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Amino Acid Substitutions in Hepatitis C Virus Core Region Predict Hepatocarcinogenesis Following Eradication of HCV RNA by Antiviral Therapy

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Substitution of amino acid (aa) 70 and/or 91 in the core region of HCV genotype 1b (HCV-1b) is an important predictor of hepatocarcinogenesis, but its impact on the development of hepatocellular carcinoma (HCC) following eradication of HCV RNA by antiviral therapy is not clear. 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of interferon monotherapy or interferon plus ribavirin combination therapy, were included in a follow-up study to evaluate the impact of aa substitution in the core region on hepatocarcinogenesis. Twenty six patients developed HCC during the follow-up. The cumulative rates of new HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively. The rates in patients infected with HCV-1b/Gln70(His70) [glutamine (histidine) at aa 70] were significantly higher than in patients infected with HCV-1b/Arg70 (arginine at aa 70) ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). The rates in patients infected with HCV-1b/Arg70 were not significantly higher than in those infected with HCV-2a/2b ($P = 0.617$; log-rank test). Multivariate analysis identified HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced fibrosis (HR 9.03, $P = 0.002$), and old age (HR 3.09, $P = 0.066$) as determinants of hepatocarcinogenesis. In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of HCC following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy. ***J. Med. Virol. 83:1016–1022, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: HCV; genotype; sustained virological response; hepatocellular carcinoma; core region; glutamine

INTRODUCTION

Infection with hepatitis C virus (HCV) is often persistent and can progress to chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. At present, interferon (IFN), in combination with ribavirin, is the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) and high viral loads account for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Tsubota et al., 2005].

Despite numerous lines of epidemiological evidence of an association between HCV infection and the development of HCC, it remains controversial whether the virus itself plays a direct role or an indirect role in the pathogenesis of HCC [Koike, 2005]. It has become evident that the HCV core region is potentially oncogenic in transgenic mice, but the clinical impact of the core region on hepatocarcinogenesis is still unclear [Moriya et al., 1998]. Previous reports indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b are pretreatment predictors of poor virological response to pegylated IFN (PEG-IFN)/ribavirin combination therapy and triple therapy of

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telaprevir/PEG-IFN/ribavirin [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also affect hepatocarcinogenesis [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. These reports support the oncogenic potential of the core region from the clinical aspect. However, hepatocarcinogenesis still occurs even after eradication of HCV RNA by antiviral therapy [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008], though whether substitutions of aa 70 and/or 91 in the core region also affect hepatocarcinogenesis following eradication of HCV RNA await further investigation.

The present study included 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy). The aims of this study were to evaluate the impact of aa substitutions in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

PATIENTS AND METHODS

Patients

Among 4,570 consecutive patients infected with HCV, in whom antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy) was initiated between February 1987 and June 2010 at the Toranomon Hospital, 1,273 were selected for the present study. We included patients who fulfilled the following criteria: (1) Patients positive for anti-HCV (by a third-generation enzyme immunoassay, Chiron Corp., Emerville, CA) and for HCV RNA by qualitative or quantitative analysis, before antiviral therapy. (2) Patients with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy, based on HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim, Germany) or by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (3) Patients without HCC, before and during IFN therapy. (4) Patients infected with a single genotype of HCV-1b, 2a, or 2b. (5) Patients negative for hepatitis B surface antigen (by radioimmunoassay, Dainabot, Tokyo). (6) Patients free of coinfection with the human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, and without hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee.

Table I summarizes the profile and laboratory data at the start of antiviral therapy of 1,273 patients with sustained virological response. They included 783 males and 490 females, aged 15–83 years (median, 53 years). The median follow-up time, from the end of antiviral therapy until the last visit, was 1.1 years (range, 0.0–18.0 years).

Laboratory Investigations

Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [Chayama et al., 1993]. HCV RNA was quantitated by branched DNA assay version 2.0 (Chiron Corp.), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems, Inc., Pleasanton, CA), or COBAS TaqMan HCV test (Roche Diagnostics). A high viral load was defined as branched DNA assay value of

TABLE I. Clinical Profile and Laboratory Data at the Start of Antiviral Therapy

Demographic data	
Number of patients	1,273
Sex (male/female)	783/490
Age (years)*	53 (15–83)
Body mass index (kg/m^2)*	22.7 (14.4–38.0)
Laboratory data	
Serum aspartate aminotransferase (IU/L)*	48 (11–1,386)
Serum alanine aminotransferase (IU/L)*	68 (10–2,009)
Total cholesterol (mg/dl)*	168 (79–328)
Fasting plasma glucose (mg/dl)*	93 (69–290)
HCV genotype (1b/2a/2b)*	664/433/176
Level of viremia (high viral load/low viral load)	838/415
Treatment regimen	
IFN monotherapy/IFN plus ribavirin	545/728
Histological findings	
Stage of fibrosis (F1/F2/F3/F4)	508/224/62/47
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 [arginine/glutamine (histidine)]	348/127
Core aa 91 (leucine/methionine)	321/156

The enrolled patients had sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy.
Data are numbers and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

≥1.0 Meq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay value of <1.0 Meq/ml, AMPLICOR GT HCV Monitor <100 × 10^3 IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim) was 100 copies/ml, and that of COBAS TaqMan HCV test was 1.2 log IU/ml. Samples with undetectable HCV RNA at 24 weeks after cessation of antiviral therapy by qualitative analysis or COBAS TaqMan HCV test were defined as HCV RNA-negative.

Detection of Amino Acid Substitutions in the Core Regions of HCV-1b

In the present study, aa substitutions in the core region of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of antiviral therapy and reverse transcribed with a random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids of the core region were amplified by nested PCR using the following primers. The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134–153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3′, nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234–253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934–953) primers. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Using HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed using 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. Thus, patients were classified into three HCV subgroups according to the HCV genotype and aa substitutions in the HCV-1b core region: (1) HCV-1b with Arg70, (2) HCV-1b with Gln70(His70), and (3) HCV-2a/2b.

Liver Histopathological Examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The samples were fixed in 10% formalin and then stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Each specimen submitted for examination contained ≥6 portal areas. Histopathological diagnosis was made by an experienced liver pathologist (HK) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994] for histopathological assessment.

Follow-Up and Diagnosis of Hepatocellular Carcinoma

Hematological, biochemical, and virological tests were performed at least once every month until the virological response was determined. When sustained virological response was confirmed, blood tests and imaging studies (computed tomography or ultrasonography) were conducted once or twice per year in the majority of patients, except those lost to follow-up. When HCC was suspected, additional procedures, such as magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy when necessary, were used to confirm the diagnosis.

Statistical Analysis

The cumulative rate of new cases of HCC was calculated using the Kaplan–Meier technique, and differences between the curves were tested using the log-rank test. Differences in the proportion of new cases of HCC according to groups were analyzed according to the period between the end of antiviral therapy and appearance of HCC. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with the development of HCC. The hazard ratio (HR) and 95% confidence interval (95%CI) were also calculated. Potential predictive factors associated with the development of HCC included the following variables: sex, age, body mass index, AST, ALT, total cholesterol, fasting plasma glucose, HCV genotype, level of viremia, treatment regimen, stage of fibrosis, and HCV subgroup according to HCV genotype in combination with aa substitutions in the core region. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL). All P values of less than 0.05 by the two-tailed test were considered significant.

RESULTS

Rate of New Cases of HCC in Patients With Sustained Virological Response

During the follow-up, 26 patients (2.0%) developed HCC. The median interval between the end of antiviral therapy and detection of HCC (latency to HCC) was 2.5 years (range, 0.0–15.9 years). The cumulative rates of new cases of HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively.

HCC Rate According to HCV Genotype and Amino Acid Substitutions in the Core Region of HCV-1b

During the follow-up, 7 (5.5%), 5 (1.4%), and 12 (2.0%) patients developed HCC in the HCV-1b with Gln70(His70), HCV-1b with Arg70, and HCV-2a/2b groups, respectively. The median latency to HCC was 1.1 years (range, 0.0–14.0 years), 3.9 (range, 0.0–15.9), and 2.8 (range, 0.0–12.9), respectively, and the cumulative rates of new cases of HCC were 10.6%, 3.6%, 3.0% at the end of 5 years; 10.6%, 6.3%, 5.2% at the end of 10 years; and 62.7%, 6.3%, 7.2% at the end of 15 years, respectively. The rates were significantly different among the three HCV subgroups ($P < 0.001$; log-rank test; Fig. 1). Especially, the rates for HCV-1b with Gln70(His70) were significantly higher than those for HCV-1b with Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). However, the rates for the HCV-1b with Arg70 group were not significantly higher than those for the HCV-2a/2b group ($P = 0.617$; log-rank test).

