± 13.0	59.6 ± 13.1	53.5 ± 12.0	< 0.0001	< 0.005	< 0.005
Gender male (%)	77 (57.0%)	93 (40.1%)	38 (67.9%)	< 0.005	< 0.0005	NS
Hypertension	5 (3.7%)	49 (21.1%)	5 (9.1%)	< 0.0001	NS	NS
Hyperlipidaemia	32 (23.9%)	33 (14.2%)	17 (30.4%)	< 0.05	< 0.01	NS
Obesity	19 (17.4%)	40 (19.1%)	19 (38.8%)	NS	< 0.01	< 0.01
BMI (kg/m^2)	22.7 ± 3.3	22.8 ± 3.1	23.8 ± 3.3	NS	< 0.05	NS
Clinical stage (ASC/CH/cirrhosis)	40/77/18	37/140/55	−/−/−	< 0.005		
FPG (mg/dl)	96 ± 8	99 ± 10	100 ± 9	< 0.005	NS	< 0.01
IRI (μU/L)	8.7 ± 5.6	9.5 ± 5.3	7.9 ± 5.0	NS	< 0.05	NS
HOMA-IR	2.1 ± 1.4	2.4 ± 1.4	2.0 ± 1.3	NS	NS	NS
AST (IU/L)	42 ± 64	49 ± 27	23 ± 7	NS	< 0.0001	< 0.05
ALT (IU/L)	53 ± 121	49 ± 34	19 ± 9	NS	< 0.0001	< 0.05
γ-GTP (IU/L)	38 ± 68	40 ± 44	30 ± 22	NS	NS	NS
Platelet (× 10^9/L)	201 ± 62	168 ± 69	213 ± 61	< 0.0001	< 0.0001	NS
Total cholesterol (mg/dl)	192 ± 32	176 ± 34	197 ± 32	< 0.0001	< 0.0001	NS
Triglyceride (mg/dl)	92 ± 59	91 ± 46	103 ± 46	NS	NS	NS
HCV-RNA genotype 1/2	−	142/46	22/24		< 0.001	

ALT, alanine aminotransferase; ASC, asymptomatic carrier; AST, aspartate aminotransferase; BMI, body mass index; CH, chronic hepatitis; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus; HCV-RNA, hepatitis C virus ribonucleic acid; HOMA-IR, homeostasis model of insulin resistance; IRI, immunoreactive insulin; NS, not significant.

patients with DM (19–21). Most previous studies have provided evidence of a positive association between them, with a few exceptions (22–24).

In this cross-sectional study, we investigated the prevalence of DM and IR in patients with chronic hepatitis C, and compared it with that in patients chronically infected with HBV and those who cleared HCV after interferon treatment as control.

Materials and methods

Diabetes study

Among the 1163 outpatients recruited from patients seropositive for the hepatitis B surface antigen (HBsAg) or the anti-hepatitis C virus antibody (HCV-Ab) who visited the First Department of Medicine, Chiba University Hospital, between January 2003 and December 2004, 87 patients were excluded because 60 patients had HCC and 27 were HCV ribonucleic acid (HCV-RNA) seronegative without previous interferon treatment. Among the remaining 1076 patients, the plasma glucose level and/or the HBA1C level were investigated retrospectively in 952 patients (88.5%), consisting of 544 patients chronically infected with HCV (HCV+chronic), 122 whose HCV had cleared after interferon treatment (HCV+cleared) and 286 chronically infected with HBV (HBV+chronic) (Table 1) (Fig. 1). One hundred and twenty-four patients whose plasma glucose level was not available were excluded: 50 patients chronically infected with HCV, 22 whose HCV had cleared after interferon treatment and 52 chronically infected with HBV. They were younger (45.0 ± 16.5 vs 53.8 ± 14.5, $P < 0.01$) with ASC more prevalent (42.9 vs 21.4%, $P < 0.05$) and cirrhosis less prevalent (3.8 vs 16.6%, $P < 0.05$), and the proportion of males was

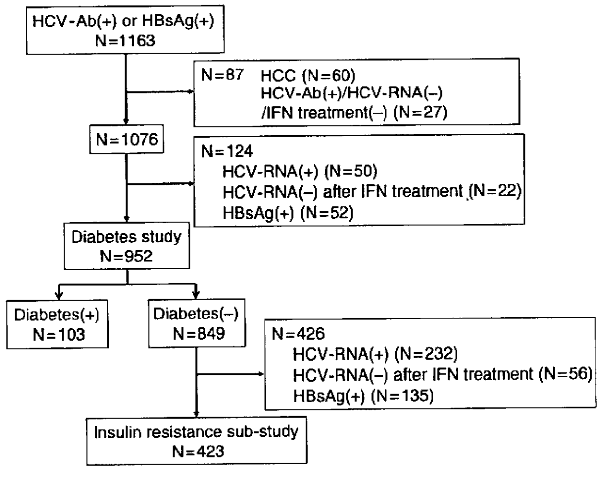

Fig. 1. Flow chart of patients analysed in diabetes study and insulin resistance substudy. IFN, interferon.

almost similar (44.4 vs 52.8%, $P = 0.09$) compared with the 952 patients (Fig. 1). More in-depth data including body mass index (BMI), a key confounder, were not available in the diabetes study because it was a retrospective one.

Patients in the HCV+cleared group had completed interferon therapy at least more than 6 months before entry. Patients in the HCV+chronic group were seropositive for HCV-RNA and HCV-Ab, those in the HCV+cleared group were seropositive for HCV-Ab but seronegative for HCV-RNA, and those in the HBV+chronic group were seropositive for HBsAg. Patients seropositive for HBsAg and HCV-RNA were not included and those with autoimmune hepatitis, primary biliary cirrhosis, haemochromatosis, Wilson's disease, excessive alcohol intake of more than 50 g/day, HCC assessed by imaging examinations such as ultrasonography and computed tomography, and with a history of pancreatitis or pancreatic tumours were also excluded from this study. The definition of an asymptomatic carrier depends on normal alanine aminotransferase (ALT) levels in blood examinations at least two times per year for more than 3 years. The diagnosis of cirrhosis was based on histological findings by liver biopsy in 66 of 138 patients (48%) or on clinical features such as the presence of oesophageal varices, platelet counts $< 100 \times 10^9$/L because of hypersplenism and abdominal ultrasonographical findings (25) in the remaining patients. All cirrhotic patients were of Child-Pugh classification A (86%) or B (13%), except for one patient with HCV.

This study received ethics committee approval according to the 1975 Declaration of Helsinki, and informed consent was obtained from each patient.

Insulin resistance substudy

Four hundred and twenty-three patients were enrolled, selected randomly from 849 patients diagnosed as non-diabetic among the above 952(Fig. 1). Among them, 232 patients were chronically infected with HCV (HCV+chronic), 56 patients had HCV cleared after interferon treatment (HCV+cleared) and 135 patients were chronically infected with HBV (HBV+chronic) (Table 1). The remaining 426 patients not recruited into this study, including 238 patients chronically infected with HCV, 55 patients whose HCV had cleared and 133 patients chronically infected with HBV, showed similar distribution in terms of male gender (52.3 vs 49.2%, $P = 0.37$) and no difference in age distribution (52.3 ± 15.0 vs 54.0 ± 14.6, $P = 0.10$), with cirrhosis being less prevalent (10.0 vs 19.9%, $P < 0.05$).

