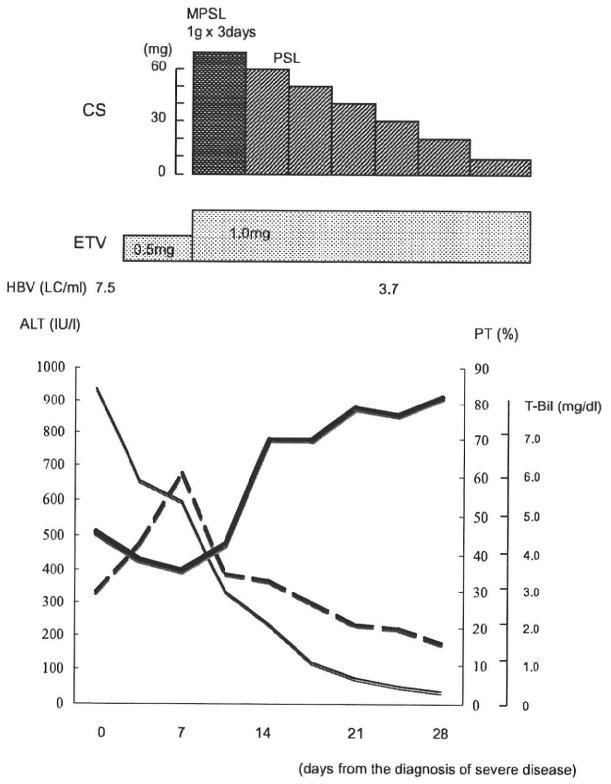

Fig. 5. Clinical course of a 51-year-old male patient. He suffered from chronic hepatitis B with HBeAg. He had natural exacerbation, and entecavir (*ETV*) was administered the day after admission, but he did not show a trend toward remission in PT or T-Bil. A double dose of ETV, together with CS, were introduced 5 days after the diagnosis of severe disease, and he responded to the therapy. *Thick solid*, *thin solid*, and *dashed lines* denote PT, ALT, and T-Bil, respectively. *MPSL*, methylprednisolone

although CS and NA therapy was started within 10 days after this diagnosis. These results emphasize that an even earlier diagnosis of severe disease is required.

Our results highlight the importance of immunosuppressive therapy for preventing the progression to liver failure. As Tsubota et al.[21] reported that an effective therapeutic strategy should be aggressively combined with LMV because LMV lacks the capability of suppressing a hyperimmune reaction, it seems that antiviral therapy is not sufficient to stop progressive deterioration and additional therapy to suppress liver cell degeneration may be necessary. Combination treatment with early high-dose CS and NA might be effective in suppressing the excessive host immune response in the early period. Additionally, NA can make it possible to shorten the term of CS therapy.

In summary, our study demonstrated that the early introduction of high-dose CS treatment in combination with NA may be beneficial for cases of clinically severe acute exacerbation of chronic hepatitis B. We were unable to include placebo-controlled patients, considering the current knowledge of the poor prognosis of such patients and our historical control patients between 1982 and 1996. Nevertheless, delay in treatment may result in fatal liver failure even when these treatment protocols are used, suggesting that an early diagnosis of such patients is urgently required.

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BRIEF REPORT

A mutational shift from domain III to II in the internal ribosome entry site of hepatitis C virus after interferon–ribavirin therapy

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Abstract We focused on the relationship between variation in the IRES of hepatitis C virus (HCV) genotype 1b and clinical outcome, since the internal ribosome entry site (IRES) has a comparatively low heterogeneity and it might be easy to find unique substitutions. Patients infected with HCV were selected using strict criteria, and unique mutations in the IRES were extracted by the subtraction of common mutations. We found that most mutations accumulated in domain III (dIII) of IRES in sustained virological responders (SVRs) and non-SVRs before therapy. However, these mutations were exclusively observed in domain II (dII) in non-SVR at 2 weeks after the start of therapy.

Hepatitis C virus (HCV) is an enveloped RNA virus of the genus *Hepacivirus* in the family *Flaviviridae* [2]. The genomic RNA is a plus strand consisting of approximately 9,600 nucleotides, which contains a large open reading frame and two untranslated regions. The untranslated regions (UTR) are present at each end of the genome (5′ and 3′ termini) and are involved in not only the translation of viral proteins but also genomic replication. An especially highly conserved region (about 341 nucleotides) in the 5′ UTR is known to act as an internal ribosome entry site (IRES), which is essential for the translation of viral proteins [17, 18]. The IRES forms a tertiary structure for ribosome binding and subsequent protein synthesis [4]. An artificial alteration of the sequence can severely affect translational activity [6].

HCV is a significant cause of morbidity and mortality, infecting over 170 million people worldwide. The majority (about 80%) of individuals with HCV infection develop chronic hepatitis, which can progressively lead to cirrhosis (10–20%), and eventually to hepatocellular carcinoma (5%). Despite recent efforts, the current therapy [pegylated-interferon (IFN) with ribavirin] for HCV infection remains inadequate for approximately half of all patients. The mechanisms of tolerance against this therapy are still unknown. HCV is genetically heterogeneous, and it circulates as a population of closely related genomes, referred to as quasispecies [12]. Previous studies have shown that specific regions in the HCV genome, such as the IRES and the NS5B coding region, accumulate nucleotide substitutions in patients receiving antiviral therapy [8, 16]. These results suggest that some mutants show tolerance against the current therapy. However, the significance of genetic variations in these regions is still not fully understood either biochemically or clinically. The quasispecies, which normally appear during treatment, consist of many heterogeneous clones. This heterogeneity makes the interpretation of the relationship between clinical outcome and resistance mutations difficult.

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In this study, we examined the relationship between the IRES of HCV from the pre- and in-treatment sera of the patients (sustained virological responder (SVR) and non-SVR to IFN–ribavirin therapy) and its clinical outcome, because there is said to be a comparatively lower mutant spectrum complexity in the IRES in comparison to the coding region. Previous reports have suggested that no clinically significant variations exist in the IRES [16, 19]. However, we found a significant importance of IRES mutations in resistant HCV clones by selecting patients carefully and isolating the specific mutations for SVRs or non-SVRs.

Among the patients hospitalized at the Kurume University Hospital from 2001 to 2003, seven patients demonstrating HCV genotype 1b with a high viral load (>100 Kilo International Units/ml (KIU/ml) by Amplicor-HCV monitor ver. 2; Roche Molecular Diag. Co., Tokyo, Japan) were included in this study. These patients were carefully selected according to the selection standard, i.e. the patients underwent the standard treatment protocol of IFN–ribavirin therapy. There were no patients with a reduction of drug or a discontinuation of the therapy. Informed consent for this therapy was obtained from every patient, and the study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. We measured the amount of HCV RNA from patients' sera regularly during the treatment for 6 months (Fig. 1) and divided them into two groups, SVR and non-SVR. An SVR was defined as a patient in whose serum HCV RNA was not detected for at least 6 months after the end of the treatment. A non-SVR was defined as a patient in whom HCV RNA levels were reduced slightly but a high level was retained in the serum within 6 months of the end of the treatment. Three of the patients (Pt4, Pt5, and Pt6) were SVRs, while the other patients (Pt1, Pt2, Pt3, and Pt7) were non-SVRs. The HCV RNA levels in the SVRs decreased dramatically to around the lower detection limit within a week. Although the HCV RNA levels in non-SVRs decreased slowly, they remained high for at least 2 weeks.