During the follow-up, 4 (2.6%) and 7 (2.2%) patients with HCV-1b/Met91, and HCV-1b/Leu91 developed HCC, respectively. In these two subgroups, the respective median latency to HCC was 3.4 years (range, 0.0–14.0 years) and 1.1 (range, 0.0–12.4), and the cumulative rates of new cases of HCC were 1.3%, 8.6% at the end of 5 years; 5.4%, 8.6% at the end of 10 years; and 36.9%, 14.7% at the end of 15 years. The rates for the HCV-1b/Met91 group were not significantly different from those for the HCV-1b/Leu91 group ($P = 0.908$; log-rank test).

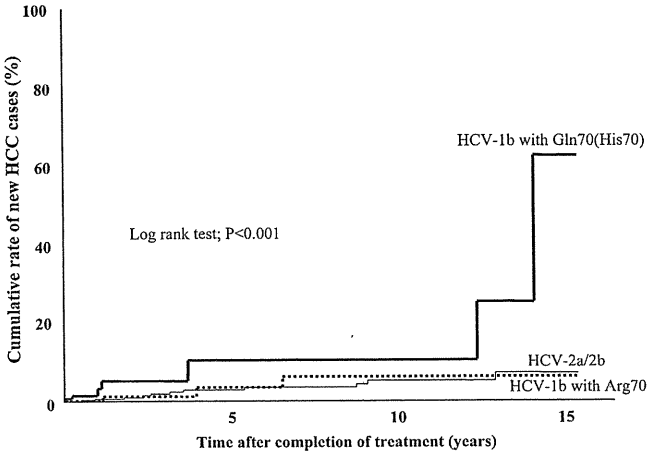

Fig. 1. Cumulative rates of new cases of HCC according to HCV genotype and amino acid substitutions in the core region of HCV-1b. The rates were significantly different among the three HCV groups ($P < 0.001$; log-rank test). Especially, the rate in patients with HCV-1b/Gln70(His70) was significantly higher than those of patients with HCV-1b/Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). Furthermore, the rate in patients with HCV-1b/Arg70 was not significantly higher than that in HCV-2a/2b ($P = 0.617$; log-rank test).

Predictive Factors Associated With the Development of HCC in Patients of Sustained Virological Response

Next, we analyzed the predictor of HCC using data of the entire group. There were significant relationships between the rate of new cases of HCC and male sex ($P = 0.003$), severe fibrosis (F3,4) ($P < 0.001$), old age (≥55 years) ($P = 0.002$), high levels of AST (≥39 IU/L) ($P = 0.023$), and HCV-1b/Gln70(His70) (log-rank test). These five factors were entered into multivariate analysis, which then identified three parameters that independently tended to or significantly influenced the development of HCC; HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced stage of fibrosis (F3,4; HR 9.03, $P = 0.002$), and old age (≥55 years; HR 3.09, $P = 0.066$; Table II).

Predictors of HCC in HCV-1b Patients With Sustained Virological Response

Finally we analyzed the data of 664 patients with HCV-1b to determine the predictors of HCC with sustained virological response. Univariate analysis identified three parameters that significantly correlated with the development of HCC: male sex ($P = 0.005$), old age ($P = 0.020$), and HCV-1b with Gln70(His70) ($P = 0.007$; log-rank test). These three factors were entered into multivariate analysis, which then identified HCV-1b with Gln70(His70) as the single parameter that significantly influenced the development of HCC (HR 8.19, $P = 0.034$).

DISCUSSION

Previous studies reported that the risk factors for hepatocarcinogenesis after elimination of HCV RNA

TABLE II. Results of Multivariate Analysis (Cox Proportional Hazard Model) for Factors Associated With Hepatocarcinogenesis in Patients With Sustained Virological Response

Factors and categories	Hazard ratio (95%CI)	*P*-Value
HCV group		
HCV-2a/2b	1	
HCV-1b with Arg70	1.15 (0.24–5.56)	0.863
HCV-1b with Gln70(His70)	10.5 (2.89–38.2)	<0.001
Fibrosis stage		
F1,2	1	
F3,4	9.03 (2.32–35.2)	0.002
Age (years)		
<55	1	
≥55	3.09 (0.93–10.3)	0.066

were severe fibrosis, male sex, and old age at the start of IFN treatment [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008]. In the present study, multivariate analysis identified HCV-1b with Gln70(His70), advanced fibrosis stage, and old age as determinants of HCC in patients with a sustained virological response. The present study is the first report to indicate that aa substitution in the core region at the start of antiviral therapy also influences hepatocarcinogenesis following eradication of HCV RNA. This result should be interpreted with caution since races other than the Japanese and patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies of patients of other races and those infected with HCV-1a.

Despite numerous lines of epidemiological evidence linking HCV infection to the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Evidence suggests that the HCV core region is potentially oncogenic in the transgenic mice [Moriya et al., 1998], though the clinical impact of the core region on hepatocarcinogenesis remains unclear. Previous reports indicated that aa substitutions in the core region of HCV-1b are pretreatment predictors of poor virological response to antiviral therapy [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also are etiological factors in HCC [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. Importantly, the present study indicated that aa substitution in the core region at the start of antiviral therapy also affects the development of HCC even after the eradication of HCV RNA, and this is the first report to suggest the persistent oncogenic potential of the core region regardless of HCV RNA persistence. Previous reports identified the PA28γ-dependent pathway as one of the mechanisms of HCV-associated hepatocarcinogenesis. Moriishi et al. [2003, 2007] reported that knockout of the PA28γ gene induces accumulation of HCV core protein in the nuclei of hepatocytes of HCV core gene transgenic mice and disrupts the development of both hepatic steatosis and HCC. Furthermore, the HCV core protein also enhances the binding of liver X receptor α (LXRα) and retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, it seems that PA28γ plays a crucial role in the development of HCV-associated steatosis and HCC. However, these basic studies were performed under the state of HCV RNA persistence [Moriya et al., 1998; Moriishi et al., 2003, 2007; Koike, 2005], and further studies should be performed to investigate the oncogenic potential of aa substitution in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

The association between HCV genotype and the risk of HCC is not clear. A study of Italian cohort indicated that the rate of HCC in patients infected with HCV-1b was significantly higher than that of patients infected with HCV-2a/2c [Bruno et al., 2007]. On the other hand, the present study of Japanese patients indicated that the rates in patients infected with HCV-1b were not significantly higher than those in those infected with HCV-2a/2b. The discrepancy between the present result and the above Italian study may be explained by differences in host factors [Montes-Cano et al., 2010], and/or differences in viral factors, such as the distribution of HCV-1b with Arg70 or Gln70(His70), and geographic diversities of HCV-1b [Nakano et al., 1999].

Previous studies showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin achieved sustained virological response rates of 35–60% and 61–69% in patients infected with HCV-1, respectively [Hézode et al., 2009; McHutchison et al., 2009; Akuta et al., 2010]. Furthermore, the PROVE3 study also showed that the 24- and 48-week regimen of triple therapy achieved sustained virological response rates of 51% and 53%, respectively, in patients infected with HCV-1 who had been unsuccessfully treated with PEG-IFN/ribavirin [McHutchison et al., 2010]. While it is anticipated that larger numbers of HCV-1 patients will achieve sustained virological response in response to telaprevir/PEG-IFN/ribavirin, a larger proportion of patients could develop HCC following eradication of HCV RNA by antiviral therapy. Hence, our study indicated that aa substitutions in the core region of HCV-1b should be detected before eradication of HCV RNA by antiviral therapy. Especially, even if patients of HCV-1b with Gln70(His70) could achieve sustained virological response, blood tests and imaging studies should be conducted at regular intervals in this high risk group for early detection and treatment of HCC.

