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Abstract

Background and Aim: Hepatocellular carcinoma (HCC) can develop in patients with chronic hepatitis C after they have achieved a sustained virologic response (SVR) to antiviral therapy, i.e., eradication of hepatitis C virus (HCV). Thus, surveillance for HCC remains necessary after SVR. We investigated factors that are predictive of HCC in HCV-infected patients who achieved SVR. *Methods*: The incidence and risk factors for HCC were evaluated in 522 patients who achieved SVR with interferon-based antiviral therapy for HCV. Patients maintained regular follow-up every 6 months for HCC surveillance. The FIB-4 index and aspartate aminotransferase to platelet count ratio index calculated based on laboratory data at the time that SVR was documented (SVR24). *Results*: Patients continued follow-up visits for 1.0 to 22.9 years (median, 7.2 years) after SVR. HCC developed in 18 patients. The incidence of HCC was 1.2% at five years and 4.3% at ten years. Use of peginterferon or ribavirin for treatment and a history of antiviral therapy prior to the course when SVR was achieved were not associated with the incidence of HCC after SVR. Presence of diabetes mellitus (risk ratio 2.08; p=0.0451) and FIB-4 index calculated at the time of SVR24 (risk ratio 1.73;

p=0.0198) were associated with a higher likelihood of HCC after SVR by multivariate analysis. *Conclusions*: Patients with diabetes mellitus and patients with the elevation of FIB-4 index at SVR24 are at higher risk of HCC after SVR. Surveillance for HCC should be continued in this patient subpopulation.

Key words: chronic hepatitis C, antiviral therapy, sustained virologic response, hepatocellular carcinoma, risk factors

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide (1), and its incidence is predicted to increase in Western countries (2,3). Chronic hepatitis C virus (HCV) infection is a major cause of HCC (3-5), and the prevention of HCC is a major goal of antiviral therapy in patients with chronic hepatitis C.

Sustained virologic response (SVR) is defined as the eradication of HCV with antiviral therapy. The benefit of HCV eradication is the prevention of the progression of chronic hepatitis and associated complications (6). Several studies have confirmed that achievement of SVR results in the resolution of liver fibrosis (7-9) and a decreased incidence of HCC (10-14). However, the development of HCC is sometimes observed in patients who achieve SVR (15-19). Some cases involved very advanced HCC with very poor prognosis at the time of detection. Due to the decreased risk of HCC development in patients with SVR, they are less likely to participate in surveillance for HCC after SVR (20). This may result in a failure to detect small HCCs, allowing the tumor to grow to a large size before detection and diagnosis at an advanced stage.

The emergence of new direct-acting antiviral drugs (DAAs) against HCV will

dramatically increase the number of patients who achieve SVR (21-24). Given the marked increase in the number of patients who achieve SVR, there will be an increase in the number of patients who develop HCC after SVR in the near future. Therefore, understanding the incidence and risk factors for the development of HCC in patients after SVR will be important for the management of this patient subpopulation.

In the present study, we investigated the incidence and risk factors for HCC in 522 patients with chronic HCV infection who achieved SVR with interferon (IFN)-based antiviral therapy.

Patients and Methods

Patients and Follow-up

Between 1990 and 2012, a total of 1285 patients with chronic HCV infection underwent IFN-based antiviral therapy at our institution. Patients were excluded if they had antibodies against human immunodeficiency virus or hepatitis B virus surface antigen or other forms of liver disease (e.g., autoimmune hepatitis, alcoholic liver disease, or hemochromatosis). Patients with cirrhosis were not included because

IFN-based antiviral therapy is not permitted by the Japanese National Medical Insurance System for patients who had cirrhosis at the start of the antiviral therapy. Of these, 522 patients achieved SVR. HCV infection was confirmed by positive HCV antibody titers and the presence of serum HCV RNA before treatment. SVR was confirmed by the absence of serum HCV RNA 24 weeks after the end of treatment (SVR24). No patient had a history of HCC. The absence of HCC was confirmed by imaging studies in all patients at the start of antiviral therapy and when SVR was documented (i.e., SVR24). Liver biopsy was performed in 494 patients prior to the start of antiviral therapy. Liver histology was classified according to the METAVIR score (25).