Presence of diabetes mellitus, hypertension, hyperlipidaemia and obesity and definition of insulin resistance

Patients were considered to have diabetes if they used insulin or hypoglycaemic drugs at the time of the survey or had a fasting plasma glucose level of 126 mg/dl or more, a non-fasting plasma glucose level of 200 mg/dl or more or a haemoglobin A1C level of 6.5% or more. IR was evaluated by the homoeostasis model of IR (HOMA-IR, fasting plasma glucose (mg/dl) $\times$ insulin (μU/ml) $\div$ 405) (26) in patients without overt diabetes and was defined as a HOMA-IR of 2.0 or more. The presence of hypertension was ascertained based on medication history or systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg. The diagnosis of hyperlipidaemia was made on the basis of the medication history and a total cholesterol level above 220 mg/dl or a triglyceride level above 150 mg/dl, and that of obesity was by a BMI (kg/m^2) of more than 25.0. The use of medications to establish diabetes, hyperlipidaemia and hypertension was not only based on patient self-report but was also confirmed by a medical record review.

Laboratory examination

Anti-hepatitis C virus antibody and HCV-RNA were determined by a second-generation enzyme-linked immunosorbent assay (Ortho Diagnostics, Tokyo, Japan) and the reverse-transcriptional polymerase chain reaction method (Amplicore HCV monitor assay version 2.0; Roche, Tokyo, Japan; lower detection limit, 500 copies/ml) (27). The HCV genotype was determined by serological grouping of serum antibody

(28), assuming that genotypes 1a and 1b correspond to group 1 and genotypes 2a and 2b correspond to group 2. Serum blood chemistries including haematological variables were obtained by a standard method using an autoanalyser. HBsAg was measured by an enzyme-linked immunosorbent assay (Abbott Laboratory, North Chicago, IL, USA).

Statistical analysis

Student's *t*-test and Fisher's exact test were used to analyse quantitative and qualitative data respectively. Multivariate logistic regression analysis was used to determine the adjusted odds ratios (ORs) of type 2 diabetes or IR with respect to HCV infection. Variables considered to be potential confounders in multivariate analysis were age, gender and clinical stage of liver disease for the development of diabetes, and age, gender, clinical stage of liver disease, hypertension, BMI, aspartate aminotransaminase (AST), ALT, γ-glutamyl transpeptidase and triglyceride for the development of IR. A *P*-value of < 0.05 was considered to indicate statistical significance.

Results

Diabetes study: prevalence of diabetes mellitus

Patient characteristics

The characteristics of the patients enrolled in this study are shown in Table 1. The mean age was statistically different among the three groups of patients with HBV infection (HBV+chronic), those with HCV infection (HCV+chronic) and those whose HCV had cleared after interferon treatment (HCV+cleared), and the proportion of male gender was also statistically different among the HCV+chronic and HBV+chronic, and HCV+chronic and HCV+cleared groups (Table 1). The clinical stages differed between the HBV+chronic and HCV+chronic groups, with more asymptomatic carriers in the HBV+chronic group and more cirrhotic patients in the HCV+chronic group (Table 1).

Prevalence of diabetes mellitus with various clinical backgrounds

The prevalence of DM was 18/286 (6.3%) in the HBV+chronic group, 74/544 (13.6%) in the HCV+chronic group and 11/122 (9.0%) in the HCV+cleared group, with the prevalence in the HCV+chronic group being significantly greater than that in the HBV+chronic group ($P < 0.005$) (Table 2). This result was also applicable when considering males and females separately. According to stratified age, the prevalence of DM was higher in the HCV+chronic group than in the HBV+chronic group without statistical significance. The prevalence of DM increased according to the progression of liver disease, but the prevalence was similar in asymptomatic carrier patients between the HBV+chronic and HCV+chronic groups (Table 2).

Table 2. Prevalence of diabetes mellitus with various clinical backgrounds in diabetes study

	HBV+chronic (Group B1)	HCV+chronic (Group C1)	HCV+cleared (Group CC1)
Number	286	544	122
Diabetes mellitus	18 (6.3%)***	74 (13.6%)***	11 (9.0%)
Gender			
Male	14 (8.5%)**	48 (18.7%)**	10 (12.2%)
Female	4 (3.3%)*	26 (9.1%)*	1 (2.5%)
Age			
≤ 49	4 (2.4%)	6 (4.8%)	3 (6.8%)
50–59	10 (11.8%)	23 (18.0%)	4 (12.1%)
≥60	4 (10.8%)	45 (15.4%)	4 (8.9%)
Clinical stage			
ASC	6 (6.0%)	5 (6.4%)	–
CH	9 (5.6%)*	44 (12.5%)*	–
Cirrhosis	3 (12%)	25 (22.1%)	–

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.005$.
ASC, asymptomatic carrier; CH, chronic hepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus.

Clinical factors associated with the development of diabetes mellitus in the HBV+chronic and HCV+chronic groups

Multivariate logistic regression analysis revealed male gender, older age and the presence of cirrhosis as independent risk factors for the development of DM, but not HCV infection (Table 3).

Clinical backgrounds and the prevalence of diabetes mellitus in asymptomatic carrier patients in the HCV+chronic and HCV+cleared groups

The prevalence of DM was 5/78 (6.4%) patients who were HCV-infected asymptomatic carriers and 11/122 (9.0%) patients with eradicated HCV after interferon treatment, showing no difference between them ($P = 0.6$). Multivariate logistic regression analysis with gender, age and HCV infection as confounding factors did not show ongoing HCV infection as an independent risk factor for developing DM (data not shown).

Insulin resistance substudy: prevalence of insulin resistance in non-diabetic patients

Patient characteristics

The characteristics of the patients enrolled in the IR substudy are shown in Table 1. Age distribution, proportion of male gender and proportion of clinical stage in the HBV+chronic, HCV+chronic and HCV+cleared groups were similar in the diabetes study and the IR substudy (Table 1). Hypertension was most prevalent in the HCV+chronic group, hyperlipidaemia was less prevalent in the HCV+chronic group owing to a lower total cholesterol level, and obesity was most prevalent in the HCV+cleared group.

Table 3. Clinical factors associated with development of diabetes mellitus in HBV+ or HCV+chronic patients using multivariate logistic regression analysis in diabetes study

Variables	Odds ratio	95% confidence interval	*P*-value
Male (vs female)	2.404	1.497–3.861	0.0003
Age (vs ≤ 49)			
50–59	4.484	2.119–9.434	< 0.0001
≥60	3.774	1.792–7.937	0.0005
Clinical stage (vs asymptomatic carrier)			
Chronic hepatitis	1.408	0.698–2.841	0.3393
Cirrhosis	2.273	1.048–4.926	0.0376
HCV (vs HBV)	1.669	0.917–3.040	0.0936

HBV, hepatitis B virus; HCV, hepatitis C virus.