Genetic variations near the IL28B gene are pretreatment predictors of poor virological response to the combination therapy of PEG-IFN/ribavirin and triple therapy of telaprevir/PEG-IFN/ribavirin [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Akuta et al., 2010; Rauch et al., 2010], but their impact on hepatocarcinogenesis are unknown at this stage. In this study, 387 of 1,273 patients were evaluated for HCC according to genetic variation in rs8099917 (data not shown). A preliminary study based on a small number of patients showed that the HCC rate in genotype TT of treatment sensitive type (2.2%) was not significantly different from that in genotype non-TT of treatment resistant type (1.6%). Unfortunately, we could analyze the effect of rs8099917 on HCC following eradication of HCV RNA by antiviral therapy. Further studies of larger patient populations should be performed to investigate the relationship between genetic variations near the IL28B gene and HCC.

The limitations of the present study were that viral factors associated with hepatocarcinogenesis were incompletely investigated. Ogata et al. [2003] reported that HCV-1b strains might be associated with HCC

on the basis of the secondary structure of the amino-terminal portion of the HCV NS3 protein. Giménez-Barcons et al. [2001] reported that high amino acid variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis. In the present study, the clinical impact of other regions on hepatocarcinogenesis could not be investigated, except for aa 70 and 91 in the HCV core region. The results should also be interpreted with caution since patients infected with HCV-1a were not included. Other limitations include lack of analysis of the effects of life-style related diseases (such as diabetes, insulin resistance or non-alcoholic steatohepatitis) on hepatocarcinogenesis, except for fasting plasma glucose and total cholesterol [Sumida et al., 2010a,b]. The impact of viral factors and life-style related diseases on hepatocarcinogenesis should also be investigated in future studies.

In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of hepatocarcinogenesis following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy.

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CLINICAL STUDIES

Stage progression of small hepatocellular carcinoma after radical therapy: comparisons of radiofrequency ablation and surgery using the Markov model

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hepatocellular carcinoma – Markov model – radiofrequency ablation – recurrence – surgery – survival

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Abstract

Background: Stage progression of 374 small hepatocellular carcinomas (HCC) was retrospectively analysed. *Patients and methods:* During 8 years, 236 patients with the early stage of HCC received radiofrequency ablation (RFA), and 138 underwent surgery as an initial therapy. More patients of young age and with better liver function tended to undergo surgical treatment. Based on 1892 patient-year data, the Markov model analysed the stepwise progression of early stage (multiple up to three nodules, 3 cm or less each) to intermediate stage (four nodules or more, or larger than 3 cm), to advanced stage (portal invasion, extrahepatic metastasis or Child–Pugh C) and to death. *Results:* The recurrence rates after RFA and surgery were 53.3 and 40.6% in the third year. The annual progression rates from the early stage to the intermediate stage, advanced stage and death were 5.40, 1.63 and 1.73% in the RFA group and 3.90, 1.87 and 0.62% in the surgery group respectively. The progression rate from the early to the intermediate stage was significantly lower (2.34% annually) in the younger patient group (< 60 years) than that in the older group (≥60 years, 5.70%, $P = 0.0053$). In contrast, the progression rate from the intermediate to the advanced stage was significantly higher in the younger patient group (< 60 years, 37.50% annually) than that in the older groups (60–69 years, 30.30%, 70 years or older 22.09%, $P = 0.0011$). Multivariate hazard analysis showed that initial treatment did not significantly affect the stage progression rate (hazard ratio of RFA 1.09, $P = 0.70$) and the survival rate (hazard ratio of RFA 1.09, $P = 0.73$). *Conclusion:* Although the recurrence rate was slightly higher in the RFA group, additional ablation procedures could control the progression of HCC, with a rate comparable to the surgical group.

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in the world today (1). Although routine imaging check-ups can often detect a small HCC at an early stage in high-risk patients with chronic hepatitis and cirrhosis, surgical resection is performed only in 20% or less of the cases because of the association of cirrhosis and tumour multiplicity (2–5). In the management of patients with HCC associated with cirrhosis, treatment repetition is common and inevitable for newly appearing multicentric tumours (6–8), and many practitioners hope each ablation procedure to be less invasive, less expensive and with a shorter hospitalization period.

Radiofrequency ablation (RFA) is currently considered the most effective percutaneous therapy for small HCCs, and certain centres now use it as a first-line treatment option (9), even in patients suitable for surgery. Indeed, RFA is sometimes considered as a less radical therapy compared with surgical resection because of the relatively high rate of local recurrence (10–12), but most of the local tumour progression can be completely treated through an additional RFA procedure. Surgical therapy, on the other hand, is an invasive mode of treatment with a higher cost (10), but achieves a lower recurrence rate. Only a few studies have evaluated the long-term outcome and prognostic factors of percutaneous RFA in comparison with surgical therapy (12–14).

When a recurrent tumour shows relatively advanced characteristics at an intermediate stage with a large tumour or multiples of four or more, transcatheter arterial chemoembolization (TACE) is preferred to surgical therapy or local ablation (15). We introduced the

Markov model to simulate the steps of stage progression of patients with small HCC under an intensive medical intervention. Here, we retrospectively evaluated the progression of HCC and the long-term prognosis of patients who had undergone RFA or surgical resection as the initial therapy for small HCCs, and assessed the prognostic factors of those patients.

The purposes of this study were, therefore, (i) to compare the recurrence rates, progression of tumour stage and survival rates between those patients who received percutaneous RFA and those who underwent surgery and (ii) to elucidate the significance of the selection of initial therapy for small HCCs from the viewpoints of stage progression and prognosis.

Patients and methods

Patients

A total of 468 patients were diagnosed as having a small HCC 3 cm or less in diameter, from March 1999 to April 2006, at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these 468 patients, 236 patients (50.4%) underwent percutaneous RFA therapy as a curative mode of treatment and the remaining 138 patients (29.5%) received surgical resection, 52 had TACE and the remaining 42 patients were treated with ethanol injection, microwave coagulation or other palliative methods of treatment.

A total of 374 consecutive patients with a small HCC, who underwent either RFA or surgery, were analysed in this study. None had been treated previously for HCC, and all had single or multinodular (up to three) HCCs 3 cm or less in diameter each, absence of portal venous thrombosis and known extrahepatic metastases, and Child–Pugh class A or B liver function.

The patients included 246 men and 128 women, and ranging in age from 29 to 87 years, with a median age of 65 years. The demography, laboratory data and features of cancer were compared between the two therapy groups (Table 1). Patients' age was lower in the surgery group by 4.5 years. The rate of HBV-positive disease was significantly higher in the surgery group, and liver function tests were also significantly better in the surgery group.

Hepatocellular carcinoma

Patients were required to have HCC with a definitive diagnosis by either typical hypervascular radiological features or histology through needle biopsy. Tumours had to be measurable by ultrasonography (US), computerized tomography (CT) and digital subtraction angiography. In order to elucidate the detailed characteristics of the HCC, CT during arterial portography and CT hepatic arteriography were performed in all the patients. Among 374 patients, HCC was confirmed by a resected specimen in 138 patients, by typical hypervascular characteristics on at least two modalities of imagings in 219 and by a fine-needle biopsy in 17.

Most patients (82.2%, 309 of 376) had a single tumour, and the median tumour diameter was 19 mm, ranging from 5 to 30 mm. The characteristics of the tumour in the subgroup of RFA and surgery are given in Table 1. The median size of the largest tumour was 18 mm in the RFA group and 20 mm in the surgery group ($P < 0.001$).

Table 1. Clinical features of the patients with small liver cancer

Initial therapy	Radiofrequency ablation ($n = 236$)	Hepatic resection ($n = 138$)	P
Demography			
Men:women	145:91 (38.6%)	101:37 (26.8%)	0.0021
Age (median, range)	67 (38–87)	62.5 (29–80)	< 0.001
Decompensated cirrhosis	16 (6.8%)	5 (3.6%)	0.20
HBsAg	24 (10.2%)	46 (33.3%)	< 0.001
Antibody to HCV	197 (83.5%)	84 (60.9%)	< 0.001
History of alcohol intake > 500 kg	21 (8.9%)	16 (11.6%)	0.40
Observation period (year)	3.7 (0.1–9.9)	4.5 (0.1–10.0)	0.041
Laboratory data (median, range)			
ICG R15 (%)*	28 (1–100)	21 (3–68)	< 0.001
Bilirubin (mg/dl)	1.0 (0.2–3.1)	1.0 (0.3–2.2)	0.003
Albumin (g/dl)	3.5 (2.2–4.2)	3.6 (2.8–4.4)	< 0.001
Aspartic transaminase (IU)	55 (17–311)	45 (17–386)	0.006
Platelet count ($\times 10^3/mm^3$)	97 (19–253)	127 (38–272)	< 0.001
Prothrombin time (%)	84 (31–125)	91 (59–115)	0.001
Liver cancer			
Median size (mm)	18 (8–30)	20 (5–30)	< 0.001
Single/multiple	195/41 (17.4%)	114/24 (17.4%) 1.00	
α-fetoprotein (ng/ml)	19 (1–2080)	17 (1–2610)	0.84
PIVKA-II (AU/L)†	17 (7–1470)	20 (9–1650)	0.008

*ICG R15, indocyanine green retention rate at 15 min.