In order to address the question of whether the IRES correlates with the clinical outcome of HCV, we compared the nucleotide sequence of the IRES between the SVRs and non-SVRs. The patients' sera were collected at pre-treatment 0 and 2 weeks after the start of therapy. Viral RNA was extracted from the serum, and IRES cDNA was obtained by nested RT-PCR. To detect all variations of the IRES, primer cocktail (5′-GCACACCCAACCTGGGGC CC-3′, 5′-CGAGGTTGCGACCGCTCGGAAG-3′, 5′-GA AGCCGCATGTGAGGGTATCGATGAC-3′) was used for reverse transcription. PCR was performed for 35 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 60 s) after pre-heating (94°C, 2 min) using an outer primer pair (5′-GGGGCGA CACTCCACCATAG-3′, 5′-GATCTGACCACCGCCCGG

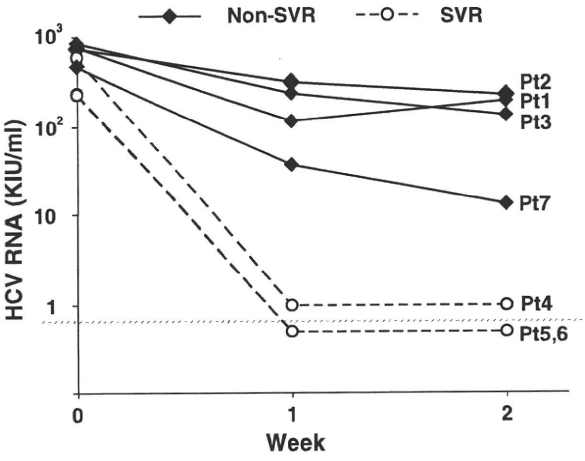

Fig. 1 Viral RNA kinetics in chronically HCV-infected patients undergoing interferon–ribavirin therapy. The *solid* and *dashed lines* indicate the kinetics of the amount of RNA from non-SVRs (*solid diamond*) and SVRs (*open circle*), respectively. The *dashed lines* from two patients' data at the bottom are overlapping. *Horizontal dotted line* shows the limit of detection by Amplicor-HCV monitor ver. 2. All patients received intramuscular IFNα-2b (Intron, Schering-Plough, Kenilworth, NJ) in combination with a daily oral 600–800 mg dose of ribavirin for 24 weeks. For the first 2 weeks of the combination therapy, 6 MU of IFNα-2b were given daily. The IFN dosing frequency was then reduced to 6 MU three times a week for the remaining 22 weeks. The ribavirin dosage was 600 mg daily for the patients who weighed less than 60 kg and 800 mg daily for patients who weighed between 60 and 80 kg

GAAC-3′), and then incubated at 72°C for 10 min. This cycle was again performed under the same conditions using an inner primer pair (5′-GTTTTTCTTTGAGGTTTAGG-3′, 5′-ACACTCCACCATAGATCACTC-3′). The final PCR product of 352 bp was cloned using TA cloning vector (pT7Blue-2, Novagen, USA), and three independent clones from each patient were sequenced (sequencer Model 310, ABI, USA).

IRES sequences of SVRs at 1 and 2 weeks after the start of therapy were unavailable because the amount of RNA was below or near the detection limit, and RNA could not be isolated, although the RNAs were detected by Amplicor-HCV monitor ver. 2, which is one of the qualitative assays with high sensitivity. The mutations specific to the non-SVRs, at pre-treatment (0W) and 2 weeks after the start of therapy (2W), are shown in Fig. 2. The distribution of mutations is summarized in Table 1, including the mutations specific to the SVRs at pre-treatment (0W). For pre-treatment (0W), the distribution of mutations was similar when SVRs and non-SVRs were compared (Table 1), with the exception that mutations were also found in dII in all clones from patient 3 (non-SVR). Previous therapy with IFN might be related to mutation in dII, since patient 3 has a history of IFN monotherapy. In comparison with pre-treatment (0W) and 2 weeks (2W)

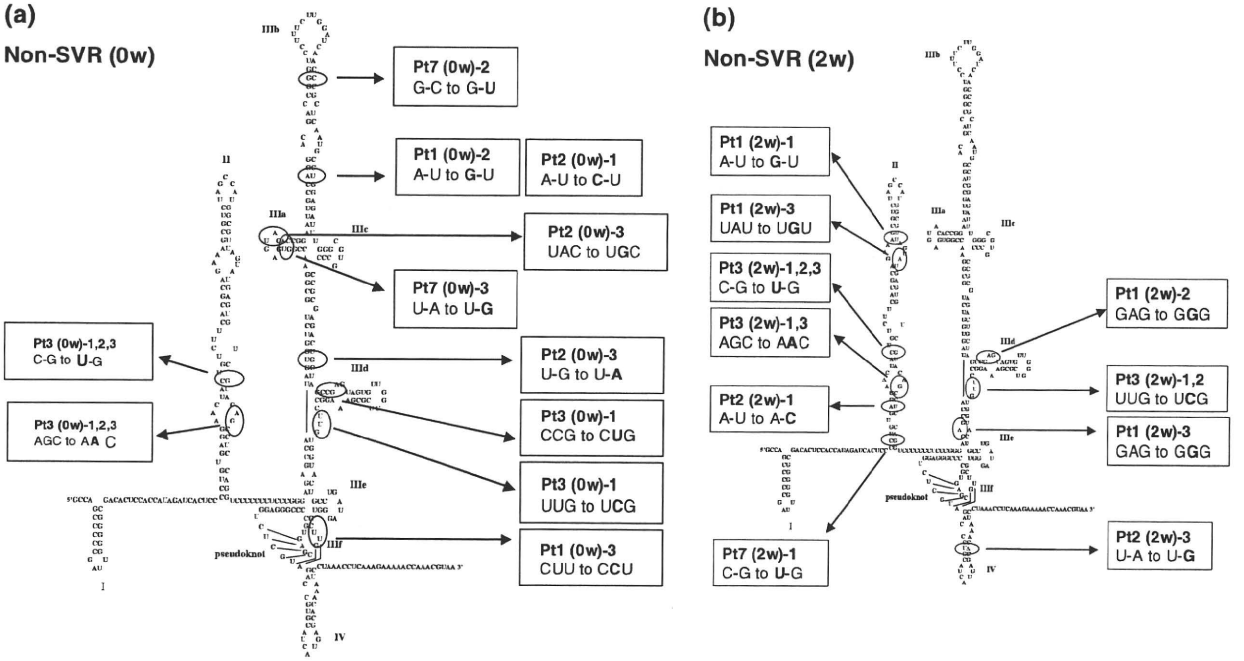

Fig. 2 The nucleotide sequence and predicted secondary structure of the HCV IRES. The nucleotide sequence shows the consensus sequence which was found among SVRs 0 weeks, non-SVRs 0 weeks and non-SVRs 2 weeks. The secondary structure is based on the HCV-JS strain [5]. The original structure is not changed for simplification of the figure, although several base pairings are partly disrupted due to the mutations. The numbers following Pt indicate the patients and each clone number from same patient. The number in brackets shows the week after the start of treatment. The position of mutations found is indicated in the *circle*. **a** Non-SVR-specific heterogeneity at pre-treatment 0 weeks. **b** Non-SVR-specific heterogeneity at 2 weeks after the start of therapy

Table 1 Number of mutations in non-SVRs and SVRs

		SVRs	Non-SVRs	
		0 weeks[a]	0 weeks	2 weeks
dII	UM[b]	0	1	4
	NM	0	1	2
	SM	0	0	0
	Total	0	2	6
dIII	UM	4	5	0
	NM	3	3	3
	SM	0	1	0
	Total	7	9	3
dIV	UM	0	0	1
	NM	1	0	0
	SM	0	0	0
	Total	1	0	1

[a] Week after the start of therapy

[b] *UM* unstable mutation, *NM* null mutation or *SM* (stable mutation) mean mutations that would disrupt the base pairing, not affect the base pairing, or form potential base pairing, respectively

after the start of therapy, in non-SVRs, the number of mutations in dII increased from 2 to 6 (Table 1, and compare Fig. 2a, b). Conversely, the number of mutations in dIII decreased from 9 to 3. These results indicate that mutation was preferentially shifted from dIII to II in non-SVRs during the therapy. It was also noted that the number of mutations which would disrupt the base pairing (referred to as UM in Table 1) in dII increased from 1 to 4, whereas those in dIII decreased from 5 to 0 (Table 1). This suggests that the mutational shift from dIII to II might disrupt several base pairings in dII but restore the base pairing in dIII. Non-SVR clones at 2 weeks after the therapy showed a decreased translational activity using a luciferase reporter gene assay (data not shown). This indicates that the mutational shift from dIII to II in non-SVRs leads to a decrease in viral translational activity.