Patients continued to follow-up every six months after SVR with laboratory testing and ultrasonography at every visit. The absence of serum HCV RNA was reconfirmed annually (at every two visits). Laboratory tests included complete blood cell count, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and tumor markers for HCC (alpha-fetoprotein [AFP] and des-gamma-carboxy prothrombin [DCP]). If a nodular lesion was detected by ultrasonography or an elevation in a tumor

marker was observed, additional imaging studies (computed tomography, magnetic resonance imaging, or both) were performed. The diagnosis of HCC was based on appropriate imaging characteristics according to criteria in the guidelines of the American Association for the Study of Liver Diseases (26,27) with the findings of arterial hypervascularity and venous or delayed phase washout by contrast-enhanced dynamic computed tomography or magnetic resonance imaging. In addition, HCC was confirmed histologically based on the resected specimen when patients underwent surgical resection as a treatment.

The entire protocol was approved by the hospital institutional review board and carried out in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participated patients before the enrollment of the study.

Estimation of Liver Fibrosis when SVR was Achieved with FIB-4 Index and APRI

Liver fibrosis was estimated at SVR24 using the FIB-4 index (28) calculated as AST [IU/L] × age [years]/ platelet count [10^9/L] × ALT [IU/L]$^{1/2}$, and using aspartate aminotransferase to platelet count ratio index (APRI) (29) calculated as AST [IU/L]

(/upper limit of normal AST [IU/L]) × 100 / platelet count [10^9/L].

Statistical Analysis

SVR24 was defined as time zero for calculating the incidence of HCC. In the analysis of HCC incidence, patients who developed HCC were non-censored and those who did not were censored. The Kaplan-Meier method was used to calculate survival rates, and the log-rank test was used to analyze differences in incidence. A Cox proportional hazards model was used for univariate and multivariate analysis of factors related to the development of HCC. Univariate analysis was first performed for all variables analyzed. Variables that reached statistical significance (P <0.05) in the univariate analysis were subsequently included in the multivariate analysis. Data analysis was performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC, USA). All P values were derived from two-tailed tests, with P <0.05 accepted as statistically significant.

Results

Patients Characteristics

Table 1 shows the baseline patient characteristics before antiviral therapy. Patients consisted of 292 (55.9%) males and 230 (44.1%) females, with a mean age of 50.6 ± 11.8 years. Approximately 20% of patients reported habitual alcohol intake (more than 50 g per day for more than 5 years) and 8.2% of patients had type 2 diabetes mellitus, defined based on the American Diabetes Association revised criteria (30). Less than half of the patients achieved SVR with use of PEG-IFN or use of ribavirin for therapy. More than half of the patients had been infected with HCV genotype 2 (2a or 2b) before the eradication, although genotype 1b is predominant in Japan. This is because patients with genotype 2 were more likely to achieve SVR than those with genotype 1. No patients had F4 fibrosis due to the disability for patients with cirrhosis to undergo IFN-based antiviral therapy by National Insurance System. The FIB-4 index and APRI at SVR 24 was 1.99 ± 1.52 and 0.45 ± 0.52, respectively. The FIB-4 index at SVR 24 was increased with the increase of pretreatment liver fibrosis grade as assessed in liver biopsy specimens despite the normalization of serum transaminase activity (Figure 1).

Incidence of HCC and Risk Factors Associated with the Development of HCC after SVR

During a median follow-up of 7.2 years (range, 1.0-22.9 years) in patients with SVR, HCC was diagnosed through screening in 18 patients with the median interval of 6.6 years (range, 1.5-17.1 years). 51 of 522 patients (9.8%) were lost for follow-up during the study period after 2.3-18.2 years' follow-up, who were treated as censored cases. HCC was treated by surgical resection according to treatment guidelines for HCC in Japan (31) and diagnosis of HCC was confirmed also histologically based on the resected specimen after treatment in 15 patients. The incidence of HCC at five and ten years was 1.2 % and 4.3 %, respectively.