†PIVKA-II, protein induced by vitamin K antagonist-II.

HCV, hepatitis C virus.

Treatment for initial hepatocellular carcinoma

Physicians and surgeons usually held a conference about the choice of therapy in individual patients. RFA or surgical therapy were selected considering the site, size and number of tumours, liver function and the patient's general status. Both RFA and the surgical procedure were explained fully to all the patients, and informed consent was obtained. Despite the feasibility and availability of surgery, some patients voluntarily preferred RFA under informed consent.

Radiofrequency ablation therapy was performed percutaneously under US or CT guidance, under conscious sedation with fentanyl citrate (0.1–0.2 mg, Fentanyl; Daiichi-Sankyo, Tokyo, Japan) or pethidine hydrochloride (35–70 mg, Opystan; Tanabe-Mitsubishi, Osaka, Japan) administered intravenously. RFA was performed using three kinds of apparatus: a radiofrequency interstitial tumour ablation system (RITA, RITA Medical Systems Inc., Mountain View, CA, USA), a cool-tip system (Tyco Healthcare Group LP, Burlington, VT, USA) and a radiofrequency tumour coagulation system (RTC system, Boston-Scientific Japan Co., Tokyo, Japan).

Hepatic resection was performed under intra-operative US monitoring and guidance. In the cases of small and superficial HCC, arterial and portal vein clumping at the hepatic hilum was not usually performed for maintenance of liver perfusion.

Evaluation of the therapeutic effect

To evaluate the efficacy of local ablation, a dynamic CT was performed at 2–7 days after treatment with RFA, and 8–21 days after surgery. CT findings were confirmed by consensus among at least two hepatologists and radiologists. On dynamic CT images, the non-enhancing area was measured as the ablated area. When the diameter of the non-enhancing area was greater than that of the ablated nodule, RFA was considered to have had a complete effect, and the treatment was terminated. When patients had a smaller ablated area or a positively enhanced area in the original tumour based on CT results after RFA therapy, they usually underwent an additional RFA within several days.

Follow-up of patients

Physicians observed the patients every 4–8 weeks after the first treatment. Liver function test, haematology and tumour markers were measured every 1–2 months. After the completion of eradication of HCC, recurrence was surveyed with CT or magnetic resonance imagings (MRI) every 3–4 months. Serum α-fetoprotein (AFP) and des-γ-carboxy prothrombin were also measured every 1–2 months to detect recurrence as early as possible.

During a median observation period of 4.2 years, four patients (1.1%) were lost to follow-up.

Statistical analysis and the Markov model

Standard statistical measures and procedures were used. The χ^2-test, Fisher's exact test and Mann–Whitney's *U*-test were used to analyse the differences in the demography, laboratory findings and tumour characteristics between the RFA group and the surgery group. The recurrence rate, progression rates and survival rate were analysed using the Kaplan–Meier technique (16) with the log-rank test. Cox's proportional hazard analysis was performed to evaluate independent predictors of the outcomes.

The Markov model (17) was adopted to analyse the transition rates from the early stage to the intermediate stage of HCC, intermediate to advanced stage and advanced stage to death. A homologous Markov chain consisted of four states (Fig. 1). These were the early stage of HCC (solitary or multiple up to three nodules, 3 cm or less each), the intermediate stage (four nodules or more, or larger than 3 cm), the advanced stage (portal vein

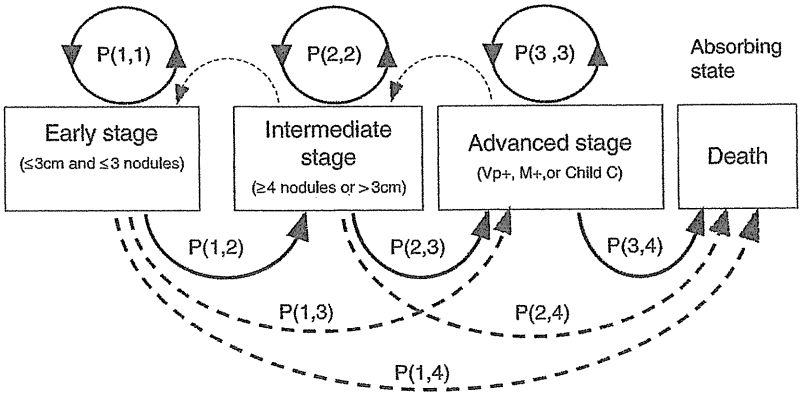

Fig. 1. The Markov state transition diagram of hepatocellular carcinoma. Four states were defined: early stage (solitary or multiple up to three nodules, 3 cm or less in diameter each), intermediate stage (multiple nodules of four or more, or 3.1 cm or more), advanced stage (main portal vein invasion, extrahepatic metastasis or Child–Pugh C) and death. Of these, death was the absorbing state from which no transitions to the other states occurred. The transition in one cycle (1 year) is shown. Arrows connecting two different states indicate the transitions observed.

invasion, extrahepatic metastasis or Child–Pugh score C) and death as an absorbing state from where no transitions to the other states occurred. The model was based on the following principles: (i) the four states are mutually exclusive and collectively exhaustive; (ii) the Markov assumption for the current state without any memories of prior states; (iii) time intervals are uniform; and (iv) transition probabilities are constant and time independent. Items (i) and (ii) define a Markov chain, whereas items (iii) and (iv) characterize a homogenous Markov chain (18).

A *P*-value of < 0.05 in a two-tailed test was considered significant. Data analysis was performed using the computer program IBM SPSS STATISTICS ver. 18 (19).

Results

Effect of initial treatment

After the initial session of RFA or surgery, complete ablation for entire tumour nodules was obtained in 232 patients (98.3%) in the RFA group and in 138 patients (100%) in the surgery group. Among four patients (1.7%) with incomplete ablation after the initial session of RFA, two achieved complete necrosis by re-RFA performed after a few months, and the other two underwent TACE for the residual tumour nodules.

Complications of treatment (Table 2)

After the initial therapy with RFA or surgery, 12 patients developed major complications after treatment: seven in the RFA group and five in the surgery group. There was no treatment-related death within 6 months after therapy in any of the patients in the RFA and surgery groups. Although abdominal pain, mild aggravation of liver function test, low-grade fever, transient elevation of aminotransferases and bilirubin values were often found after RFA therapy, significant deterioration of performance status and prolonged stay in the hospital were not observed.

Cumulative recurrence rates and treatment for recurrent hepatocellular carcinoma

The initial recurrence rates were compared between the two groups according to the initial therapy. The initial recurrence rates after treatment in the RFA and the surgery group were 11.3 and 14.2% at the end of the first year, 40.4 and 29.3% in the second year, 53.3 and 40.6% in the third year, 65.0 and 48.8% in the fourth year and 69.5 and 53.7% in the fifth year respectively. The recurrence rate in the RFA group was significantly higher than that of the surgery group (log-rank test, $P = 0.015$) (Fig. 2).

For the treatment of a recurrent tumour, we fundamentally adopted RFA or surgical treatment when patients had an early stage of HCC with sufficient liver function. Although initial therapy included surgery, patients with a recurrent tumour tended to receive RFA therapy more frequently. When a tumour progressed to the intermediate stage with a large tumour and/or multiple nodules, TACE was usually performed using anti-tumour agents, iodinated poppy seed oil fatty acid (Lipiodol Ultra-Fluide™, Guerbet Japan, Tokyo) and gelatin sponge particles. When the tumour progressed to the advanced stage (portal invasion, extrahepatic metastasis, or Child–Pugh C) during repeated local ablation or TACE therapy, anti-tumour therapy was usually not performed, except for systemic or intra-arterial chemotherapy. Anti-molecular targeted agents were not available during the study period in Japan.