Recently, comparing the IRES sequences of non-SVRs with those of SVRs, a few specific nucleotide substitutions have been observed [14, 16, 19]. No clinically significant variations have been reported in the IRES. However, we found a relationship between IRES mutations and clinical outcome. The discrepancy between previous reports and our results may be ascribed to several methodological differences. First, we selected the patients in order to obtain a uniform background of patients using strict criteria. Second, we extracted unique mutations specific for SVRs or non-SVRs in order to identify the principal mutations. Our study demonstrates that mutation was preferentially

shifted from dIII to II in non-SVRs during IFN–ribavirin therapy. Moreover, a similar tendency of mutational shift was observed with patients currently undergoing therapy with PegIFN–ribavirin (data not shown), suggesting a relationship between IRES mutation and clinical outcome.

It is apparent that the accumulation of unstable mutations in dIII shifts to that of unstable mutations in dII during treatment (Table 1). The same result was also obtained using a new IRES structure model [10] instead of using the previous model described in this study (Fig. 2). dIII has been reported to directly bind to the ribosome to stabilize the IRES-ribosome complex, whereas dII is involved in triplet decoding and therefore does not bind to the ribosome directly [9, 15]. It is thus possible that a mutation in dIII rather than dII may dramatically change the ribosome binding activity, e.g. the release of the IRES from the ribosome, and the degradation of free IRES and HCV RNA [11] or vice versa. Why does the dIII mutant in SVRs and non-SVRs accumulate before treatment? It may be partly because dIII mutant has a dominant trait, e.g. the translational activity of the dIII mutant is higher than that of the dII mutant (data not shown). And why are dIII mutants in SVRs and non-SVRs sensitive to therapy? dIII mutant may exist at the error threshold point, as suggested by the error catastrophe theory regarding viruses [1]. Therefore, if drugs that perturb the viral load, e.g. ribavirin and IFN, are added, then the clone may rapidly disappear with, for example, catastrophic breakdown of the ribosome-dIII complex. On the contrary, dII mutant may have recessive trait, e.g. inefficient translational activity (data not shown). However, this trait, conversely, might be an advantage for escape from the immune system, because inefficient translational activity might lead to a reduction in the number of viral proteins that are recognized by immune cells. An HCV variant containing a dIII deletion in the IRES, described in a previous report [13], might be the extreme escape mutant.

It has generally been thought that the decay curve of HCV in patients undergoing combination therapy with IFN–ribavirin basically exhibits a biphasic pattern in both SVRs and non-SVRs [3]. The decay curve of HCV in this study also seems to be biphasic: the first phase with a rapid decrease during 0–7 days, and the second phase with a slower decrease after 7 days (Fig. 1) except Pt1 (non-SVR). HCV in patients is a mixture of genetically distinct variants known as quasispecies. We speculate that HCV is composed of several variants before therapy, including mutations in dII that are resistant to therapy and in dIII that are sensitive to therapy. Particularly in non-SVRs, dIII mutant might be predominant and excluded rapidly in the first phase, whereas the dII mutants may exist as a small population in the first phase, resist the therapy, become a predominant population, and show slower decrease in the second phase. Theoretically, it is unlikely that a dII mutant would be directly derived from a dIII mutant, because such mutants would require simultaneous mutations in a short period: firstly a preferential mutation in dII, and secondly a selective mutation which reverts to the original dIII. On the other hand, in SVRs, the population size of the dIII mutant would be larger than that of the non-SVRs before therapy, and thus the decay curve shows a more rapid decrease than that of the non-SVRs. The dII mutant in SVRs may be a much smaller population than that in non-SVRs, possibly below or near the detection limit by PCR.

We propose that dII might be a potential target for antiviral therapy to improve long-lasting therapy. Indeed, siRNAs specific for highly conserved regions of HCV, including dII, inhibited virus translation and subgenomic replication in cultured cells [7]. The siRNA specific for the mutated position in dII might suppress dII mutant clones and shorten the length of therapy. Also, a determination of the IRES sequence in the dII mutant before therapy should be useful for the prediction of drug response and rapid design of siRNAs.

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ORIGINAL ARTICLE

Predictive Factors Associated with the Progression to Hepatic Failure Caused by Lamivudine-Resistant HBV

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Abstract The aims of this study were to select the patients with a potential for progression to hepatic failure due to lamivudine-resistant HBV and to standardize the treatment for patients with lamivudine-resistant HBV. Patients ($n = 47$) with reactivated hepatitis due to lamivudine-resistant HBV were classified into two groups, with and without potential for progression to hepatic failure, according to the criteria using the data of serum bilirubin level and prothrombin activity after the reactivated hepatitis. Multivariate analysis showed that prothrombin activity at the initiation of lamivudine therapy was related to the deterioration of the liver function after the emergence of lamivudine-resistant HBV ($P = 0.0025$, 95%CI 0.8269–0.9601). We assume that earlier additional or substitutive treatment with other antiviral agent, such as adefovir dipivoxil, should be recommended when the lamivudine-resistant HBV is detected in patients with the history of decompensated liver disease before the administration of lamivudine, even when hepatitis has not been reactivated yet.

Keywords Hepatitis B virus · Liver cirrhosis · Lamivudine · Adefovir dipivoxil · Hepatic failure · YMDD motif

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Introduction

Hepatitis B virus (HBV) infection remains a major global health problem. Chronic HBV infection is one of the major causes of cirrhosis and hepatocellular carcinoma in endemic areas, causing more than 1 million deaths per year [1].

Until recently, interferon (IFN) was the only effective antiviral agent for patients with chronic HBV infection. Although treatment with IFN benefits some patients [2–4], the overall response rate is less than 40% [3, 5, 6]. IFN treatment is occasionally contraindicated in patients with liver cirrhosis because of the risk of potentially life-threatening complications [7, 8]. Liver transplantation with antiviral prophylaxis is available as a potential salvage therapy for some patients [9], but this is possible only in a limited number of countries and not available in the rest of the world where HBV is endemic [1].

Recently, lamivudine has become the main therapeutic option for the treatment of chronic HBV infection. Lamivudine is a potent inhibitor of HBV replication by suppressing HBV-DNA polymerase. Lamivudine leads to a rapid and profound decrease in serum HBV-DNA levels, reduces disease activity, and increases the rate of hepatitis B e (HBe)-seroconversion significantly with few adverse events [10, 11]. It is also reported that treatment with lamivudine induces histological improvement [12]. Although lamivudine is a great benefit for patients with chronic HBV infection, the emergence of lamivudine-resistant HBV is the major drawback of lamivudine treatment. Lamivudine-resistant HBV strains contain methionine (M) to isoleucine (I) or valine (V) substitutions in the YMDD motif in the C-domain of the RNA-dependent DNA polymerase [13, 14]. In recent clinical trials, the emergence of lamivudine-resistant HBV occurs in 14–32% of patients after 1 year of therapy [9, 10]. Although longer treatment with lamivudine

increases the rate of HBe-seroconversion, prolonged treatment with lamivudine has been associated with the emergence of lamivudine-resistant HBV with mutations in the YMDD motif [15]. The incidence of YMDD variants was up to 74% after 4 years of lamivudine therapy [16]. The clinical course after the selection of lamivudine-resistant HBV seems to be benign [17]. The variant HBV is replication-incompetent compared with the wild-type HBV [18]. HBV-DNA levels and alanine aminotransferase (ALT) levels remained lower by comparison with baseline values in the majority of patients with lamivudine-resistant HBV. However, in some literature, it has been reported that hepatic failure and mortality developed after the emergence of lamivudine-resistant HBV [19–22]. Therefore, it should be significant clinically to select patients with the risk of progression to hepatic failure, and it may be possible to prevent reactivated hepatitis from progressing to a fatal disease if the proper countermeasures are taken before the development of hepatic failure. In order to prevent reactivated hepatitis progressing to a fatal disease, we should select patients with the potential for developing hepatic failure due to lamivudine-resistant HBV among all patients who are treated with lamivudine.

However, there is little information about the predictive factors associated with the development of hepatic failure after the emergence of lamivudine-resistant HBV. For the purpose of detecting the predictive factors associated with the progression of the disease, we retrospectively analyzed the clinical and virological characteristics of patients with the viral breakthrough.

