Lifestyles associated with prognosis after eradication of hepatitis C virus: a prospective cohort study in Japan

Satoko Ohfuji (satop@med.osaka-cu.ac.jp)
Osaka City University Graduate School of Medicine https://orcid.org/0000-0003-3239-5249
Tomoka Matsuura
Department of Public Health, Osaka City University Graduate School of Medicine
Akihiro Tamori
Department of Hepatology, Osaka City University Graduate School of Medicine
Shoji Kubo
Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine
Satoshi Sasaki
Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo
Kyoko Kondo
Osaka City University Hospital
Kazuya Ito
College of Healthcare Management
Wakaba Fukushima
Department of Public Health, Osaka City University Graduate School of Medicine

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Abstract

Background

Hepatocellular carcinoma develops in some patients with hepatitis C virus (HCV), even after achieving sustained virological response (SVR). This prospective cohort study of SVR patients examined factors associated with liver disease progression.

Methods

Participants were patients who had visited a hospital for clinical follow-up of chronic HCV infection in 2005 and had reached SVR as a result of subsequent antiviral treatment. Baseline information including lifestyle and dietary habits before SVR were collected in 2005 using self-administered questionnaires, and clinical information before SVR was collected from medical records in 2005. Study outcome was liver disease progression such as liver cirrhosis, hepatocellular carcinoma, and/or liver disease-related death after SVR. Logistic regression models were employed to calculate odds ratios (ORs) and 95% confidence intervals for each variable.

Results

Of 180 SVR patients, 27 patients (15%) showed liver disease progression after SVR: 26 (14%) were diagnosed with liver cirrhosis, two (1%) with hepatocellular carcinoma, and/or one (0.6%) with liver-disease related death. Besides older age at SVR (OR=6.65, P=0.01) and aspartate aminotransferase-to-platelet ratio index score ≥ 1.0 before SVR (OR=5.22, P<0.01), alcohol drinkers before SVR (OR=2.83, P=0.09) appeared to be associated with liver disease progression after SVR, whereas higher consumption of vitamin B12 before SVR showed a decreased OR for liver disease progression (OR=0.35, P=0.09).

Conclusions

Alcohol drinking was associated with liver disease progression, whereas vitamin B12 intake had protective effects for liver disease progression after eradication of HCV. Further studies are needed to confirm these findings.

Background

In most cases, hepatocellular carcinoma (HCC) develops against an established background of chronic liver disease. In Japan, about 60% of HCC cases result from infection with hepatitis C virus (HCV). Likewise in North America and Europe, HCV infection is the predominant risk factor for HCC [1, 2]. A nested case-control study in Japan indicated that the relative risk of HCC incidence was increased 101-fold among patients with HCV infection, compared to those without HCV [3].

Interferon (IFN)-based regimens have been the standard of care to eradicate HCV for over two decades, and also offer a reduced risk of developing HCC, decompensated cirrhosis, and overall mortality to

patients who had received IFN treatment [4, 5]. Several IFN-free regimens with direct-acting antivirals (DAAs) have recently been developed [6], and sustained virologic response (SVR) after treatment could be achieved in approximately 90% of patients [7]. In addition, SVR patients after DAA therapy could induce a 78% reduction in the risk of HCC occurrence compared with non-SVR patients [8].

However, even after achieving SVR after treatment, HCC risk is not eliminated and HCC has been reported in some cases with SVR [9]. A prospective cohort study reported that the long-term risk of developing HCC remains for up to 8 years after SVR [10]. Another cohort study also showed that approximately one-third of patients still showed liver fibrosis even after achieving SVR [11]. These findings suggested that chronic liver disease may not be cured even if SVR is obtained, or that factors other than HCV such as lifestyle habits may affect liver disease progression among these SVR patients.