Table 2. Complications after the initial treatment

	Initial therapy	
Complication	Radiofrequency ablation ($n = 236$)	Hepatic resection ($n = 138$)
Perforation of jejunum	2	0
Biloma and/or biliary infection	3	1
Prolonged ascites	1	2
Jaundice	0	1
Haemorrhage requiring transfusion	1	1

Cumulative progression rates from the early to the intermediate stage

A total of 98 (26.2%) developed to the intermediate stage during the observation: 65 (27.5%) in the RFA group and 33 (23.9%) in the surgery group.

Crude development rates to the intermediate stage in the RFA and surgery groups were 18.2 and 13.0% in the third year, 33.1 and 22.1% in the fifth year, and 40.9 and 31.8% in the fifth year respectively. The development rate of the RFA group was slightly higher ($P = 0.14$) (Fig. 3a).

Independent factors associated with the stage development rate were explored in the patients. Multivariate hazard analysis showed that the rate is independently associated with positive HBsAg ($P = 0.041$) and a high platelet count ($P = 0.032$). The factor of initial therapy

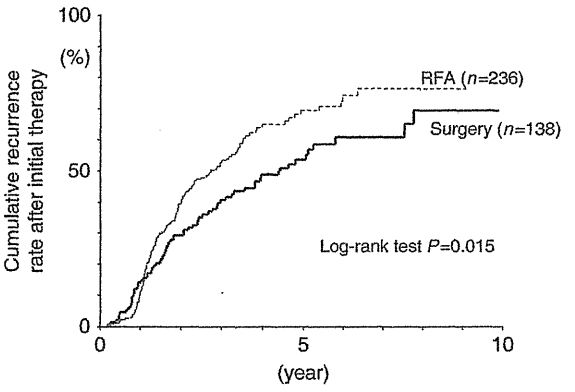

Fig. 2. Cumulative recurrence rates after therapy in patients with an early stage of hepatocellular carcinoma, according to initial therapy. RFA, radiofrequency ablation.

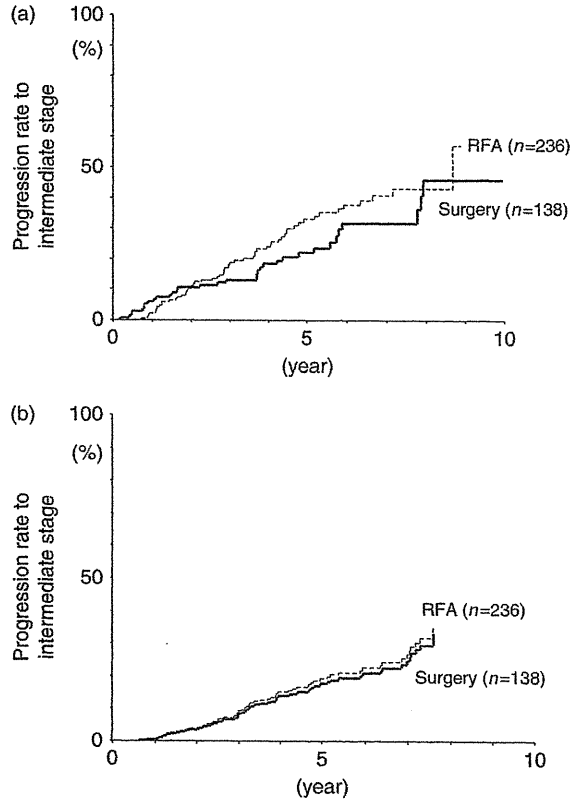

Fig. 3. (a) Crude development rates to the intermediate stage of hepatocellular carcinoma according to initial therapy. (b) Adjusted development rates to the intermediate stage, using proportional hazard analysis. RFA, radiofrequency ablation.

did not affect the eventual survival rate (hazard ratio 1.09, $P = 0.70$) (Table 3).

Cumulative progression curves from the early stage to the intermediate stage were drawn from the multivariate analysis in an imaginary RFA group and an imaginary surgery group, with an average positive rate of HBsAg and an average platelet count (Fig. 3b). Five-year progression rates to the intermediate stage were 19% in the RFA group and 18% in the surgery group. The differences in the progression rates were considered as a 'pure' impact of the difference in the initial mode of therapy on future stage progression, which was adjusted with significant covariates assuming a standardized study group.

Survival rates and predictive factors

A total of 87 (23.3%) died during the observation: 60 (25.4%) in the RFA group and 27 (19.6%) in the surgery group.

The crude survival rates in the RFA group and the surgery group were 88.5 and 92.6% in the third year, 71.7 and 80.9% in the fifth year and 60.6 and 74.6% in the seventh year respectively (Fig. 4a). The survival rate of the surgical therapy group was higher but statistical significance was not obtained ($P = 0.071$).

Table 3. Independent factors associated with the progression rate from an early stage to an intermediate stage of hepatocellular carcinoma

Factors	Category	Hazard ratio (95% confidence interval)	*P*
HBsAg	1: negative	1	
	2: positive	0.41 (0.20–0.82)	0.012
Platelet count	1: $\geq 100\,000/mm^3$	1	
	2: $< 100\,000/mm^3$	1.58 (1.04–2.39)	0.032
Initial therapy	1: surgery	1	
	2: RFA	1.09 (0.69–1.71)	0.70

RFA, radiofrequency ablation.

Independent factors associated with survival were explored in all the patients. Multivariate hazard analysis indicated that the survival rate is independently associated with a positive HBsAg ($P = 0.038$), a low indocyanine green retention rate at 15 min (ICG R15) ($P < 0.001$) and a low AFP value ($P = 0.021$). The factor of initial therapy did not affect the eventual survival rate (hazard ratio 1.26, $P = 0.35$) (Table 4).

Overall survival curves in patients with an early stage of HCC were drawn from the multivariate analysis in an imaginary RFA group and an imaginary surgery group, using an average positive rate of HBsAg, an average ICG R15 value and an average AFP value (Fig. 4b). Five-year survival rates were estimated as 80% in the RFA group and 81% in the surgery group, and 7-year rates were 71 and 72% respectively. Among 87 deaths during the observation, 70 (80.5%) died from progression of HCC, 14 (16.1%) died from liver failure without progression of HCC and the remaining three patients died from causes other than liver disease

Probabilities for transition among four disease states of hepatocellular carcinoma

The Markov model for the progression of HCC depended on the probabilities for transition among the four states at one time interval that was set at 1 year. Yearly transition probabilities were calculated based on 1892 person-year data from the 374 patients with an early stage of HCC. Figure 5 illustrates a probability diagram of the long-term progression of HCC calculated from the Markov model. All patients were at an early stage initially, but intermediate and advanced stages gradually increased with time. Approximately half of the patients died, and $< 40\%$ of the patients remained at early stage at the end of the 10th year.

The results are shown in Table 5 as a matrix of the transition probabilities for three subsets composed of three decades of their lives (< 60, 60–69 and ≥ 70 years) stratified by four states (early stage, intermediate stage, advanced stage and death).

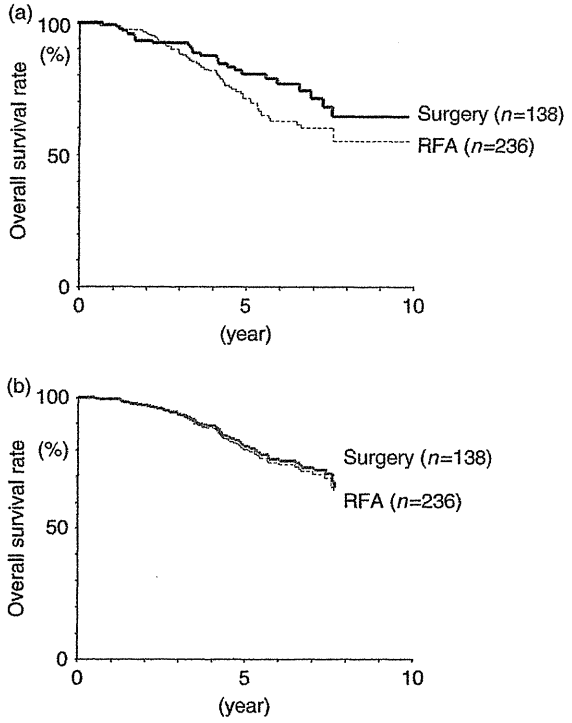

Fig. 4. (a) Crude survival rates in patients receiving radiofrequency ablation and those undergoing surgery as the initial therapy. (b) Adjusted survival rates in the radiofrequency group and surgery group, using proportional hazard analysis. RFA, radiofrequency ablation.