Methods

Patients

Forty-seven patients who were treated with lamivudine because of HBV-related chronic liver disease were studied. None of the patients enrolled in this study were treated with an antiviral agent such as IFN or other nucleoside analogues within 3 years of the initiation of the treatment with lamivudine. The patients were followed up every 2 weeks for the first 4 weeks and then every 4 weeks throughout the study. Routine liver function tests, complete blood counts and coagulation tests were determined every 4 weeks. HBV-related serological markers, including hepatitis B e antigen (HBeAg) and antibody to hepatitis B e antigen (anti-HBe), and serum levels of HBV-DNA were determined every 4 weeks during the treatment. The treatment with lamivudine was continued throughout this study. In all patients, the lamivudine-resistant HBV emerged and the elevation of serum ALT level was observed during the treatment with lamivudine. No patients were positive for antibody to hepatitis C virus (anti-HCV) and were diagnosed as having autoimmune hepatitis or drug-induced liver injury at the initiation of the treatment with lamivudine. The endpoint of this study was the last observation of patients without antiviral agents in addition to lamivudine. In patients who were treated with additional antiviral agents, such as IFN or adefovir dipivoxil, the endpoint of this study was the observation when such additional treatment started.

Serological Markers and HBV-DNA

HBeAg and anti-HBe were tested with commercial assay kits (Abbott Laboratories, North Chicago, IL). Serum HBV-DNA was quantified with commercial assay kits (Roche Amplicor Monitor polymerase chain reaction). The detection range was 2.6–7.6 log copies (LC)/ml ($10^{2.6}$–$10^{7.6}$ copies/ml). In statistical analysis, more than 7.6 LC/ml was calculated as 7.7 LC/ml. The commercially available kit, the SMITEST HBV-YMDD motif ELMA (Sumitomo Metal Industries, Tokyo, Japan), was used according to the manufacturer's instructions in order to detect the lamivudine-resistant HBV strains. The principle of this procedure is a combination of the PCR–ELISA and mini-sequence methods [23].

Classification by Serum Levels of Total Bilirubin and Prothrombin Activity

Patients enrolled in this study were placed in two categories according to the following criteria. A patient without an elevation in the serum level of total bilirubin to more than 1.5 mg/dl, but without a decline in prothrombin activity to less than 60% after the reactivation of hepatitis caused by lamivudine-resistant HBV, was defined as a patient without the potential for developing hepatic failure. On the other hand, a patient with an elevation in the serum level of total bilirubin to more than 1.5 mg/dl, and/or with a decline in prothrombin activity to less than 60% after the reactivation of hepatitis caused by lamivudine-resistant HBV, was defined as a patient with the potential for progression to hepatic failure. In our institution, the commercially available reagents, Thromborel® S (Dade Behring, Marburg, Germany) and Iatro LQ T-BIL (Mitsubishi Kagaku Iatron, Tokyo, Japan), are used for the examination of prothrombin activity and serum level of total bilirubin. The lower limit of the normal range of the prothrombin activity is 60% and the upper limit of the normal range of the total bilirubin level is 1.5 mg/dl. We used these two values of each parameter for the division of the two groups for two reasons. One reason was that we had no experiences that reactive hepatitis of patients whose prothrombin and bilirubin

level were keeping within the normal range progressed to hepatic failure. The other was that GlaxoSmithKline, U.K., had provided adefovir dipivoxil to Japanese patients with reactivated hepatitis under the condition that one or both of these two variables were out of the normal range of each institution before approval by the Ministry of Welfare and Labor of the Japanese government. This standard for supply of adefovir dipivoxil was established by the cooperation of GlaxoSmithKline and the Japan Society of Hepatology.

Variables Analyzed in this Study

Univariate and multivariate logistic regression analyses were carried out to identify the independent factors associated with the development of hepatic failure caused by lamivudine-resistant HBV. These analyses were performed on the variables at the initiation of the treatment with lamivudine in regard to patients' age, gender, leukocyte and platelet counts in peripheral blood, prothrombin activity, serum levels of albumin, serum levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), rate of serum AST to serum ALT (AST/ALT ratio), serum levels of HBV-DNA and status of HBeAg/Anti-HBe. In the same way, the values at the point of detection of lamivudine-resistant HBV, the duration of the treatment with lamivudine before viral breakthrough, leukocyte and platelet counts in peripheral blood, prothrombin activity, serum levels of albumin, serum levels of total bilirubin, AST, ALT, AST/ALT ratio, serum levels of HBV-DNA and status of HBeAg/Anti-HBe were also analyzed. The maximum levels of ALT after the reactivation of hepatitis and the duration of the follow-up periods from viral breakthrough to the endpoint were also examined. Values of prothrombin activity and the serum levels of total bilirubin at the reactivation of hepatitis were used for the definition of the two groups. But we did not exclude these two variables from logistic regression analyses. The reason was that these two variables used in the analyses were obtained at the initiation of lamivudine therapy and at the detection of lamivudine-resistant HBV before the reactivation.

Statistical Analysis

Univariate logistic regression analysis and multivariate logistic regression analysis were carried out by using the SPSS v.6.1 software for the Macintosh edition (SPSS Inc. Chicago, IL). The Mann-Whitney's U test was used to make comparisons between these two groups for certain variables. A P value of less than 0.05 was considered statistically significant.

Results

The characteristics of the 47 patients at the initiation of lamivudine therapy and those at the point of detection of lamivudine-resistant HBV are shown in Tables 1 and 2, respectively. At the initiation of lamivudine therapy, the mean age of these 47 patients was 46.4 ± 8.0 years. Among them, 33 were male and 14 were female. All had HBV-DNA with an average of 6.98 ± 1.08 LC/ml, and the average of serum ALT was 120 ± 95.7 U/l. Thirty-nine (83.0%) were HBeAg-positive and eight (17%) were HBeAg-negative. The YMDD variant HBV, YIDD or YVDD, were detected in all patients with the SMITEST HBV-YMDD motif ELMA. Twenty-one (44.7%) had an elevation in their serum level of total bilirubin to more than 1.5 mg/dl and/or a decline in the level of prothrombin activity to less than 60% after the reactivation of hepatitis caused by lamivudine-resistant HBV.

Among the variables obtained from the initiation of the treatment with lamivudine, there were significant differences in the patients' age ($P = 0.0473$), platelet count ($P < 0.0001$), prothrombin activity ($P < 0.0001$), AST/ALT ratio ($P = 0.0109$), albumin ($P < 0.0001$) and total bilirubin ($P < 0.0001$) between the two groups (Table 1). As shown in Table 3, there were univariate associations of age at the initiation of lamivudine therapy ($P = 0.0402$), platelet count ($P = 0.0006$), prothrombin activity ($P = 0.0010$), AST/ALT ratio ($P = 0.0076$), serum levels of albumin ($P = 0.0021$), and serum levels of total bilirubin ($P = 0.0039$).

In the analysis of the variables obtained from the data at the point of detection of lamivudine-resistant HBV, platelet count ($P = 0.0002$), prothrombin activity ($P = 0.0104$), serum AST ($P = 0.0084$), ALT ($P = 0.0477$), albumin ($P = 0.0119$), and total bilirubin ($P = 0.0018$) were significantly different between the two groups (Table 2). Univariate logistic regression analysis showed that the variables of platelet count ($P = 0.0013$), prothrombin activity ($P = 0.0141$), albumin ($P = 0.0154$) and bilirubin ($P = 0.0056$) were related to the deterioration of the liver function after the emergence of lamivudine-resistant HBV.