Some studies have examined predictors of HCC occurrence after SVR, revealing older age, male sex, fibrosis, albumin level, platelet count, alpha-fetoprotein level, and diabetes as risk factors for HCC [12–16]. However, since those studies focused on elucidating clinical predictors of HCC, whether lifestyle habits including smoking, alcohol drinking and dietary factors affect progression of liver disease after SVR has remained unclear. In addition, as a dietary factor, while vitamin B intake has been reported to have protective effects against the development of cancers at other sites such as breast cancer [17] and colorectal cancer [18], the association with HCC remains unclear. The present study thus aimed to clarify associations between lifestyle habits and liver disease progression using a database of chronic HCV patients established in 2005 and their follow-up data until 2017.

Methods

Study subjects

The present prospective cohort study used a database of chronic HCV patients established in the Department of Hepatology at Osaka City University Hospital in 2005. The database has been described in detail elsewhere [19]. Briefly, patients eligible for recruitment to the database were those with detectable HCV-RNA in 2005 and without other types of liver diseases such as co-infection with hepatitis B virus, HCC, autoimmune hepatitis, primary biliary cirrhosis, or idiopathic portal hypertension. A total of 509 patients were enrolled in 2005. Among those, patients who achieved SVR by subsequent antiviral treatment were extracted and included in the present study. All study patients provided written informed consent.

The study protocol was approved by the ethics committee of Osaka City University Graduate School of Medicine, and was performed in accordance with the Declaration of Helsinki.

Information Collection At Baseline

At the time of recruitment in 2005, study subjects filled out a set of two self-administered questionnaires. One questionnaire asked about demographic characteristics, height (cm) and body weight (kg), age at diagnosis of liver disease, age at diagnosis of hepatitis C, underlying illnesses including diabetes and dyslipidemia, alcohol drinking habits (never, former, or current), and smoking habits (never, former, or current). Body mass index at baseline was calculated by using height and body weight data from 2005.

The other questionnaire was a brief self-administered diet history questionnaire (BDHQ). The BDHQ asked about the frequency of consumption of selected food, but not about portion size, to estimate the dietary intake of 58 food and beverage items during the preceding month. Energy intake and nutrients intakes were also calculated from the data obtained from BDHQ using an ad-hoc computer algorithm for calculating nutrient intakes from the BDHQ. Detailed descriptions of the methods used for calculating dietary intakes and the validity of the BDHQ have been published elsewhere [20].

In addition, well-trained research nurses abstracted baseline clinical information including laboratory data and findings of imaging modalities in 2005 from the medical records of the study subjects. Laboratory data included serum albumin [g/dL], total bilirubin [mg/dL], aspartate aminotransferase [IU/L], alanine aminotransferase [IU/L], γ-glutamyl transpeptidase [IU/L], platelet count [10^4/μL], alpha-fetoprotein [ng/mL], HCV genotype, and HCV-RNA [KIU/mL]. As a fibrosis indicator at baseline, the aspartate aminotransferase-to-platelet ratio index (APRI) in 2005 was calculated as aspartate aminotransferase [IU/L]/upper limit of normal value for aspartate aminotransferase (i.e., 33 IU/L) divided by platelet count [10^9/L] multiplied by 100.

Follow-up Survey

Study subjects were followed-up using their medical records until 2017. We obtained data on virologic status in 2017 (SVR or not), antiviral treatment that led to SVR (IFN or DAA) and the date of achieving SVR for SVR patients. In addition, we also extracted information on HCC occurrence between 2005 and 2017, date of HCC diagnosis if such a diagnosis was made, the most recent laboratory data, findings from the most recent imaging modalities, date of last observation and live status as of the time of last observation, and cause of death if deceased (hepatic failure, HCC, and others). The diagnosis of HCC or liver cirrhosis was confirmed by the findings for imaging modalities or histological findings.

Statistical analysis

Study outcome was regarded as liver disease progression such as HCC occurrence, liver cirrhosis and/or liver disease-related death. Liver disease-related death included death caused by HCC, hepatic failure, bleeding from esophageal or gastric varices.