Table 4. Independent factors associated with the survival rate after the initial treatment for hepatocellular carcinoma

Factors	Category	Hazard ratio (95% confidence interval)	*P*
HBsAg	1: negative	1	
	2: positive	0.43 (0.19–0.94)	0.034
ICG R15*	1: < 30%	1	
	2: ≥30%	1.96 (1.20–3.20)	0.0070
α-fetoprotein	1: < 40 mg/ml	1	
	2: ≥40 mg/ml	1.71 (1.09–2.68)	0.020
Prothrombin time	1: < 80%	1	
	2: ≥80%	0.60 (0.37–0.96)	0.035
Initial therapy	1: surgery	1	
	2: RFA	1.09 (0.66–1.81)	0.73

*ICG R15, indocyanine green retention rate at 15 min.
RFA, radiofrequency ablation.

In the matrix of age of < 60 years, 2.34% of the patients in the early stage developed to the intermediate stage annually, 1.40% to the advanced stage and 0.93% died. The remaining 95.33% of the patients remained in the early stage after 1 year. The probability for the transition from an early stage to an intermediate stage

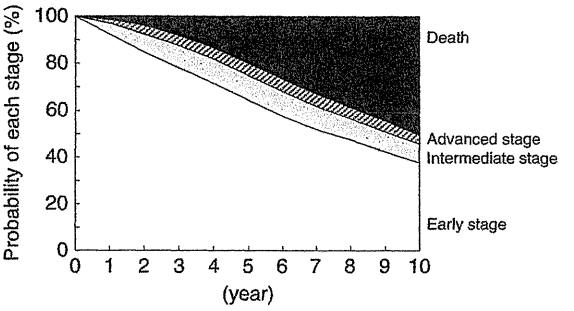

Fig. 5. Illustrated transition probabilities of patients, from the early stage of hepatocellular carcinoma, to the intermediate stage, the advanced stage and to death.

Table 5. One-year state-transition probability matrices for subsets of hepatocellular carcinoma*

	Early	Intermediate	Advanced	Death
All Patients of all age groups				
Early	92.17	4.81	1.73	1.29
Intermediate		69.32	27.27	3.41
Advanced			24.77	75.23
Death				100.00
Age < 60 years				
Early	95.33	2.34	1.40	0.93
Intermediate		58.33	37.50	4.17
Advanced			23.53	76.47
Death				100.00
Age 60–69 years				
Early	91.40	5.90	1.35	1.35
Intermediate		68.18	30.30	1.52
Advanced			22.21	78.79
Death				100.00
Age ≥70 years				
Early	90.68	5.49	2.33	1.50
Intermediate		74.42	22.09	3.49
Advanced			27.91	72.09
Death				100.00

*Early stage, solitary or multiple up to three nodules 3 cm or less each; Intermediate stage, four nodules or more, or larger than 3 cm; Advanced stage, portal vein invasion, extrahepatic metastasis, or Child–Pugh score C.

was significantly lower in young patients < 60 years of age (2.34%) than that in patients 60 years of age or older (5.70%)($\chi^2 = 7.76$, $P = 0.0053$). From the matrix stratified by three age groups, the transition probability from an intermediate to an advanced stage decreased with age: 37.50% in patients < 60 year of age, 30.30% in patients 60–69 year of age and 22.09% in patients 70 year of age or older ($\chi^2 = 10.57$, $P = 0.0011$).

Probabilities for transition according to the initial treatment

We also evaluated the transition probabilities among the four states in the subgroups of RFA and surgery as the initial mode of therapy.

In the matrix of patients receiving RFA therapy, the transition probability from early to intermediate stage was 5.40%, probability to the advanced stage was 1.63% and to death was 1.73%. In the patients undergoing surgery, the transition probability from an early to an intermediate stage was 3.90%, probability to an advanced stage was 1.87% and to death was 0.62%.

The probability for the transition from an early stage to an intermediate stage was slightly higher in the RFA group (5.40%) than that in the surgery group (3.90%), but statistical significance was not found ($\chi^2 = 1.90$, $P = 0.17$).

Discussion

Radiofrequency ablation has been considered as a less curative mode of therapy than surgical resection, because local tumour progression sometimes occurs after conservative treatment with relatively small ablative margins. As those patients with loco-regional therapy are generally followed up for tumour recurrence with a short time interval of 3–6 months using CT or MRI, we can usually ablate a newly appeared or a locally progressed tumour within a small size and few numbers. In order to elucidate the efficacies and usefulness of RFA compared with surgical resection, we analysed many HCC patients receiving RFA or surgical therapy regarding tumour progression and survival.

Fortunately, in Japan, where highly socialized medicine is practiced with everyone covered by some form of health insurance, almost all of the patients can receive any extensive medical services including surgery, RFA, embolization and repeated imaging diagnosis, regardless of the cost. Under intensive check-up and treatment repetition, the Markov model showed the probability of remaining at the early stage as 92.17% per year: the transition rate from the early to the intermediate stage was 4.81%, to the advanced stage 1.73 and to death 1.29% respectively. Similarly, the probabilities of remaining at the intermediate and advanced stages were 69.32 and 24.77% per year respectively.

Because young patients with HCC usually have better liver function and a relatively low carcinogenesis rate, younger patients are more likely to undergo radical methods of therapy for a recurrent tumour repeatedly. The reason for the low transition rate from the early to the intermediate stage was convincingly explained in the young patient group (Table 4). In contrast, the transition rate from the intermediate to the advanced stage was significantly higher in the young patient group. Although the exact reason was not known, multiple tumours of younger patients possibly progressed rapidly or were resistant to TACE. Hence, the Markov model would be eligible for simulating the outcomes of patients with the early stage of HCC. It is also helpful in planning strategies for the management of small HCC, for the eventual prolongation of a patient's life and for ideal cost-effective guidelines on a national basis, not only in Japan but also elsewhere in the world where the prevalence of HCC is increasing. Although we once generated a 'five-state model' consisting of no tumour, early stage, intermediate stage, advanced stage and death, we finally adopted the current 'four-state model' because of good mathematical fit and statistical robustness. Molinari and Helton (20) and Cho *et al.* (21) described a progression model of HCC after RFA and/or hepatectomy by the Markov model. Both authors performed a meta-analysis-like study using heterogeneous sources of patients from varied published articles, and estimated progression models of HCC in hypothetical patient cohorts. We analysed the actual clinical courses of patients in a single institution, where the same diagnostic and therapeutic procedures were adopted for every patient. Sufficient medical procedures and resources under a universal medical insurance system of the country seemed to give rise to better outcomes and survival, but an exact comparison cannot be carried out using the current data and the previous literatures.

In this study, we also compared RFA and surgery as an initial therapy for the early stage of HCC. Understandably, older patients, patients with severe cirrhosis and those with a concomitant disease other than liver disease tended to undergo non-surgical therapy. In addition, young patients with HBV-related HCC were likely to receive surgery because of good liver function, relatively low potential of recurrence and young age. Although the crude recurrence rate and the crude progression rate from the early stage to the intermediate stage were higher in patients receiving RFA therapy, multivariate analysis with adjustment of background biases showed that the initial mode of therapy did not affect the progression rate and did not affect the overall survival rate. When a regular check-up of imagings with an interval of 3–4 months was conducted, an additional ablation therapy was usually performed successfully for a small locally progressed tumour. Under intensive medical care for liver disease, the initial mode of therapy therefore did not affect the overall survival of a patient with an early stage of HCC. When a careful check-up with imagings and adequate application of repeated ablative procedures for HCC were performed, the choice of ablative manners was insignificant compared with the background liver features of aetiology of liver disease (hepatitis virus) and severity of liver disease (platelet count). The choice of ablative therapy for small-sized HCC should also be assessed from the viewpoints of conservation of liver function, cost-effectiveness and quality of life (9, 10, 12, 22).

Since it seemed to require at least 5 years to obtain a statistical difference in the recurrence rates and survival rates between RFA-treated and surgically treated groups, a prospective randomized trial is actually difficult to perform from both ethical and medical viewpoints. One of the significant results of the current study is that highly socialized medical circumstances with sufficient medical practice can attain a high survival rate of 71–80% at the end of the fifth year in patients at an early stage.