Prevalence of insulin resistance with various clinical backgrounds

The prevalence of IR was 49/135 (36.3%) in the HBV+chronic group, 126/232 (54.3%) in the HCV+chronic group and 20/56 (35.7%) in the HCV+cleared group, with the prevalence in the HCV+chronic group being significantly higher than that in the HBV+chronic group ($P < 0.005$) (Table 4). This result also applied when considering males and females separately. According to stratified age, the prevalence of IR was higher above the age of 50 years in the HCV+chronic group compared with the HBV+chronic group. According to clinical stage, IR in chronic hepatitis was more prevalent in the HCV+chronic group than in the HBV+chronic group ($P < 0.05$), but the prevalence was similar in asymptomatic carrier patients in these two groups (Table 4).

Table 4. Prevalence of insulin resistance with various clinical backgrounds in insulin resistance substudy

	HBV+ chronic	HCV+ chronic	HCV+ cleared	*P*-value HBV+chronic vs HCV+chronic	HCV+chronic vs HCV+cleared	HBV+chronic vs HCV+cleared
Number	135	232	56			
HOMA-IR≥2.0	49 (36.3%)	126 (54.3%)	20 (35.7%)	< 0.005	< 0.05	NS
Gender						
Male	30 (39.0%)	51 (54.8%)	15 (39.5%)	< 0.05	NS	NS
Female	19 (32.8%)	75 (54.0%)	5 (27.8%)	< 0.01	< 0.05	NS
Age						
≤ 49	34 (43.0%)	18 (40.9%)	6 (30.0%)	NS	NS	NS
50–59	10 (25.6%)	30 (57.7%)	4 (26.7%)	< 0.005	< 0.05	NS
≥60	5 (29.4%)	78 (57.4%)	10 (47.6%)	< 0.05	NS	NS
Clinical stage						
ASC	10 (25.0%)	12 (32.4%)	–	NS		
CH	32 (41.6%)	80 (57.1%)	–	< 0.05		
LC	7 (38.9%)	34 (61.8%)	–	NS		
Hypertension						
(+)	0 (0%)	32 (65.3%)	2 (40%)	< 0.01	NS	NS
(−)	49 (38.0%)	94 (51.4%)	18 (36.0%)	< 0.05	< 0.05	NS
Hyperlipidaemia						
(+)	18 (56.3%)	15 (45.5%)	5 (29.4%)	NS	NS	NS
(−)	31 (30.4%)	111 (55.8%)	15 (38.5%)	< 0.0001	< 0.05	NS
Obesity						
(+)	15 (78.9%)	34 (85.0%)	10 (50%)	NS	*P*< 0.01	NS
(−)	25 (27.8%)	81 (47.9%)	8 (26.7%)	*P*< 0.005	*P*< 0.05	NS

ASC, asymptomatic carrier; CH, chronic hepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostasis model of insulin resistance; LC, cirrhosis; NS, not significant.

Table 5. Clinical factors associated with insulin resistance in HBV+chronic or HCV+chronic patients using multivariate logistic regression analysis in insulin resistance substudy

Variables	Odds ratio	95% confidence interval	*P*-value
Age (vs $\leq$ 49)			
50–59	1.133	0.534–2.404	0.7444
$\geq$60	1.642	0.769–3.509	0.2003
Body mass index (vs < 25)			
$\geq$25	5.765	2.563–12.967	< 0.001
Clinical stage (vs asymptomatic carrier)			
Chronic hepatitis	1.764	0.792–3.922	0.1652
Cirrhosis	2.183	0.820–5.814	0.1180
HCV (vs HBV)	1.531	0.781–3.003	0.2154
AST$\geq$40 (vs < 40)	0.980	0.475–2.021	0.9567
ALT$\geq$40 (vs < 40)	2.595	1.279–5.265	0.0082
γ-glutamyl transpeptidase (vs < 40 IU/L)			
$\geq$40	2.100	1.108–3.981	0.0229
Triglyceride (vs < 100 mg/dl)			
$\geq$100	1.966	1.077–3.588	0.0276

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.

Clinical factors associated with insulin resistance in the HBV+chronic and HCV+chronic groups

One hundred and seventy-five of 367 patients had a HOMA-IR >2.0, were older (55.9 ± 14.1 vs 52.4 ± 15.6, $P < 0.05$), had a higher proportion of HCV infection (72.0 vs 55.2%, $P < 0.01$), had more advanced liver disease (asymptomatic carrier/chronic hepatitis/cirrhosis; 22/112/41 vs 55/105/32, $P < 0.001$) and had higher BMI (24.2 ± 3.0 vs 21.4 ± 2.7, $P < 0.0001$), fasting plasma glucose (101 ± 10 vs 96 ± 8, $P < 0.0001$), immunoreactive insulin (13.3 ± 5.2 vs 5.5 ± 1.7, $P < 0.0001$), AST (56 ± 58 vs 37 ± 23, $P < 0.0001$), ALT (66 ± 107 vs 36 ± 30, $P < 0.0001$), γ-glutamyl transpeptidase (50 ± 69 vs 30 ± 32, $P < 0.0001$) and triglyceride levels (99 ± 46 vs 84 ± 54, $P < 0.0001$) than those with a HOMA-IR < 2.0.

Multivariate logistic regression analysis showed BMI ≥25, ALT ≥40, γ-glutamyl transpeptidase ≥40 and triglyceride ≥100 as independent risk factors, with ORs of 5.765, 2.595, 2.100 and 1.966, respectively, but HCV infection compared with HBV infection was not found to be a statistically significant variable (Table 7).

Clinical factors associated with insulin resistance in the HCV+chronic and HCV+cleared groups

One hundred and forty-six patients showed a HOMA-IR >2.0 among 288 patients in the HCV+chronic and HCV+cleared groups. They were older (60.3 ± 11.3 vs 56.5 ± 14.5, $P < 0.05$), had a higher proportion of HCV infection (86.3 vs 74.6%, $P < 0.05$) and had a higher BMI (24.1 ± 2.9 vs 21.8 ± 3.0, $P <$ < 0.0001), fasting plasma glucose (102 ± 11 vs 97 ± 8, $P < 0.0001$), immunoreactive insulin (12.9 ± 4.9 vs 5.4 ± 1.7, $P < 0.0001$), AST (49 ± 27 vs 38 ± 24, $P < 0.0005$), ALT (51 ± 35 vs 35 ± 29, $P < 0.0001$) and γ-glutamyl transpeptidase levels (43 ± 43 vs 34 ± 38, $P < 0.05$) than those with a HOMA-IR < 2.0.

Multivariate logistic regression analysis showed age over 60, BMI ≥25 and ALT ≥40 as independent risk factors for IR, with ORs of 2.392, 4.749 and 4.634 respectively. However, ongoing HCV infection compared with HCV eradication was not found to be a statistically significant variable (Table 6).

Clinical factors associated with insulin resistance in asymptomatic carrier patients in the HCV+chronic and HCV+cleared groups

Thirteen variables, including HOMA-IR were compared between 37 asymptomatic carrier patients with HCV infection and 56 patients whose HCV had cleared after interferon treatment. Asymptomatic carriers with HCV infection showed a smaller proportion of male gender (27 vs 68%, $P < 0.0005$), a higher level of ALT (23 ± 9 vs 19 ± 9, $P < 0.05$) and a greater proportion of HCV genotype 1 (80 vs 47.8%, $P < 0.05$) compared with patients whose HCV infection had cleared, but the proportion of patients with a HOMA-IR above 2.0 was similar in the two groups (32 vs 36%, $P = 0.8$). These results suggest that HCV infection alone might not be associated with IR.