Risk factors associated with the development of HCC after SVR were investigated. Based on the univariate analysis, patient age (risk ratio [RR] 1.06; P = 0.0228), sex (RR 1.87; P = 0.0250), habitual alcohol intake (RR 1.72; P = 0.0484), diabetes mellitus (RR 1.91; P = 0.0277), baseline albumin (RR 0.12; P = 0.0045), baseline platelet counts (RR 0.82; P = 0.0002), liver fibrosis grade before treatment based on percutaneous liver biopsy (RR 2.35; P = 0.0005), FIB-4 index at SVR 24 (RR 1.51; P < 0.0001), and APRI at SVR24 (RR 2.04; P = 0.0031) were identified as factors

significantly associated with a likelihood of HCC after SVR (Table 2A). Pretreatment HCV RNA levels, HCV genotype, past history of antiviral treatment, and use of PEG-IFN or use of ribavirin in the treatment regimen that achieved SVR were not associated with HCC development after SVR. Presence of diabetes mellitus (RR 2.08; $P = 0.0453$) and higher FIB-4 index at SVR24 (RR 1.73; $P = 0.0198$) were selected as a factor significantly associated with a higher likelihood of HCC according to the multivariate analysis (Table 2B).

Figure 1 shows the cumulative incidence of HCC after SVR based on the FIB-4 index at SVR24. The optimal cutoff point for the FIB-4 index was determined with a Cox proportional hazards model and the distribution of the FIB-4 index in the study patients. Patients were classified as having a FIB-4 index of < 2.0 or ≥ 2.0. The incidence of HCC in patients with a FIB-4 index ≥ 2.0 was significantly higher than that of patients with FIB-4 index < 2.0 ($P = 0.0001$).

Characteristics of Patients Who Developed Hepatocellular Carcinoma after SVR

Table 4 summarizes the characteristics of 18 patients who developed HCC after

SVR. The interval between SVR24 and the diagnosis of HCC was 6.76 ± 4.19 years. The percentages of patients who achieved SVR with the use of PEG-IFN or use of ribavirin was smaller comparing the entire study patients. AFP level increased significantly at HCC development, compared to the baseline AFP level ($P = 0.0437$). Whereas APRI at HCC development was significantly lower than that at SVR24 ($P = 0.0424$), no significant decrease was observed in FIB-4 index between at SVR24 and at HCC development ($P = 0.1750$). Liver fibrosis progressed to cirrhosis at the development of HCC in 6 of 15 patients (40.0%) who underwent surgical resection as a treatment of HCC and non-cancerous liver tissue at HCC development was available (Supplementary table).

Discussion

Some HCCs detected and diagnosed after the achievement of SVR could have been minute tumors that were undetectable by imaging studies during or even before antiviral therapy that grew to a detectable size after SVR (15,32,33). However, our previous analysis of tumor volume doubling time of HCC detected after SVR suggests

that HCC does develop after the eradication of HCV (34). Indeed, there are several case reports of small HCC tumors diagnosed more than five years after SVR (16,17). These findings highlight the importance of understanding the incidence and risk factors for HCC development after SVR.

In the present study, we investigated the incidence and risk factors for HCC diagnosed after the eradication of HCV in more than 500 patients who underwent surveillance for median 7.2 years. The incidence of HCC was 1.2% at five years and 4.3% at ten years after SVR, which was significantly lower than the reported incidence of HCC in patients who underwent IFN-based antiviral therapy but failed SVR (10-14). The incidence of HCC development in non-cirrhotic patients who achieved SVR in this study was higher than the incidence of HCC in non-cirrhotic patients with SVR in Western countries and similar to those with advanced fibrosis (35). This is partly because the age of patients with chronic hepatitis C in Japan is older than those in Western countries (36,37). The use of PEG-IFN or the use of ribavirin did not affect the incidence of HCC after SVR. This means that the risk of HCC development did not differ according to the antiviral treatment regimen used if HCV was eradicated.

Although univariate analysis identified patient age, sex, habitual alcohol intake, diabetes mellitus, baseline serum albumin and platelet count, pretreatment liver fibrosis, FIB-4 index at SVR24, and APRI at SVR24 as factors associated with HCC development after SVR, only the presence of diabetes mellitus and the FIB-4 index at SVR24 were identified as risk factors independently associated with HCC development in multivariate analysis. Previous study reported the association between diabetes mellitus and HCC development in patients with chronic hepatitis C after SVR (38). More recent study reported patient age, severity of liver disease, and diabetes mellitus as risk factors of HCC development after SVR (39). Because FIB-4 index is a laboratory index for severity of liver disease (liver fibrosis) and includes age as a factor, our results were consistent with previous reports.