Explanatory variables were sex, body mass index at baseline [kg/m^2], age at diagnosis of liver disease, age at SVR, duration between liver disease diagnosis and SVR, type of treatment that led to SVR (IFN or DAA), year at SVR, duration between SVR and last observation (i.e., follow-up period), HCC before SVR,

laboratory data at baseline, diabetes and dyslipidemia as underlying illnesses, alcohol drinking and smoking status at baseline, and dietary intakes at baseline. Body mass index and laboratory data at baseline (e.g., albumin [g/dL], total bilirubin [mg/dL], aspartate aminotransferase [IU/L], alanine aminotransferase [IU/L], γ-glutamyl transpeptidase [IU/L], platelet count [10^4/μL], APRI, alpha-fetoprotein [ng/mL]) were classified into two categories using conventional cutoff points. Other continuous variables such as age, duration or HCV-RNA were classified into tertiles according to the distribution of study subjects. Regarding dietary factors, energy-adjusted intake by the density method was used for all analyses.

To examine the association with progression of liver disease, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each characteristic using logistic regression model. Trends for association were assessed by assigning ordinal scores to a single variable. Variables showing association with outcomes in age- and sex-adjusted analyses with values of $p < 0.05$ or reported associations with HCC from previous studies were considered as potential confounders for adjustment in multivariate models. Since laboratory data strongly correlated with each other, priority was given to the commonly used APRI included in the multivariate model. In addition, alpha-fetoprotein, despite being a reported risk factor for HCC, was not included in the multivariate model, since one-third of patients did not have baseline data besides a strong correlation with APRI.

All analyses were performed using Statistical Analysis System version 9.1 software (SAS Institute, Cary, NC).

Results

During follow up, a total of 180 patients achieved SVR after antiviral treatment, and comprised the subjects for analysis. Table 1 shows the characteristics of study subjects. One-third were male, and median age at achieving SVR was 65.0 (range, 27.6–88.3) years. Median age at recruitment to the 2005 database was 59.5 years (range, 26.0-78.5 years), and therefore about 8.1 years in median had passed to achieve SVR since recruitment to the 2005 database. In total, 102 patients (57%) achieved SVR with IFN treatment, and another 78 (43%) with DAAs. Before SVR, 9% of patients had a history of HCC, and about one-quarter showed an APRI of one or more.

Table 1

Background characteristics of study subjects.

Variables	Category	N	n (%)
Age at SVR (years)	Median (range)	180	65.0 (27.6–88.3)
Sex	Male	180	64 (36)
Body mass index (kg/m^2)	Median (range)	179	22.4 (16.2–30.0)
Age at diagnosis of liver disease (years)*	Median (range)	180	46 (6–78)
Duration between liver disease diagnosis and SVR (years)*	Median (range)	180	18.8 (0.4–56.0)
Type of treatment leading to SVR	IFN	180	102 (57)
	DAA		78 (43)
Year at SVR	2005–2008	180	58 (32)
	2009–2014		52 (29)
	2015–2017		70 (39)
Clinical characteristics before SVR			
History of hepatocellular carcinoma	Present	180	16 (9)
Laboratory data in 2005			
Albumin (g/dL)	< 3.5	178	4 (2)
Total bilirubin (mg/dL)	≥ 1.1	177	31 (18)
Aspartate aminotransferase (IU/L)	≥ 34	179	122 (68)
Alanine aminotransferase (IU/L)	≥ 43 (M); 28 (F)	179	122 (68)
γ-glutamyl transpeptidase (IU/L)	≥ 61	178	35 (20)

Data are presented as median (range) or number (%).

APRI, aspartate aminotransferase-to-platelet ratio index; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

* Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.

† According to baseline information collected in 2005.

Variables	Category	N	n (%)
Platelet count (× 10^4/μL)	< 18.0	178	81 (46)
APRI	≥ 1.0	178	43 (24)
Alpha-fetoprotein (ng/mL)	≥ 20.1	129	7 (5)
HCV genotype		139	114 (82)
			25 (18)
HCV-RNA (KIU/mL)	Median (range)	166	973 (0-5000)
Underlying illnesses in 2005			
Diabetes mellitus	Present	178	17 (10)
Dyslipidemia	Present	174	19 (11)
Lifestyle habits before SVR†			
Smoking	Never	180	120 (67)
	Former		37 (21)
	Current		23 (13)
Alcohol drinking	Never	180	79 (44)
	Former		49 (27)
	Current		52 (29)
Data are presented as median (range) or number (%).			
APRI, aspartate aminotransferase-to-platelet ratio index; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.			
* Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.			
† According to baseline information collected in 2005.			