Discussion

This cross-sectional study showed a weak and not statistically significant higher association of diabetes with HCV infection compared with HBV infection, and advanced liver disease such as cirrhosis and other traditional risk factors for diabetes such as older age and male gender were more closely involved than HCV infection. This result also held true for the association between HCV infection and IR in patients without overt diabetes.

The prevalence of DM increased according to the progression of liver disease in patients with either HBV or HCV infection, and it was higher in patients with HCV than in those with HBV in every clinical stage except for asymptomatic carrier (Table 2). The prevalence of DM was similar in asymptomatic carrier patients with HCV and those with HBV (6.4 and 6.0%, $P = 0.67$) and, furthermore, it was also similar in asymptomatic carrier patients with HCV and those whose HCV had cleared (6.4 and 9.0%, $P = 0.60$), with both groups showing almost normal ALT levels. These observations suggest that not HCV infection itself but the resultant ongoing inflammation and fibrosis of the liver might determine a higher risk for DM. This was also applicable to IR in patients without overt DM, as the present study showed that liver enzyme abnormalities such as elevation of ALT, γ-glutamyl transpeptidase and BMI over 25.0 were independent risk factors for IR but HCV infection itself was not. These results are discordant with the recent report of a transgenic mouse model, in which the HCV core protein was shown to contribute directly to the development of IR by disturbing tyrosine phosphorylation of insulin receptor substrate 1 without inflammation and fibrosis during the observation period (29).

Treatment with interferon or interferon plus ribavirin is now a standard regimen for hepatitis C, and IR was reported to be improved in patients whose HCV had cleared and was not affected in those with relapse or no response after interferon treatment (30). In the present study, we found the prevalence of IR to be higher in patients infected with HCV than in those whose HCV had been cleared, but multivariate logistic regression analysis did not extract HCV infection as an independent risk factor for IR after adjusting for age, BMI and ALT (Table 6). Furthermore, we found no difference in the prevalence of IR between HCV-infected asymptomatic carrier patients and patients whose HCV had cleared (32 vs 36%, $P = 0.83$), both with normal ALT levels.

Table 6. Clinical factors associated with insulin resistance in HCV+chronic or HCV+cleared patients using multivariate logistic regression analysis in insulin resistance substudy

Variables	Odds ratio	95% confidence interval	*P*-value
Male (vs female)	0.870	0.473–1.603	0.6550
Age (vs ≤ 49)			
50–59	1.715	0.700–4.202	0.2376
≥60	2.392	1.093–5.236	0.0290
Body mass index (vs < 25)			
≥25	4.749	2.170–10.393	< 0.0001
HCV-RNA (+) [vs (−)]	1.518	0.646–3.570	0.3383
AST ≥40 (vs < 40 IU/L)	1.101	0.502–2.417	0.8090
ALT ≥40 (vs < 40 IU/L))	4.634	2.153–9.973	< 0.0001
Platelet (vs ≥ 200 × 10^9/L)			
< 200	1.155	0.602–2.213	0.6647
Total cholesterol (vs ≥ 180 mg/dl)			
< 180	1.042	0.564–1.925	0.8946

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; HCV-RNA, hepatitis C virus ribonucleic acid.

There are six major genotypes of HCV classified in the world (31). Among them, genotype 3 has been reported to be closely associated with steatosis in the liver (32), but genotype 3 is not common in Japan. There have been several reports on the association between genotype 1 or 2 and DM (13, 16, 21). In the present study, we could not confirm these findings because there were no differences in the prevalence of DM or IR between patients with genotypes 1 and 2.

The diabetes study is a retrospective one, and hence some important confounding risk factors for diabetes such as BMI were defective. However, similar results were obtained in the prospective IR substudy on the prevalence of IR, antecedent to the development of diabetes, after adjustment for additional confounding risk factors. In the diabetes study, BMI data were available in 396 (48%) of 830 patients and the prevalence of diabetes in these patients was higher in those with HCV infection than in those with HBV infection (33/266 (12.4%) vs 7/130 (5.4%), $P = 0.033$). Multivariate logistic regression analysis showed that the OR of HCV infection was 2.07 after adjustment for age, gender, BMI and clinical stage without statistical significance ($P = 0.1347$).

Previous studies by Caronia *et al.* (15) and Mason *et al.* (21) demonstrated a higher prevalence of DM in patients with HCV-related disease than in those with HBV-related disease in agreement with our findings. Although the present study failed to ascertain HCV infection as an independent factor for diabetes by multivariate logistic regression analysis in discordance with the previous studies (15, 21), the relative OR for diabetes was 1.67 times higher in patients with HCV than in those with HBV. Considering that there is no normal control group, we could not deny the association of HCV infection with DM. Furthermore, HBV infections occur vertically from their mothers at birth in most Japanese patients with chronic hepatitis B, indicating that the duration of infection is almost the same as the age of the patients, but in this study of community-acquired HCV the duration of infection could not be estimated and it is difficult to establish the temporality of the development of hepatitis and diabetes.

In conclusion, this study showed a higher prevalence of DM and IR in patients with HCV infection than in those with HBV infection. However, other factors such as age, male gender, BMI and cirrhosis seemed to be more important risk factors for the development of glucose abnormalities in Japan.

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Efficacy of combination therapy of antiviral and immunosuppressive drugs for the treatment of severe acute exacerbation of chronic hepatitis B

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Background. Patients with severe exacerbation of chronic hepatitis B, sometimes developing into fulminant liver failure, are at high risk for mortality even with antiviral therapy. The efficacy of immunosuppressive therapy in clinically severe exacerbation of chronic hepatitis B has not been well demonstrated. In this study, we evaluated the efficacy of the early introduction of immunosuppressive therapy in combination with antiviral therapy in such patients. ***Methods.*** Forty-two patients, 29 men and 13 women, were defined as having severe exacerbation of chronic hepatitis B based on our uniform criteria, and were enrolled in this study. Sixteen patients between 1982 and 1996 were analyzed retrospectively. We defined the criteria of severe disease in 1997, and then began to introduce sufficient doses of corticosteroids prospectively. Nucleoside analogs were administered in combination with corticosteroids after 1999. Twenty-six patients between 1997 and 2007 were analyzed prospectively. ***Results.*** In the retrospective study between 1982 and 1996, four of 16 (25%) patients recovered. In the prospective study between 1997 and 2007, 17 of 26 (65%) patients recovered; 15 of 17 patients treated with corticosteroids with or without antiviral drugs within 10 days after the diagnosis of severe disease recovered, none of five treated similarly but later than 10 days after the diagnosis recovered, and two of three treated with antiviral drugs recovered. ***Conclusions.*** The early introduction of sufficient doses of corticosteroids and nucleoside analogs could be one option for reversing the potential deterioration of patients with clinically severe, life-threatening exacerbation of chronic hepatitis B.