In a multivariate logistic regression analysis using the variables obtained during lamivudine therapy, only prothrombin activity at the initiation of lamivudine therapy was significantly associated with the deterioration of the liver function after the reactivated hepatitis caused by lamivudine-resistant HBV ($P = 0.0025$, 95%CI 0.8269–0.9601) (Table 4). On the other hand, none of the parameters at the point of detection of lamivudine-resistant HBV were associated significantly with the deterioration caused by the drug-resistant HBV. The maximum levels of ALT after the reactivation of hepatitis, the duration of administration of lamivudine before the emergence of

Table 1 Characteristics of patients at the initiation of lamivudine therapy

	All ($n = 47$)	After the reactivation of hepatitis due to LMV-R HBV		P value[a]
		PT act < 60% and/or T-Bil > 1.5 mg/dl ($n = 26$)	PT act ≧ 60% and T-Bil ≦ 1.5 mg/dl ($n = 21$)	
Age (years old)	46.4 ± 8.0	44.2 ± 7.2	49.2 ± 8.2	0.0473
Male sex (%)	33 (70.2%)	17 (65.4%)	16 (76.2%)	0.4256
Peripheral leukocyte (/μl)	5013 ± 1783	5242 ± 1472	4730 ± 2110	0.1774
Peripheral platelet (104/μl)	13.6 ± 6.0	16.6 ± 5.1	9.7 ± 4.6	<0.0001
Prothrombin activity (%)	76.4 ± 22.3	91.7 ± 15.7	60.3 ± 16.1	<0.0001
AST (U/l)	88.6 ± 51.5	86.2 ± 57.6	91.6 ± 44.0	0.363
ALT (U/l)	120 ± 95.7	131.3 ± 103.3	105.9 ± 85.7	0.2798
AST/ALT ratio	0.89 ± 0.40	0.73 ± 0.22	1.09 ± 0.49	0.0109
Serum albumin (g/dl)	3.62 ± 0.68	3.97 ± 0.54	3.20 ± 0.60	<0.0001
Serum total bilirubin (mg/dl)	1.35 ± 1.36	0.73 ± 0.25	2.12 ± 1.74	<0.0001
HBeAg positive	39 (83.0%)	21 (80.8%)	18 (85.7%)	0.6573
HBV-DNA (LC/ml)	6.93 ± 1.08	6.85 ± 1.19	7.02 ± 1.74	0.9339

[a] Mann-Whitney's *U* test

LC/ml Log copies/ml, *PT act* prothrombin activity, *T-Bil* total bilirubin, *LMV-R* lamivudine-resistant

Table 2 Characteristics of patients during lamivudine therapy and at the point of detection of lamivudine-resistant HBV

	All ($n = 47$)	After the reactivation of hepatitis due to LMV-R HBV		P value[a]
		PT act < 60% and/or T-Bil > 1.5 mg/dl ($n = 26$)	PT act ≧ 60% and T-Bil ≦ 1.5 mg/dl ($n = 21$)	
Variables during lamivudine therapy				
Interval from the initiation of LMV therapy to the detection of LMV-R HBV (day)	523 ± 248	474 ± 250	584 ± 237	0.0642
Interval from the detection of LMV-R HBV to the last observation (day)	456 ± 299	483 ± 294	423 ± 310	0.4868
Maximum levels of ALT after Viral breakthrough (U/l)	274.9 ± 246.8	270.3 ± 215.6	280.6 ± 286.2	0.7563
Variables at the point of detection of lamivudine-resistant HBV				
Peripheral leukocyte (/μl)	4761 ± 1585	5012 ± 1792	4450 ± 1258	0.1915
Peripheral platelet (10^4/μl)	14.4 ± 5.6	17.1 ± 4.8	11.1 ± 4.7	0.0002
Prothrombin activity (%)	87.0 ± 16.6	95.1 ± 10.9	80.2 ± 17.8	0.0104
AST (U/l)	56.3 ± 70.8	58.3 ± 93.1	53.9 ± 26.2	0.0084
ALT (U/l)	65.7 ± 115.4	68.3 ± 150.7	62.5 ± 46.7	0.0477
AST/ALT ratio	1.07 ± 0.45	1.07 ± 0.46	1.06 ± 0.46	0.6454
Serum albumin (g/dl)	4.13 ± 0.76	4.40 ± 0.34	3.79 ± 0.98	0.0119
Serum total bilirubin (mg/dl)	0.95 ± 0.50	0.74 ± 0.29	1.20 ± 0.59	0.0018
HBeAg positive	38 (80.6%)	21 (80.8%)	17 (81.0%)	0.7511
HBV-DNA (LC/ml)	5.63 ± 1.24	5.68 ± 1.21	5.58 ± 1.31	0.8071

[a] Mann-Whitney's *U* test

LC/ml Log copies/ml, *PT act* prothrombin activity, *T-Bil* total bilirubin, *LMV-R* lamivudine-resistant

lamivudine-resistant HBV, and the duration of the follow-up period after the detection of lamivudine-resistant HBV were not significantly associated with the progression of the disease.

Discussion

In a clinical trial of patients with a compensated liver function, lamivudine suppressed the replication of HBV-

Table 3 Univariate logistic regression analysis

	P	Odds ratio	95% Confidence interval
Variables at the initiation of lamivudine therapy			
Age (years old)	0.0402	1.0957	1.004–1.1958
Male sex (%)	0.4228	1.694	0.4668–6.1472
Peripheral leukocyte (/μl)	0.3262	0.9998	0.9994–1.0002
Peripheral platelet (10^4/μl)	0.0006	0.738	0.6206–0.8776
Prothrombin activity (%)	0.001	0.8789	0.8137–0.9493
AST (U/l)	0.7212	1.0021	0.9908–1.0136
ALT (U/l)	0.3685	0.997	0.9906–1.0035
AST/ALT ratio	0.0076	18.1342	2.1615–152.1386
Serum albumin (g/dl)	0.0021	0.0977	0.0239–0.3986
Serum total bilirubin (mg/dl)	0.0039	23.35	2.7464–98.526
HBeAg positive	0.6548	1.4286	0.2999–6.8236
HBV-DNA (LC/ml)	0.6011	1.1614	0.6674–2.0208
Variables during lamivudine therapy			
Interval from the initiation of LMV therapy to the detection of LMV-R HBV (day)	0.1389	1.0019	0.9993–1.0045
Interval from the detection of LMV-R HBV to the last observation (day)	0.4922	0.9993	0.9973–1.0012
Maximum levels of ALT after Viral breakthrough (U/l)	0.886	1.002	0.9996–1.0043
Variables at the point of detection of lamivudine-resistant HBV			
Peripheral leukocyte (/μl)	0.2312	0.9998	0.9994–1.0002
Peripheral platelet (10^4/μl)	0.0013	0.7665	0.6521–0.901
Prothrombin activity (%)	0.0141	0.9293	0.8764–0.9854
AST (U/l)	0.8277	0.9991	0.9907–1.0076
ALT (U/l)	0.8614	0.9995	0.9944–1.0046
AST/ALT ratio	0.9111	0.9294	0.2571–3.3587
Serum albumin (g/dl)	0.0154	0.12	0.0216–0.6668
Serum total bilirubin (mg/dl)	0.0056	17.5227	2.3127–132.7638
HBeAg positive	0.7487	1.2631	0.3025–5.2742
HBV-DNA (LC/ml)	0.7911	0.9377	0.5829–1.5086

LC/ml Log copies/ml

Table 4 Multivariate logistic regression analysis of variables

Variables	*P*	Odds ratio	95% Confidence interval
Prothrombin activity at the initiation of LMV therapy (%)	0.0025	0.891	0.8269–0.9601

DNA sufficiently in more than 90% of them and improved the serum ALT levels as well as liver histology [11]. Lamivudine is generally well tolerated with few adverse events, and hepatitis flares are uncommon during treatment unless the drug-resistant HBV emerges. These features make treatment with lamivudine more feasible compared with IFN for patients with decompensated liver cirrhosis. The beneficial effects of lamivudine therapy were observed in patients with a compensated liver function as well as in patients with decompensated liver cirrhosis. Lamivudine rapidly suppressed the replication of HBV-DNA and improved the biochemical parameters in some clinical trials for HBV-related decompensated liver cirrhosis [24–29].

However, the incidence of lamivudine-resistant HBV in patients with decompensated liver cirrhosis varied from 7 to 21% [24, 25, 27, 30]. The reactivation of hepatitis due to lamivudine-resistant HBV is often associated with a fatal outcome, especially when the drug-resistant HBV emerged in patients with liver cirrhosis [24, 25, 27, 30]. The factors associated with the emergence of lamivudine-resistant HBV were commented on in many previous reports. In this study, we detected predictive factors associated with the prognosis after the emergence of lamivudine-resistant HBV, and which were the parameters obtained from the data at the introduction of lamivudine. It might be meaningful that the predictive factors were detected among the

baseline parameters, because patients with a history of a decompensated liver function were at greater risk of a fatal outcome. We showed that prothrombin activity at the initiation of lamivudine had a significant association with the deterioration of liver function caused by lamivudine-resistant HBV. The liver is the major site of synthesis of coagulation proteins. Prothrombin is the independent predictive index of hepatic fibrosis [31]. Among the variables obtained at baseline, univariate logistic regression analysis showed that platelet count, AST/ALT ratio, albumin and total bilirubin were significantly associated with hepatic failure caused by lamivudine-resistant HBV in addition to prothrombin activity, although there was no significance by multivariate logistic regression analysis. Previous studies reported that these parameters were also related to the progression of chronic liver disease [32–35]. Except for the patients' age, five of six variables that were extracted as significant variables by univariate logistic regression analysis reflected the reserve capacity of the liver rather than the activity of hepatitis.