As a study outcome, liver disease progression was able to be assessed for a mean of 4.1 years after achieving SVR. Among the 180 patients, 27 patients (15%) showed progression of liver disease, including 26 (14%) with liver cirrhosis, two (1%) with HCC, and/or one (0.6%) with liver disease-related death (Table 2). As additional information, two patients who developed HCC after SVR had no history of HCC before SVR. After considering the effect of potential confounders, older age at SVR (OR = 6.65, 95%CI = 1.49–29.8; P = 0.01), APRI ≥ 1.0 before SVR (OR = 5.22, 95%CI = 1.88–14.5; P < 0.01), and an alcohol

drinking habit before SVR (OR = 2.83, 95%CI = 0.82–9.72; P = 0.09) appeared to be associated with liver disease progression after SVR.

Table 2

Association between selected characteristics and progression of liver disease (cirrhosis, hepatocellular carcinoma and/or liver disease-related death).

Variables	Category	Incidence	Age-, sex-adjusted model	Multivariate model*
		n/N (%)	OR (95%CI)	OR (95%CI)
Total subjects		27/180 (15)		
Age at SVR (years)	< 59.5	3/59 (5)	1.00	1.00
	59.5–71.7	7/61 (11)	2.58 (0.63–10.6)	2.30 (0.50–10.5)
	≥ 71.8	17/60 (28)	7.65 (2.09-28.0)	6.65 (1.49–29.8)
			(Trend P < 0.01)	(Trend P < 0.01)
Sex	Male	12/64 (19)	1.00	1.00
	Female	15/116 (13)	0.61 (0.25–1.45)	0.60 (0.20–1.80)
Body mass index (kg/m^2)	< 25.0	20/146 (14)	1.00	
	≥ 25.0	6/33 (18)	1.81 (0.61–5.39)	
Age at diagnosis of liver disease (years)†	< 40	5/58 (9)	1.00	
	40–51	7/63 (11)	0.99 (0.26–3.76)	
	≥ 52	15/59 (25)	1.67 (0.42–6.64)	

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI score at 2005, smoking and alcohol drinking history.

† Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.

‡ According to baseline information collected in 2005.

Variables	Category	Incidence	Age-, sex-adjusted model	Multivariate model*
		n/N (%)	OR (95%CI)	OR (95%CI)
			(Trend P = 0.39)	
Duration between liver disease diagnosis and SVR (years) †	< 14.6	6/60 (10)	1.00	
	14.6–22.5	11/61 (18)	1.35 (0.44–4.17)	
	≥ 22.6	10/59 (17)	0.99 (0.30–3.26)	
			(Trend P = 0.94)	
Type of treatment that lead to SVR	IFN	2/102 (8)	1.00	
	DAA	19/78 (24)	2.07 (0.71–6.09)	
Year at SVR	2005–2008	3/58 (5)	1.00	
	2009–2014	7/52 (13)	2.36 (0.53–10.6)	
	2015–2017	17/70 (24)	3.31 (0.75–14.5)	
			(Trend P = 0.29)	
Clinical characteristics before SVR				
History of hepatocellular carcinoma	Absent	19/164 (12)	1.00	1.00
	Present	8/16 (50)	3.76 (1.13–12.6)	1.86 (0.48–7.19)

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI score at 2005, smoking and alcohol drinking history.

† Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.

‡ According to baseline information collected in 2005.