Key words: chronic hepatitis B, severe exacerbation, immunosuppressive therapy, antiviral therapy

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Introduction

It is well recognized that exacerbation of hepatitis B may occur in chronic hepatitis B virus (HBV) carriers, spontaneously or in relation to cytotoxic or immunosuppressive therapy. A clinical picture of acute hepatitis, and even severe exacerbation, and sometimes fulminant liver failure, may develop that is associated with high mortality.[1] In a retrospective survey in Japan, a 53% incidence of severe hepatitis with a 24% mortality rate (mortality rate of 45% in severe hepatitis) has been reported in relation to chemotherapy in HBV carriers with hematologic malignancies.[2] For the treatment of patients with severe exacerbation without malignancies who progress to serious deterioration, liver transplantation may be a viable option. However, the problems of a shortage of donor livers in Japan and the high cost of the procedure still remain. Thus, therapies other than transplantation must be further investigated for chronic hepatitis B patients with severe exacerbation.

In HBV infection, liver injury is considered to be induced mainly by cytotoxic T lymphocyte-mediated cytolytic pathways of infected hepatocytes.[3] Sjogren et al.[4] suggested that corticosteroids modulate the activity of chronic hepatitis B by suppression of the host immune response to hepatitis B virus antigens, based on a comparison of alanine aminotransferase (ALT) and IgM anti-hepatitis B core antibody (IgM-HBc) levels during the course of short-term high-dose prednisolone therapy. Accordingly, treating chronic hepatitis B patients with corticosteroids to inhibit excessive immune response and prevent cytolysis of infected hepatocytes is a reasonable treatment decision.

Corticosteroids have been administered to treat active chronic hepatitis B since the 1970s. However, in recent years, because the advantage of their use has not been confirmed by controlled studies, their use for the routine management of chronic hepatitis B has fallen out of favor.[5–7] However, these previous studies dealt mainly with cases of clinically nonsevere hepatitis that was not urgently life threatening, and the effects of corticosteroid treatment for severe, potentially life-threatening exacerbation of chronic hepatitis B, as well as the timing and dose of such treatment, have not been well demonstrated. Lau et al.[8] reported that the reintroduction of long-term, high-dose corticosteroids in the early phase of reactivation after withdrawal of immunosuppressive therapy prevents both progressive clinical deterioration and potentially the need for orthotopic liver transplantation.

In our previous study, we investigated the clinicopathological features of patients with severe exacerbation selected by uniform criteria and treated by the early introduction or reintroduction of sufficient doses of corticosteroids, and reported that the introduction of high-dose corticosteroids can significantly reverse deterioration in patients with clinically severe, life-threatening exacerbation of chronic hepatitis B, compared with historical controls, when used in the early stage of illness.[9] Recently, nucleoside analogs have been administered safely even to patients with severe disease,[10–15] but their benefits in terms of the clinical outcome have still to be clarified.

In this study, we treated patients with severe disease by early initiation of sufficient doses of corticosteroids and nucleoside analogs prospectively to clarify the benefits and limitations of the therapy for amelioration of clinically severe exacerbation of chronic hepatitis B.

Patients and methods

Patients

Forty-two patients with severe exacerbation of chronic hepatitis B who were admitted to our liver unit (Chiba University Hospital and related hospitals) between 1982 and 2007 were studied. The diagnosis of chronic hepatitis B viral carrier was made based on either hepatitis B surface antigen (HBsAg) positivity for at least 6 months before entry, or, in patients with a follow-up period of less than 6 months before entry, on hepatitis B surface antigen positivity, a high anti-hepatitis B core antibody (HBcAb) titer, and IgM-HBc negativity or a low titer. The patients fulfilling all of the following three criteria during the disease course were defined as having severe exacerbation: prothrombin time (PT) activity less than 60% of normal control, total bilirubin (T-Bil) greater than 3.0 mg/dl, and ALT greater than 300 IU/l. All patients were in poor general condition, including general malaise, fatigue, jaundice, edema, ascites, and encephalopathy. A histological examination was performed in patients during the convalescent phase or after their death.

All patients were negative for IgM anti-hepatitis A virus (HAV) antibody, anti-hepatitis D antibody, anti-hepatitis C virus (HCV) antibody, HCV RNA, IgM anti-Epstein-Barr virus antibody (IgM-EBV), IgM anti-herpes simplex virus antibody (IgM-HSV), IgM anti-cytomegalovirus antibody (IgM-CMV), anti-nuclear antibody, anti-smooth muscle antibody, liver kidney microsomal antibody 1, and anti-mitochondrial antibody. Patients with histories of recent exposure to drugs and chemical agents as well as of recent heavy alcohol intake were excluded. One patient was human immunodeficiency virus (HIV) positive, but had no clinical evidence of acquired immune deficiency syndrome.

Protocols for treatment

Twenty-six patients treated after 1997 were examined prospectively and 16 treated before 1996 were examined retrospectively. Of the prospectively studied group, informed consent was obtained from the patients or appropriate family members. Patients were treated by early introduction of corticosteroids (CS) (early CS) as follows: 60 mg or more of prednisolone daily was administered within 10 days after the diagnosis of severe disease using the above-mentioned criteria. This dose was maintained for a minimum of 4 days. When the patient showed a trend toward remission in PT, the dose was reduced by 10 mg at least every 4 days to 30 mg. Then, the dose was further tapered by 2.5 or 5 mg every 2 weeks or longer, depending on the decreasing trend of the ALT level, in the period of immunosuppressive monotherapy before nucleoside analogs were administered. Afterward, lamivudine (LMV), adefovir (ADV), and entecavir (ETV), nucleoside analogs with significant inhibition of HBV DNA polymerase, could be used safely in patients with severe disease.[10–15] In Japan, LMV for HIV and chronic hepatitis B became available in 1997 and 2000, respectively. The CS dose was reduced more rapidly and tapered off while monitoring the viral load reduction after 1999, when we began to administer nucleoside analogs.

Patients who had already passed more than 10 days after the diagnosis before being admitted to our unit were treated by the delayed introduction of corticosteroids (delayed CS). Patients with marked prolongation of PT were treated with 1000 mg of methylprednisolone daily for 3 days followed by the same prednisolone therapy as described above. After 1998, LMV was administered at a daily dose of 100–300 mg. ADV was administered at a daily dose of 10 mg to LMV-breakthrough hepatitis. ETV was administered at a daily dose of 0.5–1.0 mg. Patients who showed a trend toward remission or irreversible hepatic failure at admission were treated with intravenous glycyrrhizin (stronger neominophagen C, SNMC), an aqueous extract of licorice root, at 60–100 ml/day. SNMC is reported to have anti-inflammatory activity and has been used for the treatment of chronic viral hepatitis in Japan.[16]

In the retrospective study before 1996, two patients with deep hepatic coma on admission were treated with a combination therapy of CS and interferon (IFN). IFN β was administered at 3 million units/day. Cyclosporin A (CyA) was administered to one patient, and IFN monotherapy to one.

Serological markers

HBsAg, hepatitis B e antigen (HBeAg), anti-HBe antibody (HBeAb), HBcAb, IgM-HBc, IgM anti-HAV antibody, and anti-hepatitis D antibody were detected by commercial radioimmunoassay (Abbott Laboratories, Chicago, IL, USA), and HCV RNA was measured by nested reverse transcriptase-polymerase chain reaction.[17] Second generation anti-HCV antibody was measured by enzyme immunoassay (Ortho Diagnostics, Tokyo, Japan). IgM-EBV, IgM-CMV, and IgM-HSV were examined by enzyme-linked immunosorbent assay. Anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody, and anti-liver kidney microsomal 1 antibody were examined by the fluorescent antibody method. HBV DNA polymerase was assayed according to the method of Kaplan et al.[18] The HBV DNA level was measured by hybridization assay (Abbott), branched DNA hybridization assay (Chiron, Emeryville, CA, USA), transcription-mediated amplification and hybridization protection assay (Chugai Diagnosis Science, Tokyo, Japan), or Amplicor HBV monitor (Roche Diagnostics, Tokyo, Japan).