In this study, we showed that the maximum levels of ALT after the reactivation of hepatitis due to lamivudine-resistant HBV did not have significant prognostic relevance with regard to the deterioration of the liver function. In a clinical situation, the maximum levels of ALT were not always higher in patients who developed hepatic failure after the selection of lamivudine-resistant HBV. For these reasons, we estimated that these results emphasized that the deterioration of the liver function caused by lamivudine-resistant HBV was more closely related with the reserve capacity of the liver before the initiation of treatment with lamivudine. Moreover, this result suggests that the patients whose disease had already progressed to decompensated liver cirrhosis at the initiation of lamivudine therapy should be observed more carefully than patients with compensated HBV infection, even those whose liver function had improved by the administration of lamivudine.

We reached the conclusion that reactivated hepatitis was not severe in patients whose liver function was compensated at baseline. On the other hand, those with liver disease which had progressed were at risk of hepatic failure. In such patients, in order to prevent the progression to hepatic failure, additional or alternative treatment with another antiviral agent should be recommended, even though the hepatitis has not yet been reactivated.

Several new nucleoside analogues are now under development and adefovir dipivoxil is a new-arrival agent in the clinical field. So far, the approved nucleoside analogues are limited to two kinds of medicine, lamivudine and adefovir dipivoxil. The active metabolite of adefovir dipivoxil, adefovir diphosphate, displays potent antiviral activity against HBV [36, 37], and an in vitro study demonstrated the antiviral activity to be against both wild-type and lamivudine-resistant strains of HBV [38]. Recent clinical trials reported that adefovir dipivoxil showed sufficient antiviral efficacy both in patients with wild-type HBV and lamivudine-resistant HBV [39, 40] with the advantage of a low incidence of drug-resistant HBV-DNA mutations [41]. In patients with compensated liver disease, adefovir dipivoxil alone or in combination with ongoing lamivudine therapy provided sufficient antiviral effects [42], although a small portion of patients treated with adefovir dipivoxil alone had an elevation of serum ALT levels after the cessation of lamivudine therapy [42]. In patients with decompensated liver cirrhosis, a mild flare of ALT triggers hepatic failure. For these reasons, we think that the combination therapy with adefovir and lamivudine should be continued until HBV-DNA level decreases and the activity of reactivated hepatitis is suppressed sufficiently in patients with liver cirrhosis.

Entecavir is approved for the treatment of chronic hepatitis B by FDA based on the three phase III trials [43–45]. New nucleoside analogue that have sufficient antiviral effects on both wild-type HBV and lamivudine-resistant HBV, such as tenofovir, are now under clinical trials for the treatment of chronic HBV infection [46]. It was reported that these medicines had the advantage of a lower incidence of the drug-resistant HBV variants [46, 47].

In conclusion, the prognosis after the emergence of lamivudine-resistant HBV was more closely related to the reserve capacity of the liver at the initiation of lamivudine therapy than the activity of reactivated hepatitis. Therapies for HBV-related chronic liver disease should be tailored according to the stage of the liver disease as well as to viral and host factors. From the foregoing, it would appear that additional treatment with adefovir dipivoxil or another antiviral agent early after viral breakthrough should be recommended when the lamivudine-resistant HBV is detected in patients with the history of decompensated liver disease, even before ALT is elevated. And these patients should be observed more carefully and strictly, even if the liver function improves after the administration of lamivudine.

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HEPATOLOGY

Insulin resistance and lichen planus in patients with HCV-infectious liver diseases

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Abstract

Background and Aim: Hepatitis C virus (HCV) causes liver diseases and extrahepatic manifestations, and also contributes to insulin resistance and type 2 diabetes mellitus (DM). The aims of the present study were to examine the incidence of extrahepatic manifestations including lichen planus in HCV-infected patients and to evaluate the relationship between lichen planus and insulin resistance.

Methods: Of 9396 patients with liver diseases presenting to the study hospital, 87 patients (mean age 60.0 ± 11.5 years) with HCV-related liver diseases were identified and examined for the incidence of extrahepatic manifestations. Insulin resistance and the presence of *Helicobacter pylori* antibodies were also measured.

Results: The prevalence of DM was 21.8% (19/87), hypertension was 28.7% (25/87), thyroid dysfunction was 20.7% (18/87), and extrahepatic malignant tumor was 9.2% (8/87). The prevalence of lichen planus at oral, cutaneous, pharyngeal, and/or vulval locations was 19.5% (17/87). Characteristics of 17 patients with lichen planus (group A) were compared with 70 patients without lichen planus (group B). Prevalence of smoking history, presence of hypertension, extrahepatic malignant tumor, and insulin resistance (HOMA-IR) were significantly higher in group A than in group B. Significant differences were not observed for age, sex, body mass index, diagnosis of liver disease, alcohol consumption, presence of DM, thyroid dysfunction, liver function tests, or presence of *H. pylori* infection between the two groups.

Conclusions: Infection with HCV induces insulin resistance and may cause lichen planus. It is necessary for an HCV-infected patient to be assayed for insulin resistance, and to be checked for different extrahepatic manifestations of this infection, particularly lichen planus.

Introduction

The number of fatalities due to hepatocellular carcinoma (HCC) in Japan continues to increase, and it is estimated that this tendency will continue at least until 2015. Of the HCC cases in Japan, approximately 16% are caused by hepatitis B virus (HBV) infection and approximately 80% by hepatitis C virus (HCV) infection.[1] The average prevalence of HCV carriers in Japan is about 2%, with the absolute number estimated at 2 million.[2] The increase in HCC in Japan depends on the spread of HCV infection.[2]

Infection with HCV induces various extrahepatic manifestations as well as chronic liver diseases.[3,4] HCV infects cells or organs except hepatocytes and multiplies. Representative extrahepatic manifestations of HCV infection include lichen planus, diabetes mellitus (DM), malignant lymphoma, Sjögren's syndrome, cryoglobulinemia, and membranoproliferative glomerulonephritis. It has been reported that combined therapy using interferon and ribavirin is effective for different extrahepatic manifestations that are apt to be overlooked.[5,6]

At present, it has been shown that HCV multiplies in skin and oral mucosa leading to HCV-related lichen planus,[7,8] and that the risk of malignant transformation is higher in lichen planus with HCV infection than in lichen planus without HCV.[9] However, a mechanism for these extrahepatic manifestations has not been elucidated. Recently it was reported that there is a significant correlation between lichen planus and HCV and DM in southern Taiwan, particularly in HCV patients with elevated serum alanine aminotransferase (ALT) levels and atrophic-erosive oral lichen planus (OLP).[10] In our previous report, patients with lichen planus having DM were all found to be HCV-infected.[11]

In addition, it has been reported that DM is a risk factor for HCV-related hepatocarcinogenesis[12] and for decreased survival

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among liver cirrhosis patients.[13] In addition, the incidence of diabetes in patients having HCV-related liver cirrhosis is higher than that in patients with HBV-related liver diseases.[14]

We recently showed molecular mechanisms for HCV core-induced insulin resistance.[15] HCV core up-regulates the suppressor of cytokine signaling (SOCS) 3, and inhibits insulin signaling by down-regulation of insulin receptor substrate (IRS) -1 and IRS-2 in hepatocytes. Moreover, in an epidemiological survey, we demonstrated that a significant increase in the incidence of diabetes occurs in subjects with high titers of HCV core compared to subjects who are negative for anti-HCV antibody[16] and concluded that HCV infection induces insulin resistance, which causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.[17]

In the current study, we surveyed the incidence of abnormal glucose tolerance in patients with or without lichen planus in a study population with HCV-related chronic liver disease, and investigated the relationship between lichen planus and insulin resistance.

Methods

Patients

A total of 105 984 consecutive patients had checkups for chronic liver disease for the first time in the Digestive Disease Center at Kurume University Hospital from April 1988 to August 2005. In the Digestive Disease Center, physicians, surgeons, radiologists, and an oral surgeon hold full-time positions. One of us (M.S.) is a hepatologist and examined 9396 of these 105 984 patients. There were 522 patients who were HCV antibody positive and who thereafter continued with regular hospital visits until April 2006.