Variables	Category	Incidence	Age-, sex-adjusted model	Multivariate model*
		n/N (%)	OR (95%CI)	OR (95%CI)
Laboratory data in 2005				
Albumin (g/dL)	< 3.5	2/4 (50)	3.92 (0.42–36.3)	
	≥ 3.5	25/174 (14)	1.00	
Total bilirubin (mg/dL)	< 1.1	22/146 (15)	1.00	
	≥ 1.1	5/31 (16)	0.97 (0.31–2.98)	
Aspartate aminotransferase (IU/L)	< 34	4/57 (7)	1.00	
	≥ 34	23/122 (19)	2.29 (0.72–7.25)	
Alanine aminotransferase (IU/L)	< 43 (M); 28 (F)	9/57 (16)	1.00	
	≥ 43 (M); 28 (F)	18/122 (15)	0.89 (0.34–2.30)	
γ-glutamyl transpeptidase (IU/L)	< 61	16/143 (11)	1.00	
	≥ 61	11/35 (31)	4.33 (1.55–12.1)	
Platelet count (× 10^4/μL)	< 18.0	20/81 (25)	4.03 (1.55–10.5)	
	≥ 18.0	7/97 (7)	1.00	
APRI	< 1.0	12/135 (9)	1.00	1.00

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI score at 2005, smoking and alcohol drinking history.

† Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.

‡ According to baseline information collected in 2005.

Variables		Category	Incidence	Age-, sex-adjusted model	Multivariate model*
			n/N (%)	OR (95%CI)	OR (95%CI)
		≧ 1.0	15/43 (35)	5.13 (2.07–12.7)	5.22 (1.88–14.5)
	Alpha-fetoprotein (ng/mL)	< 20.1	18/122 (15)	1.00	
		≧ 20.1	4/7 (57)	7.10 (1.20–41.8)	
	HCV genotype	□	19/114 (17)		
		□	0/25 (0)	Not applicable	
	HCV-RNA (KIU/mL)	< 650	9/55 (16)	1.00	
		650–1419	12/56 (21)	1.87 (0.66–5.34)	
		≧ 1420	4/55 (7)	0.46 (0.13–1.68)	
				(Trend P = 0.30)	
Underlying illnesses in 2005					
	Diabetes mellitus	Absent	22/155 (14)	1.00	
		Present	5/17 (29)	1.40 (0.39–5.01)	
	Dyslipidemia	Absent	22/128 (17)	1.00	
		Present	5/19 (26)	2.25 (0.68–7.45)	

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI score at 2005, smoking and alcohol drinking history.

† Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.

‡ According to baseline information collected in 2005.

Variables	Category	Incidence	Age-, sex-adjusted model	Multivariate model*
		n/N (%)	OR (95%CI)	OR (95%CI)
Lifestyle habits before SVR‡				
Smoking	Never	19/120 (16)	1.00	1.00
	Former	5/37 (14)	0.51 (0.15–1.71)	0.35 (0.09–1.33)
	Current	3/23 (13)	0.79 (0.19–3.29)	0.57 (0.12–2.78)
			(Trend P = 0.50)	(Trend P = 0.26)
Alcohol drinking	Never	8/79 (10)	1.00	1.00
	Former	9/49 (18)	1.55 (0.50–4.79)	1.57 (0.45–5.45)
	Current	10/52 (19)	2.43 (0.77–7.64)	2.83 (0.82–9.72)
			(Trend P = 0.13)	(Trend P = 0.099)

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI score at 2005, smoking and alcohol drinking history.

† Missing information for age at diagnosis of liver disease (2 subjects) were complemented by age at diagnosis of hepatitis C.

‡ According to baseline information collected in 2005.

Regarding dietary factors (Table 3), higher consumptions of niacin and vitamin B12 seemed to be associated with decreased risk of liver disease progression after SVR in age- and sex-adjusted analyses, with marginal significance. In the multivariate analysis, however, only higher consumption of vitamin B12 before SVR remained associated with a marginally significant decrease in OR for liver disease progression after SVR (OR = 0.35, 95%CI = 0.10–1.17; P = 0.09).

Table 3

Association between daily intake of selected nutrients* and progression of liver disease (cirrhosis, hepatocellular carcinoma and/or liver disease-related death).