Further studies are required to determine the relationship between patient's age and stage transition. Because HCV-related chronic hepatitis often progresses to HCC during the clinical course, this kind of staging model with analyses of medical intervention will be necessary in the future from the viewpoints of epidemiological assessment and medical politics, together with patient's quality of life and feeling of satisfaction.

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Original Article

Efficacy and safety in sitagliptin therapy for diabetes complicated by chronic liver disease caused by hepatitis C virus

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Aim: Diabetes is present in patients with chronic liver disease caused by hepatitis C virus (HCV). The aim of this case–control study is to assess the efficacy and safety of dipeptidyl peptidase-4 inhibitor (sitagliptin) for type 2 diabetes mellitus (T2DM) with chronic liver disease caused by HCV.

Methods: Sixteen HCV positive patients with T2DM treated by sitagliptin were retrospectively enrolled. These patients were given sitagliptin between December 2009 and January 2010. Another 16 HCV patients with T2DM treated only with diet and excise for 48 weeks were selected as the control group. Serum levels of fasting plasma glucose (FPG), hemoglobin A1C (HbA1C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured before and 12, 24, 36 and 48 weeks after the initiation of treatment.

Results: In the sitagliptin group, the average HbA1C level decreased approximately 0.8% at 48 weeks after the initiation of sitagliptin. Next, the average FPG level decreased approximately 20 mg/dL during follow up after the initiation of sitagliptin. All the patients were able to take sitagliptin of 50 mg/day without reduction because of sitagliptin-related side-effects. On the other hand, in the control group, the average HbA1C and FPG level did not change with statistical significance during follow up of 48 weeks. Regarding aminotransferase, there were no significant changes of average AST and ALT level during follow up of 48 weeks in both the sitagliptin group and control group.

Conclusion: Our results indicate that sitagliptin is effective and safe for the treatment of T2DM complicated with HCV positive chronic liver disease.

Key words: hepatitis C virus, sitagliptin, type 2 diabetes mellitus

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of the more common causes of chronic liver disease in the world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20–50% of cases over a period of 10–30 years.[1,2] In addition, HCV is a major risk for hepatocellular carcinoma (HCC).[3–7] Lately, it has been reported that chronic HCV infection is associated with type 2 diabetes mellitus (T2DM)[8–14] Moreover, T2DM has been suggested to enhance with the development of HCC and poor prognosis of liver transplantation.[15–19] Thus, in patients with chronic liver diseases, the management of T2DM is very important to improve the prolonged prognosis.

However, most oral hypoglycemic agents are metabolized in the liver and often induce the liver damage. Thus, it is difficult to treat the patients who have T2DM complicated with chronic liver disease.[20] A new oral hypoglycemic agent, dipeptidyl peptidase-4 (DPP-4)

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inhibitor (sitagliptin), is minimally metabolized.[21,22] Hence, sitagliptin raises the possibility for use in patients with T2DM complicated with chronic liver disease.

With this background in mind, the case–control study was initiated to investigate the efficacy and safety of DPP-4 inhibitors for T2DM patients with HCV positive chronic liver disease.

METHODS

Patients

SIXTEEN PATIENTS WITH T2DM complicated with HCV positive chronic liver disease started the treatment with oral DPP-4 inhibitor (sitagliptin; MDS, Tokyo, Japan) of 50 mg/day from December 2009 to January 2010 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. These 16 consecutive patients treated with sitagliptin of 50 mg/day were regarded as the sitagliptin group. Inclusion criteria of DPP-4 inhibitor administration were as follows: (i) evidence of diabetes mellitus (i.e. plasma glucose concentration of ≥126 mg/dL [6.9 mM) in the fasting state, ≥200 mg/dL [11.0 mM] in casual state and/or 2 h after a 75-g oral glucose load; (ii) a diabetic history of less than 2 years; (iii) features of chronic hepatitis or cirrhosis diagnosed by ultrasonography and/or computed tomography; (iv) positive for serum HCV RNA; (v) negativity for hepatitis B surface antigens (HBsAg), antinuclear antibodies or anti-mitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (vi) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; and (vii) no underlying systemic disease, such as systemic lupus erythematosus and rheumatic arthritis. The distinction between chronic hepatitis and liver cirrhosis in patients was done by discriminant function using platelet, hyaluronic acid, and γ-globulin.[23] Patients with either of the following criteria were excluded from the study: (i) they were taking medicines except DPP-4 inhibitors known to alter glucose tolerance; and/or (ii) they had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial. Patients in the sitagliptin group exercised and participated in diet therapy in addition to administration of sitagliptin. In the same period, 303 patients with T2DM and chronic liver disease type C were not treated with antidiabetic drugs. These patients exercised and participated in diet therapy for T2DM. Seventy-three of these 303 patients were applied with seven inclusion criteria and two exclusion criteria as described above. Sixteen subjects in the control group were selected from these 73 patients by matching 1:1 with the sitagliptin group for age and sex. Patients who belonged to the control group or sitagliptin group had been subjected to lifestyle intervention of diet and physical exercise after the diagnosis of T2DM. The diet prescription included daily calorie intake of 125.6 kJ/ideal bodyweight (kg), a protein energy fraction of 15% and a fat energy fraction of 25%. Physical activity was recommended as at least 120 min of aerobic exercise a week. The physicians in charge explained the methods and side-effects of sitagliptin therapy to each patient and/or patient's family before sitagliptin therapy. Informed consent was obtained from 16 patients of the sitagliptin group before the initiation of sitagliptin therapy. All of the studies in the control group were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by the Institutional Review Board of Toranomon Hospital.

Outcome measures

Type 2 diabetes mellitus was diagnosed by the 2003 criteria of the American Diabetes Association:[24] (i) casual plasma glucose of 200 mg/dL or more; (ii) fasting plasma glucose (FPG) of 126 mg/dL or more; and/or (iii) 2-h post-glucose (oral glucose tolerance test) of 200 mg/dL or more. Hemoglobin A1c (HbA1c) was measured using a high-performance liquid chromatography method.

Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test v2.0; Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society, JDS) + 0.4%, considering the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard materials and measurement methods and HbA1c (NGSP).[25] Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (kg) / height (m^2).

Follow up

The starting time of follow up in the sitagliptin group was the initiation of sitagliptin therapy. That is, the time

Table 1 Clinical characteristics at the starting time of follow up

	Sitagliptin group	Control group	*P*-value
n	16	16	
Age (years)	65.3 ± 9.1	65.2 ± 9.5	1.0
Sex (male/female)	8/8	8/8	1.0
Chronic hepatitis/liver cirrhosis	13/3	13/3	1.0
BMI	23.0 ± 3.5	23.5 ± 2.9	0.713
BMI (post-intervention)	22.4 ± 2.4	22.6 ± 2.3	1.0
AST (IU/L)	43 ± 34	34 ± 21	0.170
ALT (IU/L)	45 ± 31	40 ± 31	0.423
Albumin (g/dL)	3.8 ± 0.4	3.9 ± 0.4	0.873
Total bilirubin (mg/dL)	0.9 ± 0.5	0.8 ± 0.3	0.167
Platelets ($\times 10^4$/mm^3)	15.1 ± 5.3	17.0 ± 6.7	0.208
Hyaluronic acid (ng/mL)	132 ± 80	112 ± 62	0.637
HbA1c (NGSP value)	7.4 ± 0.8	7.2 ± 0.9	0.552
FPG (mg/dL)	142.1 ± 24.1	140.0 ± 25.7	0.951

Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; NGSP, National Glycohemoglobin Standardization Program.

was from December 2009 to January 2010. The starting time of follow up in the control group was the same as that in the sitagliptin group. Patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check up. An overnight (12 h) fasting blood sample and HbA1C sample were taken for routine analyses. These included transaminase activities.

Statistical analysis

Clinical differences between the sitagliptin group and control group were evaluated by Wilcoxon rank sum test or Fisher's exact test. Changes in serum HbA1C and FPG level between the sitagliptin group and control group during follow up were analyzed by one-way repeated measurement ANOVA. Next, predictive factors for responders were assessed. A $P < 0.05$ was considered to be statistically significant. SPSS ver. 11.5 for Windows was used to perform statistical analysis.