Exclusion criteria were the following: (i) other causes of chronic liver disease or disease other than chronic HCV infection; (ii) liver disease related to HBV infection; and (iii) patients treated with interferon therapy at the time of study inclusion.

We examined the presence of extrahepatic manifestations of chronic HCV infection in 87 patients. Informed consent was obtained from all patients after the purpose and methods of the study were explained. The 87 patients were 44 men and 43 women with a mean age of 60.0 ± 11.5 years.

The patients were monitored for the presence of extrahepatic manifestations of HCV infection such as lichen planus, DM, hypertension, thyroid dysfunction, and extrahepatic malignant tumor as well as liver disease. Biochemical tests were done and insulin values, blood glucose levels, and *Helicobacter pylori* antibody were measured in patient blood samples. Life histories were taken.

Clinical examinations

Patients received oral mucosa and cutaneous medical examinations by an oral surgeon and a dermatologist. The diagnosis of OLP was made on the basis of clinical and histopathological features. Diagnosis of type 2 DM was based on the American Diabetic Association (ADA) criteria of 1997.[18] Persons in whom diabetes was diagnosed before 30 years of age and who used insulin were categorized as type 1 DM and were excluded from our study.

The following definitions of cardiovascular disease were employed. Obesity was defined as a body mass index (BMI) >25 kg/m^2 or higher. Hypertension was defined as a systolic blood pressure (SBP) of 140 mmHg or higher, or a diastolic blood pressure (DBP) of 90 mmHg or higher according to the criteria of JNC-VI of the International Hypertension Society.[19] Thyroid hormones such as FT3, FT4 and thyroid stimulating hormone were measured for all patients, and thyroid echography examination was performed for some patients. Examination of the upper gastrointestinal tract or lower digestive tract was performed on patients for whom it was deemed clinically necessary.

We also took a history of smoking and alcohol consumption.

Serological assays

Serum samples from the 87 patients were collected and tested for platelets (PLT) and for the following liver function tests: serum ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bilirubin (TBil), direct bilirubin (DBil), thymol turbidity test (TTT), zinc sulfate turbidity test (ZTT), total cholesterol (TC), total protein (TP), and albumin (Alb). Sera were also examined for the presence or absence of HCV or HBV infection. Anti-HCV was measured by a chemiluminescent enzyme immunoassay kit (Lumipulse II HCV, Fujirebio, Tokyo, Japan). HCV RNA in serum was detected using the Amplicore HCV test (Roche, Tokyo, Japan). Hepatitis B virus surface antigen (HBsAg) was assayed using a chemiluminescent immunoassay kit (Architect, HBsAg QT, Dainabot, Tokyo, Japan). Ultrasonographic examination for all patients was performed in order to investigate the shape of the liver and lesions occupying the liver. Computed tomography and liver biopsy were performed in some patients. Most patients underwent endoscopy for detection of esophagogastric varices. We used other possible predictors of liver cirrhosis progression, including serum albumin, TBil, prothrombin time, and PLT.

Plasma glucose levels were measured by a glucose oxidase method for all subjects and serum insulin levels were measured using a sandwich enzyme immunoassay kit (Eiken Chemical, Tokyo, Japan). Insulin resistance (IR) was calculated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA-IR) method.[20] The formula for the HOMA-IR is: HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.

The presence of serum IgG antibodies against *H. pylori* antibody were measured by the SRL (Tokyo) using E Plate *H. pylori* antibody produced by Eiken Chemical.

Statistical analysis

The chi-squared test and the unpaired Student *t*-test were used for statistical analyses. Differences were judged significant for $P < 0.05$ (two-tailed). This study was approved by the Institutional Review Board/Ethics Committee of our Institution.

Results

Among 87 patients with HCV-related liver diseases, the prevalence of lichen planus was 19.5% (17/87), DM was 21.8% (19/87),

Table 1 Clinical characteristics of 87 patients with HCV-related liver diseases according to presence of lichen planus (LP)

Clinical characteristic	All patients	Group A (with LP)	Group B (without LP)	*P*-value (A *vs* B)
No. subjects	87	17	70	–
Age (years)	60.0 ± 11.5	63.7 ± 10.6	59.1 ± 11.6	NS
Sex (M/F)	44/43	11/6	33/37	NS
BMI (kg/m^2)	22.8 ± 2.9	23.9 ± 2.8	22.5 ± 2.9	NS
Smoking history	32 (36.8)	10 (58.8)	22 (31.4)	0.0356
Alcohol consumption percentage	50 (57.5)	10 (58.8)	40 (57.1)	NS
Diagnosis of liver disease				
Past history of HCV infection	1	0	1	NS
Chronic hepatitis C	69	11	58	
HCV-related liver cirrhosis	9	3	6	
HCV-related HCC	8	3	5	
Comorbidities				
Diabetes mellitus	19 (21.8)	4 (23.5)	15 (21.4)	NS
Hypertension	25 (28.7)	10 (58.8)	15 (21.4)	0.0022
Thyroid dysfunction	18 (20.7)	5 (29.4)	13 (18.6)	NS
Extrahepatic malignant tumor	8 (9.2%)	5 (29.4)†	3 (4.3)‡	0.0013

Values shown as *n* (%) or mean ± SD. BMI, body mass index; F, female; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, male; NS, not significant.
†Tumors were: gastric cancer (two), tongue cancer (one), larynx cancer (one), and renal and colon cancer (one). ‡Tumors were: gastric cancer (one), colon cancer (one), and gallbladder cancer (one).

hypertension was 28.7% (25/87), thyroid dysfunction was 20.7% (18/87), and extrahepatic malignant tumor was 9.2% (8/87).

We compared characteristics of 17 patients who had lichen planus (group A) and 70 patients who did not have lichen planus (group B). The mean age in group A was 63.7 ± 10.6 years; there were 11 men and six women. The mean age in group B was 59.1 ± 11.6 years; there were 33 men and 37 women. Table 1 shows clinical features of groups A and B. The diagnoses of liver diseases in group A were chronic hepatitis C infection (11 patients), HCV-related liver cirrhosis (three patients), and HCV-related HCC (three patients). Those of group B were chronic hepatitis C infection (58 patients), HCV-related liver cirrhosis (six patients), HCV-related HCC (five patients) and past history of HCV infection (one patient) (Table 1).

The prevalence of smoking history ($P = 0.0356$), hypertension ($P = 0.0022$), and extrahepatic malignant tumor ($P = 0.0013$) were significantly higher in group A than in group B (Table 1). Diagnoses of extrahepatic malignant tumors in group A were: tongue cancer (one squamous cell carcinoma), larynx cancer (one squamous cell carcinoma), gastric cancer (one adenocarcinoma, one signet ring cell carcinoma), renal and colon cancer (one renal cell carcinoma). Diagnoses of extrahepatic tumor in group B were: gastric cancer (one adenocarcinoma), colon cancer (one adenocarcinoma), and gallbladder cancer (one adenocarcinoma). Significant differences were not observed for age, sex, BMI, liver disease, alcohol consumption, presence of DM, or thyroid dysfunction between these two groups.

We analyzed for differences between these two groups in liver assays, blood platelets, insulin, blood glucose, HOMA-IR, and presence of *H. pylori* infection. The laboratory data of both groups are shown in Table 2. Prevalence of insulin ($P = 0.0076$) and HOMA-IR ($P = 0.0113$) were significantly higher in group A than in group B (Table 2). Significant differences were not observed for serum AST, ALT, LDH, γGTP, TP, Alb, TBil, DBil, TTT, ZTT, TC, blood platelets, blood glucose, or presence of *H. pylori* infection between these two groups.

Seventeen patients had OLP at a total of 24 sites. The site of occurrence was: buccal mucosa in 13 (76.5%), lower lip in six (35.3%), upper lip in two (11.8%), gingiva in one (5.9%), tongue in one (5.9%), and floor of mouth in one (5.9%) (Table 3). The sites of lichen planus except oral mucosa were lower leg in four (23.5%), antebrachium in one (5.9%), skin extremities in two (11.8%), hypopharynx in one (5.9%), and vulva in one (5.9%). Biopsies of hypopharyngeal lichen planus were performed by an otolaryngologist, and of vulvar lichen planus by a gynecologist. The erosive and reticular variety, respectively, was found to be the prevalent form (Table 3).