Statistical analysis

Differences in proportions among the groups were compared by Fisher's exact probability test, Student's t test, or Welch's t test.

Results

Clinical features of severe chronic hepatitis B patients on admission

Of the 42 patients fulfilling the criteria of severe exacerbation, 29 were men and 13 were women. Mean age at the time of admission was 50.3 ± 13.5 years. Mean PT activity was 32 ± 14%, mean ALT was 820 ± 860 IU/l, and mean T-Bil was 12.1 ± 7.5 mg/dl. HBeAg/HBeAb status was +/– in 15, –/+ in 23, +/+ in two and –/– in two. Sixteen patients (38%) had primary diseases or conditions (five, non-Hodgkin's lymphoma; two, ulcerative colitis; and one each, acute lymphocytic leukemia, breast cancer, rheumatoid arthritis, pemphigoid, aplastic anemia, alcoholic hepatitis, HIV-positive, schizophrenia, Down's syndrome, and mental retardation), and 11 (26%) had been treated with immunosuppressive or cytotoxic drugs and suffered exacerbation after their withdrawal.

In the 1982–1996 period, the main treatment was delayed CS (period I), retrospectively. We defined the criteria of severe disease in 1997, and in the 1997–1998 period began to introduce CS as soon as possible after reaching a diagnosis; the main treatment was early CS (period II). After 1999, we administered LMV in combination with CS, and ETV after 2007; the main treatment was early CS and nucleoside analogs (NA) (period III). The three periods included 16, 10, and 16 patients, respectively (Table 1). Mean ages were 53.7 ± 14.0 years in 1982–1996, 43.0 ± 13.6 in 1997–1998, and 51.4 ± 12.0 in 1999–2007. Mean PT activities were 28 ± 14%, 33 ± 12%, and 36 ± 14%, mean ALT levels were 593 ± 853 IU/l, 1290 ± 1005, and 753 ± 693, and mean T-Bil levels were 15.8 ± 7.8 mg/dl, 9.9 ± 5.4, and 9.8 ± 7.2, respectively. Differences in age, sex, PT, ALT, HBe Ag/Ab status, and use of preimmunosuppressive or cytotoxic therapies for primary diseases were not significant in any of the periods. The T-Bil level was higher in period I than in period II ($P = 0.047$) or period III ($P = 0.03$). The time between the diagnosis of severe disease and the introduction of immunosuppressive drugs was longer in period I than in period II ($P = 0.02$) or period III ($P = 0.02$) (Table 1).

All surviving patients except one in period III were free of hepatic encephalopathy during the treatment course. Six patients (two in period I and four in period III) had hepatic encephalopathy at admission, and five did not respond to any therapy, including artificial liver supports such as plasma exchange and hemodiafiltration. One surviving patient in period III had grade II encephalopathy at admission. Twenty failed to respond to any therapy, including artificial liver supports, and gradually developed hepatic failure and died. One with mental retardation and recurrent pneumonia was treated with SNMC in period I and died of sepsis.

Therapies in each period

During 1982–1996, seven patients were treated with delayed CS, three with SNMC, two with delayed CS and IFN, two with early CS, one with CyA, and one with IFN. During 1997–1998, seven patients were treated with early CS, two with delayed CS, and one with delayed CS and LMV. During 1999–2007, seven patients were treated with early CS and LMV, two with early CS

Table 1. Clinical and biochemical features of patients according to study period

	1982–1996 (period I) Retrospective study	1997–1998 (period II) Prospective study	1999–2007 (period III) Prospective study
n	16	10	16
Age[a]	53.7 ± 14.0	43.0 ± 13.6	51.4 ± 12.0
Sex (M/F)	10/6	7/3	12/4
PT (%)[a]	28 ± 14	33 ± 12	36 ± 14
ALT (IU/l)[a]	593 ± 853	1290 ± 1005	753 ± 693
T-Bil (mg/dl)[a]	15.8 ± 7.8*	9.9 ± 5.4*	9.8 ± 7.2*
HBeAg/Ab	5/11[d]	5/5[d]	7/9[d]
Duration[a,b]	34.8 ± 43.0**	6.3 ± 5.4**	6.1 ± 4.1**
Pretherapy[c]	4	3	5
Recovery	4***	7***	10***

PT, prothrombin time; ALT, alanine aminotransferase; T-Bil, total bilirubin; HBeAg, hepatitis B e antigen; Ab, antibody
* $P = 0.047$, between periods I and II; $P = 0.03$ between periods I and III; Student's t test
** $P = 0.02$ between periods I and II, and between periods I and III; Welch's t test
*** $P = 0.03$ between periods I and II; $P = 0.04$ between periods I and III; Fisher's exact probability test
[a] Mean ± SD
[b] Time between the diagnosis of severe disease and introduction of corticosteroids
[c] Use of preimmunosuppressive or cytotoxic therapies for primary diseases
[d] Statistically not significant

Table 2. Therapies and clinical outcomes in each period

Period	Therapy Early CS	Non-early CS	Recovery rate
I. 1982–1996 (retrospective)			4/16
	Early CS		2/2
		Delayed CS	0/7
		Delayed CS + IFN	0/2
		Cyclosporine	0/1
		IFN	0/1
		SNMC	2/3
II. 1997–1998 (prospective)			7/10
	Early CS		7/7
		Delayed CS	0/2
		Delayed CS + LMV	0/1
III. 1999–2007 (prospective)			10/16
	Early CS + LMV		6/7
	Early CS + ETV		2/2
	Early CS + IFN, LMV		0/1
		Delayed CS + LMV	0/2
		LMV	1/2
		IFN, ADV	1/1
		SNMC	0/1

CS, corticosteroid; IFN, interferon; SNMC, stronger neominophagen C; LMV, lamivudine; ETV, entecavir; ADV, adefovir

and ETV, two with delayed CS and LMV, two with LMV, one with early CS, IFN, and LMV, one with IFN and ADV, and one with SNMC (Table 2).

Clinical outcome according to period

Overall, 21 (50%) of 42 patients survived. Four (25%) patients survived among those in 1982–1996 (period I), seven (70%) in 1997–1998 (period II), and ten (63%) in 1999–2007 (period III). The recovery rate was lower in period I than in period II ($P = 0.03$) or period III ($P = 0.04$) (Tables 1 and 2, Fig. 1).

Clinical outcomes according to therapy

In the retrospective study between 1982 and 1996, none of the ten patients survived who received delayed immunosuppressive therapy with or without IFN; two (100%) who received early CS, none who received IFN, and two (67%) who received SNMC survived. In the prospective study between 1997 and 2007, 15 (88%) patients survived who were treated with early CS with or without antiviral drugs, but none of five who were treated with delayed CS with or without antiviral drugs, two (67%) who received antiviral drugs, and none who received SNMC (Table 2) survived.