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics before follow up in the 32 patients with T2DM and HCV positive chronic liver disease. There were no significant differences in clinical profiles between the sitagliptin group and control group.

Change of HbA1c and FPG

Change of average HbA1C and FPG level are plotted in Figures 1 and 2 in the sitagliptin group and control group. In the sitagliptin group, average HbA1C level decreased from 7.4% to 6.5% at 48 weeks after the initiation of sitagliptin. Moreover, average FPG level could be deduced at approximately 20 mg/dL during follow up after the initiation of sitagliptin. The HbA1C and FPG level in the sitagliptin group were statistically lower than those in the control group.

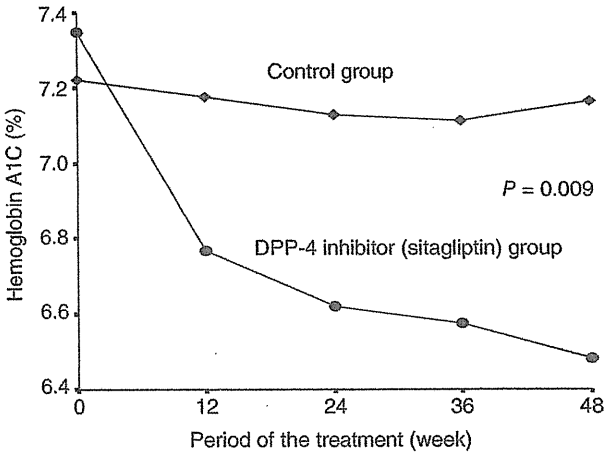

Figure 1 Change of average hemoglobin A1c (HbA1c) level during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group.

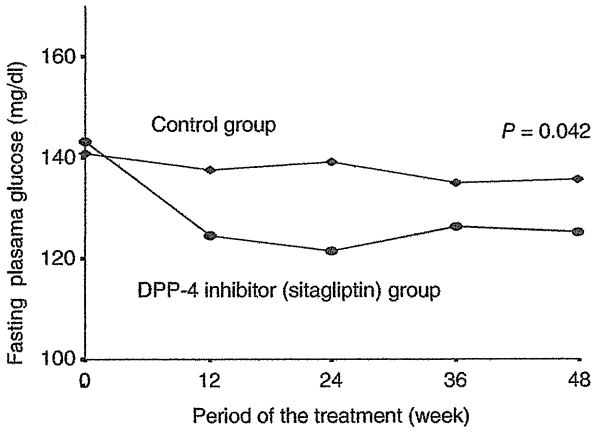

Figure 2 Change of average fasting plasma glucose during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group.

Adverse events of sitagliptin

Regarding side-effects, none of the patients treated with DDP-4 inhibitor had sitagliptin-related episodes severe enough to stop the treatment of sitagliptin. Thus, all the patients were able to take sitagliptin 50 mg/day for 48 weeks without reduction. Next, changes of average AST and ALT level during follow up are plotted in Figure 3. There were no significant changes of average AST and ALT level during follow up in either the sitagliptin or control group.

DISCUSSION

WE HAVE DESCRIBED the efficacy and side-effects of sitagliptin for T2DM patients with HCV positive chronic liver disease in the present study. The present study was limited by being a case–control study. Another limitation of the study was that patients were treated with different types of diet and different excise. This heterogeneity makes it slightly difficult to interpret the results of the study.

On the other hand, the present study shows several findings with regard to the efficacy and side-effects of sitagliptin for T2DM patients with HCV positive chronic liver disease. First, in the sitagliptin group, average HbA1C and FPG levels after the initiation of sitagliptin were statistically lower than those at the starting time of DPP-4 inhibitor. It is suggested that sitagliptin increases active glucagon-like peptide-1, stimulates insulin secretion and inhibits glucagon secretion.[21,22] Thus, it is accepted that sitagliptin could improve both HbA1C and glucose level in patients with T2DM and HCV positive chronic liver disease.

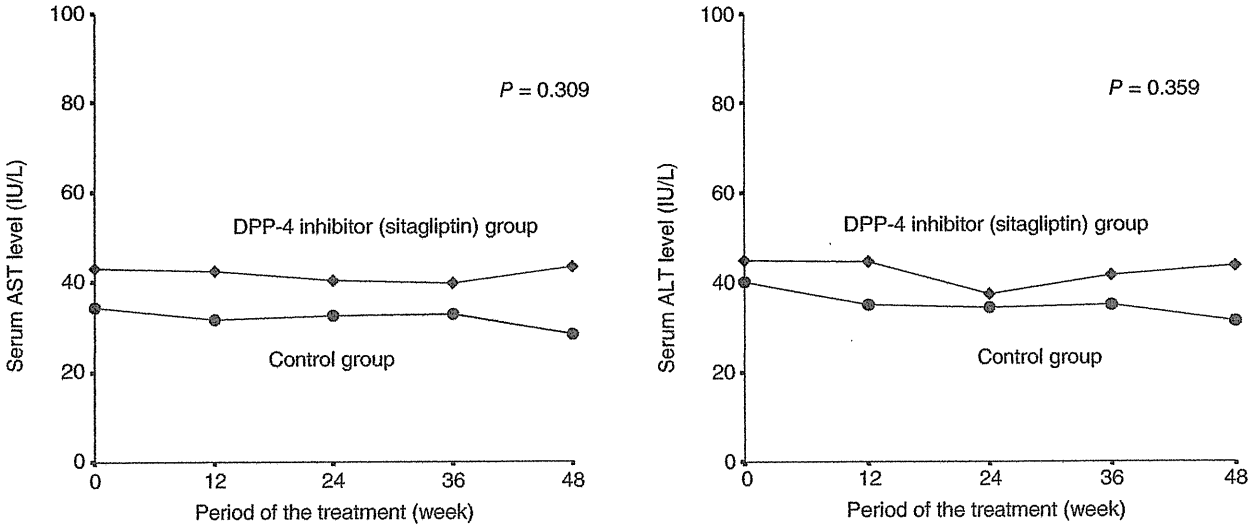

Figure 3 Change of average aminotransferase level during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group. (a) Change of average aspartate aminotransferase (AST) level during follow up was plotted in both the DPP-4 inhibitor group and control group. (b) Change of average alanine aminotransferase (ALT) level during follow up was plotted in both the DPP-4 inhibitor group and control group. Patients who belonged to the control group or sitagliptin group were subjected to lifestyle intervention of diet and physical exercise. The diet prescription included daily calorie intake of 30 kcal/ideal bodyweight, a protein energy fraction of 15% and a fat energy fraction of 25%.

Second, administration of sitagliptin is minimal risk and highly tolerable for T2DM patients with HCV positive chronic liver disease. In the present study, none of the patients treated with DDP-4 inhibitor had sitagliptin-related episodes severe enough to stop the sitagliptin therapy. Thus, all the patients could take sitagliptin of 50 mg/day over 48 weeks without reduction or stopping. This new oral hypoglycemic agent, sitagliptin, is minimally metabolized and over 80% of it is excreted in the urine. It seems not to alter pharmacokinetics in hepatic insufficiency.[22] Thus, sitagliptin has few possibilities to cause the aggravation of the chronic liver damage. In fact, in the present study, three patients with liver cirrhosis did not have elevation of aminotransferase during the treatment by sitagliptin. This result indicates that sitagliptin is valuable for treating T2DM with HCV positive liver cirrhosis.

Type 2 diabetes mellitus has been increasing dramatically in many nations including Japan over the past decades.[26] It is widely accepted that approximately 7–8 million people are affected by DM in Japan. Approximately 8–10% of adults in Japan have T2DM. Recently, it has been reported that T2DM has occurred in HCV positive chronic liver disease.[8–13] Moreover, HCV patients with T2DM are at major risk for HCC.[15–17] So, in patients with T2DM and HCV positive chronic liver diseases, the management of DM is very important to improve the prolonged prognosis. However, most oral hypoglycemic agents (thiazolidines, sulfonylurea and biguanides) are metabolized in the liver. Thus, it is suggested that most oral hypoglycemic agents often induce liver damage. The new oral hypoglycemic agent, DPP-4 inhibitor (sitagliptin), is minimally metabolized. Hence, this drug raises the possibility of being used for T2DM patients with HCV positive chronic liver disease.

In conclusion, our retrospective study suggests that sitagliptin is effective and safe for the treatment of T2DM complicated with HCV positive chronic liver disease.

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