Discussion

We performed an epidemiological survey for extrahepatic manifestations and HCC in an HCV hyperendemic area in Japan.[21,22] Anti-HCV positivity among residents of this area in 1990 was 23.6%.[23] We found that the prevalence of extrahepatic manifestations among individuals with HCV infection was higher than among those without HCV,[22] and found an association between HCV core, insulin resistance, and the development of type 2 DM.[16] Recently, we reported that insulin resistance in inhabitants who have an extrahepatic manifestation including OLP with HCV infection shows significantly greater increases than for inhabitants who have neither an extrahepatic manifestation nor HCV infection.[17] By the results of these epidemiological surveys we think that insulin resistance induced by HCV infection causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.

In this study, we did long-term follow up for insulin resistance from the standpoint of lichen planus among patients who we identified as having HCV-related chronic liver disease at our hos-

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Table 2 Laboratory data of 87 patients with HCV-related liver diseases according to presence of lichen planus (LP)

Laboratory assay	All patients	Group A (with LP)	Group B (without LP)	*P*-value (A *vs* B)
AST (IU/L)	61.1 ± 38.1	60.9 ± 33.5	61.2 ± 39.3	NS
ALT (IU/L)	68.2 ± 46.7	62.4 ± 39.6	69.6 ± 48.5	NS
LDH (IU/L)	216.8 ± 62.8	205.8 ± 72.1	219.6 ± 60.6	NS
γ-GTP (IU/L)	64.1 ± 68.4	63.5 ± 50.0	64.2 ± 72.5	NS
TP (g/dL)	7.7 ± 0.5	7.7 ± 0.5	7.7 ± 0.5	NS
Alb (g/dL)	4.1 ± 0.5	3.9 ± 0.5	4.2 ± 0.5	NS
PLT (/mm^3)	13.8 ± 5.1	12.5 ± 5.0	14.1 ± 5.09	NS
TBil (mg/dL)	1.1 ± 0.6	1.2 ± 0.9	1.0 ± 0.5	NS
DBil (mg/dL)	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	NS
TTT	16.2 ± 6.7	18.4 ± 4.7	15.8 ± 7.0	NS
ZTT	20.6 ± 6.9	21.8 ± 5.8	20.3 ± 7.2	NS
TC (mg/dL)	172.3 ± 35.8	164.3 ± 41.9	174.1 ± 34.4	NS
Insulin (μU/L)	23.3 ± 42.0	47.3 ± 87.8	17.4 ± 15.4	0.0076
Blood glucose (mg/dL)	97.4 ± 30.1	103 ± 33.2	96.1 ± 29.5	NS
HOMA-IR	7.1 ± 18.8	17.4 ± 40.0	4.6 ± 6.0	0.0113
Helicobacter pylori antibody (*n* (%))	58 (66.7)	10 (58.8)	48 (68.6)	NS

Values shown as mean ± SD. Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DBil, direct bilirubin; γ-GTP, gamma-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment; LDH, lactate dehydrogenase; NS, not significant; PLT, platelets; TBil, total bilirubin; TP, total protein; TTT, thymol turbidity test; TC, total cholesterol; ZTT, zinc sulfate turbidity test.

Table 3 Location of lichen planus in 17 patients with hepatitis C virus-related liver diseases

No	Sex	Age (years)	Liver disease	Lichen planus location			Type
				Cutaneous	Oral	Other	
1	M	71	CH	Antebrachium	–	–	–
2	M	60	CH	Extremities	–	–	
3	F	70	LC	–	Gingiva	–	Erosive
4	M	72	LC	–	Lower lip	–	Reticular
5	F	64	LC	Leg	Buccal mucosa, upper lip, lower lip	–	Erosive
6	M	66	CH	Leg	Buccal mucosa, upper lip, lower lip	–	Erosive
7	M	59	CH	–	Buccal mucosa (reticular)	Pharynx (erosive)	Erosive + reticular
8	M	66	CH	Leg	Buccal mucosa, lower lip	–	Reticular
9	M	57	CH	–	Buccal mucosa	–	Reticular
10	M	50	CH	–	Buccal mucosa, tongue, lower lip	–	Erosive
11	F	77	CH	–	Buccal mucosa	–	Atrophic
12	F	75	CH	–	Buccal mucosa	–	Reticular
13	M	62	HCC	–	Buccal mucosa, lower lip	–	Erosive
14	F	83	HCC	Leg	Buccal mucosa (atrophic)	Vulva (erosive)	Atrophic + erosive
15	M	41	CH	–	Buccal mucosa	–	Reticular
16	M	58	HCC	Extremities	Buccal mucosa, floor of mouth	–	Erosive
17	F	53	CH	–	Buccal mucosa	–	Reticular

CH, chronic hepatitis C; F, female; LC, HCV-related liver cirrhosis; HCC, HCV-related hepatocellular carcinoma; M, male.

pital. Although there was no significant difference in fasting glucose levels and BMI between patients with and without lichen planus, fasting insulin levels and HOMA-IR values, an indicator of insulin resistance, were significantly higher in patients who had lichen planus than in those who did not.

In the present study, insulin levels (17.4 ± 15.4 μU/L) and HOMA-IR values (4.6 ± 6.0) in patients having HCV infection without lichen planus (group B) were higher than the normal range. Normal values for insulin are 3.06–16.9 μU/L, and for HOMA-IR are less than 2. Therefore, the significantly higher insulinemia in patients such as those in group A (among HCV infectious patients) might cause lichen planus.

In Japan, it is known that the prevalence of HCV infection in patients with lichen planus is high;[11] therefore, interferon therapy is often administered to patients with lichen planus and a persistent HCV infection. However, it has been reported that patients cannot

complete interferon therapy because of aggravation of lichen planus.[24,25] The measurement of insulin resistance as well as a search for lichen planus may be useful before performing interferon therapy. A large series of patients with OLP was evaluated for extraoral involvement by Eisen *et al.*[26] They concluded that any patient with OLP should undergo a through history and examination as part of an investigation of potential extraoral manifestations, because a high percentage of patients with OLP develop extraoral manifestations. In our 17 cases of lichen planus, cutaneous lichen planus was diagnosed in seven (41.2%), hypopharynx in one (5.9%), and vulva in one (5.9%). The simultaneous appearance of extraoral and oral lesions was noted among six (35.3%). Because the majority of OLP patients suffer from lichen planus of the genitalia,[27] clinicians should follow OLP patients with sufficient attention to the presence of extraoral manifestations.

Sikuker *et al.* evaluated an association between HCV infection and extrahepatic malignancies. Extrahepatic malignancies were found in 14.6% of anti-HCV positive patients.[28] The incidence of extrahepatic malignant tumor in our subjects was 9.2% (8/87). The insulin-like growth factor family of proteins plays a key role in cellular metabolism, differentiation, proliferation, transformation and apoptosis, during normal development and malignant growth.[29] The hyperinsulinemia that HCV infection causes may induce an extrahepatic malignant tumor as well as HCC.

Many studies have shown that *H. pylori* is involved in the pathogenesis of gastric cancer.[30] The seroprevalence of *H. pylori* is 71% in Japanese aged 50–59 years, and is 81% in those aged 60–69 years.[31] This is almost the same as the seroprevalence of our patients, which was 66.7% (58/87) overall and 82.6% (19/23) in those aged 60–69 years. Seroprevalence of *H. pylori* in our three subjects with gastric cancer was 66.7%. In our study, we did not find an association between *H. pylori* and lichen planus in patients with HCV-infectious liver diseases.

In conclusion, we investigated the association of insulin resistance and lichen planus among patients with HCV-infected chronic liver diseases. The significant factors for development of lichen planus were smoking history, presence of hypertension, extrahepatic malignant tumor, and insulin resistance (HOMA-IR). This supports our previous conclusion that insulin resistance in patients who have an extrahepatic manifestation of HCV infection increases more than insulin resistance of patients who have neither an extrahepatic manifestation nor HCV infection. HCV-infected patients with lichen planus should pay attention to the development of an extrahepatic malignancy. Cooperation with an oral surgeon and a hepatologist is vital for early diagnosis and treatment of any extrahepatic manifestations.

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