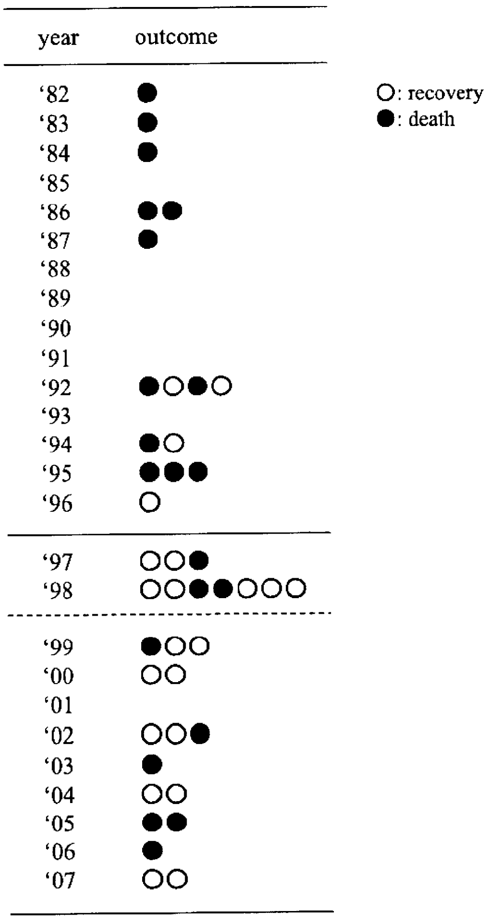

Fig. 1. Serial changes and clinical outcomes of patients

Comparison of CS monotherapy and CS and NA combination therapy in the prospective study

In the prospective study, the clinical features of patients treated with CS monotherapy and CS and NA combination therapy were compared. The differences in age, sex, PT, ALT, T-Bil, HBe Ag/Ab status, time between the diagnosis of severe disease and introduction of corticosteroids, and recovery rate were not significant between these groups (Table 3).

Clinical outcomes according to the time between the diagnosis of severe disease and the introduction of immunosuppressive drugs

Overall, immunosuppressive drugs were introduced to 19 patients within 10 days after the diagnosis of severe disease, and 17 (89%) recovered. In contrast, when they were introduced later than 10 days after the diagnosis of severe disease, none of 12 recovered (Fig. 2). The exact number of days before immunosuppressive drug introduction was not known in three patients, but they were obviously introduced later than 10 days after the diagnosis.

In the retrospective study between 1982 and 1996, both of two patients in whom immunosuppressive drugs were initiated within 10 days after the diagnosis of severe disease recovered, and all eight with initiation 11 days or longer after diagnosis died. In the prospective study between 1997 and 2007, 15 of 17 patients administered immunosuppressive drugs within 10 days after the diagnosis recovered, and all four treated 11 days or longer after diagnosis died.

Viral kinetics during therapies in CS and NA combination therapy

Among eight patients receiving early CS and NA therapy of the prospective study group, the hepatitis B

Table 3. Comparison of CS monotherapy and CS and NA combination therapy in the prospective study

	CS monotherapy	CS and NA combination therapy
n	9	13
Age[a]	44.6 ± 13.4	47.6 ± 13.3
Sex (M/F)	6/3	10/3
PT (%)[a]	34 ± 12	35 ± 14
ALT (IU/l)[a]	1392 ± 1010	863 ± 691
T-Bil (mg/dl)[a]	9.7 ± 5.7	8.2 ± 5.2
HBeAg/Ab	4/5	6/8
Duration[a,b]	6.3 ± 5.4	5.8 ± 4.0
Recovery	7	8

No statistically significant differences were observed
NA, nucleoside analog
[a]Mean ± SD
[b]Time between the diagnosis of severe disease and introduction of corticosteroids

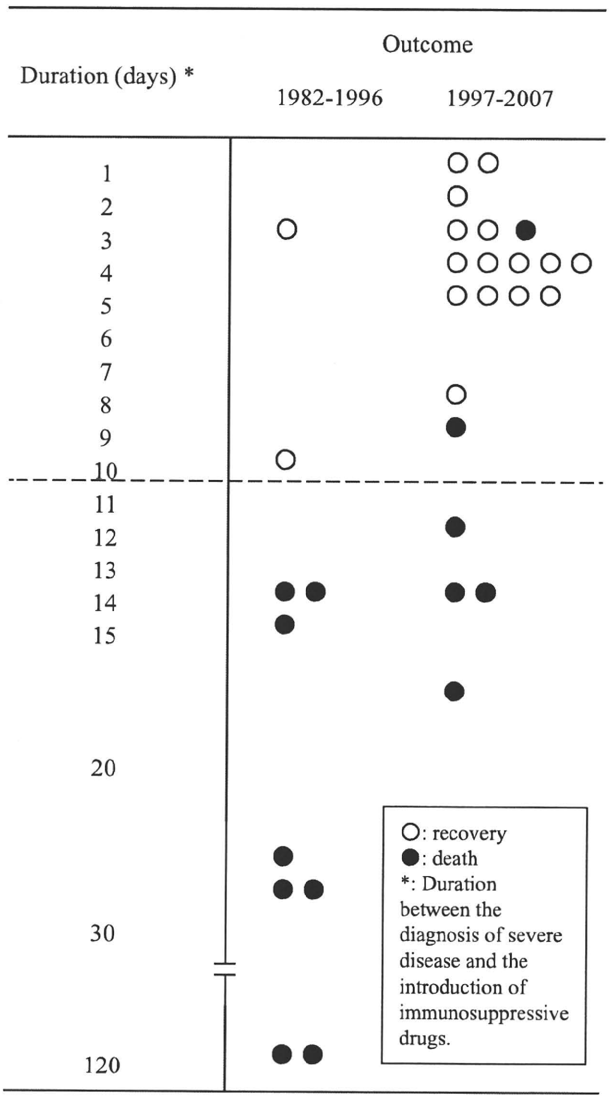

Fig. 2. Clinical outcomes according to the time between the diagnosis of severe disease and the introduction of immunosuppressive drugs

viral load was 6.5 ± 1.7 log copies/ml before treatment initiation (at week 0), 5.1 ± 1.2 at 2 weeks after treatment initiation, and 4.0 ± 1.3 at 4 weeks. The difference between the load at week 0 and at 4 weeks was significant ($P < 0.01$) (Fig. 3). Figure 3 shows the viral kinetics in the eight patients receiving early CS and NA therapy, one receiving delayed CS and NA therapy, and two receiving NA therapy.

Long-term outcomes of survivors

Of the nine survivors in the early CS group, two had LMV introduced afterward and three did not (four

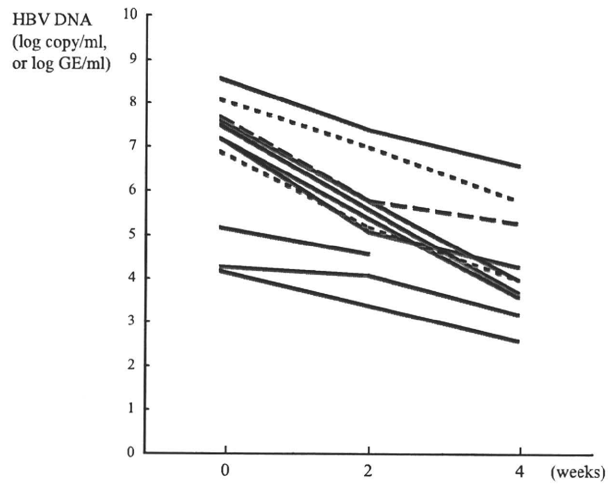

Fig. 3. Viral kinetics during therapies in the prospective study. *Solid*, *dashed*, and *dotted* lines denote early corticosteroid (CS) and nucleoside analog (NA) therapy, delayed CS and NA therapy, and NA therapy alone, respectively. *HBV*, hepatitis B virus; *GE*, genome equivalents

unknown), and corticosteroids were tapered to zero within a year in all five of these patients.