Liver fibrosis is an important risk factor for the development of HCC in patients with HCV infection (12), and liver biopsy is the gold standard for assessing liver fibrosis (40). However, it is associated with rare but lethal complications such as hemorrhage (41), and it is impractical to perform serial liver biopsies after the achievement of SVR. Several laboratory indices of liver fibrosis have been reported

(28,29,42). Vallet-Pichard et al. reported that the FIB-4 index is concordant with liver fibrosis based on pathological evaluation of liver biopsy specimens in patients with persistent HCV infection (43). The results of the present study showed that the FIB-4 index could predict the likelihood of HCC development focusing on patients who achieved SVR, reflecting the degree of liver fibrosis before antiviral therapy. In addition, needle biopsy has a risk of sampling error (25); pathological assessment is not always objective and not necessarily reflect the fibrosis status of the entire liver when based on needle-biopsied specimens. In addition, one recent study reported poor association between histological liver fibrosis and laboratory indices of liver fibrosis in HCV-infected patients after SVR (44). These might be why pretreatment histological liver fibrosis based on the biopsied liver tissue was not identified as a factor associated with HCC development after SVR in multivariate analysis. In contrast, FIB-4 index was associated with HCC development after SVR, including risk factors for HCC after SVR as factors for calculation.

APRI, another laboratory index of liver fibrosis had a less predictive ability of HCC development after SVR, showing superiority of FIB-4 index as a laboratory

predictive index of HCC development after SVR. In patients who achieved SVR, AST and ALT usually normalized because of the eradication of HCV. Whereas FIB-4 index includes AST/ALT$^{1/2}$ ratio, APRI includes only AST. In addition, APRI does not include patient age, an important risk factor of HCC development. These may be reasons of the lower ability of APRI to predict HCC development after SVR in comparison to FIB-4 index.

There are several issues that should be further studied in the future. This study is retrospective based on the laboratory data and medical records, although study patients underwent regular follow-up prospectively after the achievement of SVR. Therefore, the prospective validation of this result will be necessary. It is reported that imaging techniques to assess liver fibrosis such as transient elastography have the ability to predict the risk of HCC development in patients with persistent HCV infection (45). It is necessary to clarify whether these imaging techniques can predict the risk of HCC also in patients after SVR, comparing the ability with laboratory fibrosis indices. The eradication of HCV was achieved with IFN-based antiviral therapy using IFN or PEG-IFN with or without ribavirin in all patients in the present study. Although the use

of PEG-IFN, ribavirin, or both was not associated with the incidence of HCC after SVR, it should be re-evaluated in patients who achieve SVR with newly emerging IFN-free DAAs, with a very high rate of SVR (21-24). The risk of HCC after SVR may depend on the kind of antiviral drugs used to induce SVR when DAAs are included in the analysis. In addition, genotype 2 was the most common genotype among patients in the present study. With the use of DAAs, it is presumed that there will be more patients with genotype 1 infection resistant to IFN-based antiviral therapy who achieve SVR, including those bearing an unfavorable interleukin-28B polymorphisms genotype (46,47), HCV with histidine at residue 70 of the HCV core region (48), and liver steatosis, all of which have reportedly been also associated with a higher likelihood of HCC development (49,50). Re-appraisal of the predictive value of the FIB-4 index for HCC after SVR will be necessary as patients with factors associated with resistance to IFN-based antiviral therapy and a higher likelihood of developing HCC achieve SVR through DAAs in the future.

In conclusion, the incidence of HCC was 1.2 % at five years and 4.3 % at ten years in non-cirrhotic patients with chronic HCV infection who achieved the eradication

of HCV with IFN-based antiviral therapy in Japan. The risk of HCC after SVR was not associated with the antiviral treatment regimen that eradicated HCV. Presence of diabetes mellitus and the elevation of FIB-4 index at SVR24 are at risk factors of HCC after SVR.

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