One patient with liver cirrhosis in the LMV group was introduced to ADV 2 years later, and the viral load decreased, but liver failure developed gradually. He received a living donor-liver transplantation from his son 3 years later.

Discussion

The prognosis of severe exacerbation of chronic hepatitis B is poor if signs of liver failure appear.[1,2,19] Recently, nucleoside analogs (NA), which exhibit a strong inhibitory effect on HBV replication, have been administered to patients with chronic hepatitis B, and dramatic improvements have been achieved. NA can be administered safely even to patients with severe disease,[10–15] but mortality is still high in patients demonstrating liver failure. A nationwide survey of fulminant hepatitis and late-onset hepatic failure between 1998 and 2003 in Japan revealed that the prognosis was especially poor in HBV carriers even after the introduction of LMV.[20] Tsubota et al.[21] reported that LMV monotherapy conferred no significant protection against rapid progression of the disease to liver failure in cases of severe acute exacerbation of chronic hepatitis B. Similar results were reported by Chan et al.[22] and Chien et al.[23] With the administration of NA, HBV DNA is reduced rapidly, but the improvement in liver function is delayed by a few weeks to a few months.[12] During the time lag, excessive immunological reaction may continue and liver cell injury may progress. If in this phase effective therapeutic approaches were available, they would certainly be beneficial for these patients.

In our previous study, we described that the introduction of high-dose CS can reverse deterioration in patients with clinically severe, life-threatening exacerbation of chronic hepatitis B, when used in the early stage of illness.[9] We defined the criteria of severe disease in 1997, treated patients with severe disease with early initiation of CS of sufficient doses after 1997 prospectively, and after 1999 we used the combination of early and sufficient doses of CS and NA. In the present study, we examined the effect of the combination therapy of CS and NA.

One reason for not using CS for the treatment of chronic hepatitis B is that CS might enhance HBV replication through a steroid responsive element in the HBV genome.[24] In our previous study, none of the patients given high doses of CS showed increases in HBV replication during short-term observation periods.[9] In this study, HBV DNA decreased significantly during the 4-week period from the start of the CS and NA therapy. Gregory et al.[25] reported that in their study steroids would likely have proved beneficial if treatment had been started "much earlier" in the course of the illness. We are also convinced that timing is very important for optimum CS treatment.

In the 1982–1996 period, CS was introduced in the advanced stage of liver failure in most patients, but the recovery rate was low (25%). After we established the criteria of severe disease in 1997, CS was introduced at an earlier stage and the recovery rate improved (70% in 1997–1998). After we could use NA in combination with CS from 1999, we shortened the treatment period of CS while monitoring the viral load, but, contrary to our expectations, the recovery rate did not improve (63%).

Regarding the timing of the therapies, none of the patients recovered when delayed immunosuppressive therapy, with or without antiviral drugs such as NA, were implemented. In contrast, 89% of patients who received early immunosuppressive therapy with or without NA recovered ($P < 0.001$). The importance of the early introduction of immunosuppressive therapy was shown again in the presence of effective NA. Regarding the time between the diagnosis of severe disease and the start of immunosuppressive therapy, 89% of patients administered immunosuppressive therapy within 10 days recovered, but none administered the therapy 11 days or longer after diagnosis recovered. When the start of the treatment is delayed beyond 10 days, large numbers of hepatocytes are likely already destroyed and inhibition of the inflammatory reaction might not be effective. Our cutoff point of 10 days to define "early introduction" seems to be very close to the mark.

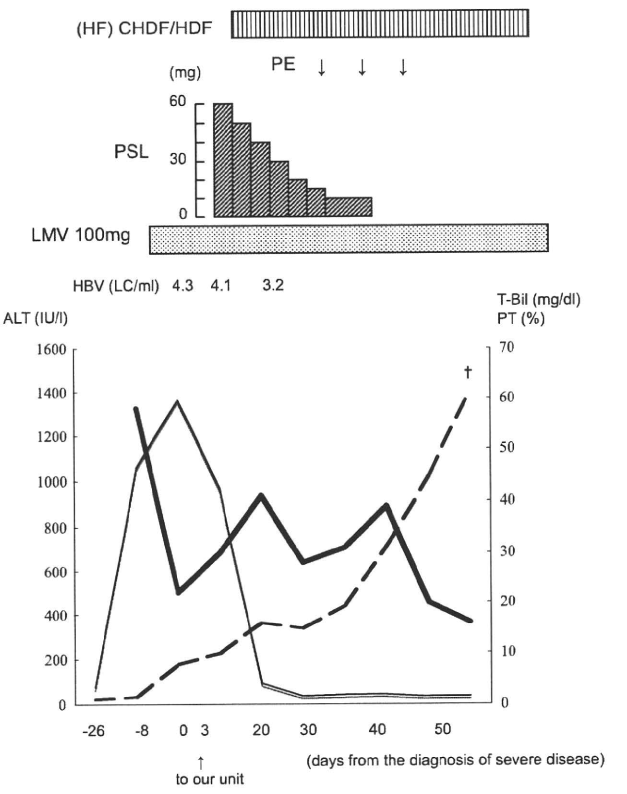

Fig. 4. Clinical course of a 63-year-old female patient. She suffered from chronic hepatitis B with hepatitis B e antigen (HBeAg). She had natural exacerbation, and lamivudine (*LMV*) was administered before the criteria of severe disease were fulfilled. Eight days after the start of LMV, she showed hepatic encephalopathy, marked prolonged prothrombin time (*PT*) activity, and elevated alanine transaminase (*ALT*) and total bilirubin (*T-Bil*). A corticosteroid was administered 9 days after the diagnosis of severe disease, but she did not respond to the therapies. *Thick solid*, *thin solid*, and *dashed lines* denote PT, ALT, and T-Bil, respectively. *PE*, plasma exchange; *(HF) CHDF*, (high flow) continuous hemodiafiltration; *HDF*, hemodiafiltration; *PSL*, prednisolone

Two patients died despite early CS and NA. One was an HIV-positive man who was hospitalized 10 days after the onset of jaundice. Three days after the diagnosis of severe disease, CS and NA were administered, but he did not respond to the therapy. The other was a woman suffering from acute exacerbation in a related hospital, and LMV was administered before the criteria of severe disease were fulfilled. Eight days after the start of LMV, she showed hepatic encephalopathy, marked prolonged PT activity, and elevated ALT and T-Bil. CS was administered 9 days after the diagnosis of severe disease, but she did not respond to the therapy. In both cases, the timing of the diagnosis of severe disease was delayed,