Variables	Daily intake (Tertile)	Incidence	Age-, sex-adjusted model	Multivariate model†
		n/N (%)	OR (95%CI)	OR (95%CI)
Protein (g/4184 kJ)	< 34.3	13/60 (22)	1.00	1.00
	34.3–40.5	6/60 (10)	0.38 (0.13–1.15)	0.49 (0.15–1.61)
	≥ 40.6	8/60 (13)	0.59 (0.20–1.69)	0.54 (0.16–1.79)
			(Trend P = 0.27)	(Trend P = 0.29)
Fat (g/4184 kJ)	< 24.47	13/60 (22)	1.00	1.00
	24.47–29.99	9/60 (15)	0.70 (0.26–1.89)	0.77 (0.25–2.36)
	≥ 30.00	5/60 (8)	0.38 (0.12–1.23)	0.54 (0.15–1.93)
			(Trend P = 0.11)	(Trend P = 0.34)
Carbohydrate (g/4184 kJ)	< 133.69	5/60 (8)	1.00	1.00
	133.69-149.82	11/60 (18)	2.21 (0.68–7.17)	1.75 (0.50–6.21)
	≥ 149.83	11/60 (18)	1.66 (0.51–5.41)	2.11 (0.55–8.07)
			(Trend P = 0.48)	(Trend P = 0.28)
Cholesterol (mg/4184 kJ)	< 150	12/60 (20)	1.00	1.00
	150–217	5/60 (8)	0.41 (0.13–1.33)	0.39 (0.11–1.47)
	≥ 218	10/60 (17)	0.84 (0.31–2.27)	0.81 (0.26–2.50)
			(Trend P = 0.72)	(Trend P = 0.76)

CI, confidence interval; OR, odds ratio.

* According to baseline information collected in 2005.

† Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI at 2005, smoking and alcohol drinking history.

Variables	Daily intake (Tertile)	Incidence	Age-, sex-adjusted model	Multivariate model[†]
		n/N (%)	OR (95%CI)	OR (95%CI)
Vitamin B1 (mg/4184 kJ)	< 0.4225	12/60 (20)	1.00	1.00
	0.4225–0.5149	7/60 (12)	0.66 (0.22–1.99)	1.06 (0.30–3.66)
	≥ 0.5150	8/60 (13)	0.64 (0.21–1.94)	0.75 (0.22–2.58)
			(Trend P = 0.43)	(Trend P = 0.63)
Vitamin B2 (mg/4184 kJ)	< 0.627	11/60 (18)	1.00	1.00
	0.627–0.766	10/60 (17)	0.87 (0.31–2.48)	1.16 (0.36–3.74)
	≥ 0.767	6/60 (10)	0.52 (0.16–1.67)	0.68 (0.18–2.55)
			(Trend P = 0.27)	(Trend P = 0.57)
Niacin (mg/4184 kJ)	< 6.455	13/60 (22)	1.00	1.00
	6.455–8.299	10/60 (17)	1.03 (0.38–2.83)	1.21 (0.38–3.84)
	≥ 8.300	4/60 (7)	0.33 (0.09–1.15)	0.31 (0.08–1.27)
			(Trend P = 0.096)	(Trend P = 0.12)
Vitamin B6 (mg/4184 kJ)	< 0.764	9/60 (15)	1.00	1.00
	0.764–0.892	8/60 (13)	1.04 (0.34–3.19)	1.18 (0.33–4.16)
	≥ 0.893	10/60 (17)	1.12 (0.37–3.36)	1.34 (0.37–4.81)
			(Trend P = 0.85)	(Trend P = 0.65)
Vitamin B12 (μg/4184 kJ)	< 3.86	14/60 (23)	1.00	1.00
	3.86–5.84	7/60 (12)	0.46 (0.16–1.29)	0.51 (0.16–1.66)

CI, confidence interval; OR, odds ratio.

[*] According to baseline information collected in 2005.

[†] Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI at 2005, smoking and alcohol drinking history.

Variables	Daily intake (Tertile)	Incidence	Age-, sex-adjusted model	Multivariate model†
		n/N (%)	OR (95%CI)	OR (95%CI)
	≥ 5.85	6/60 (10)	0.41 (0.14–1.22)	0.35 (0.10–1.17)
			(Trend P = 0.09)	(Trend P = 0.08)
Folate (μg/4184 kJ)	< 146.5	8/60 (13)	1.00	1.00
	146.5-192.9	11/60 (18)	1.63 (0.57–4.69)	2.10 (0.59–7.44)
	≥ 193.0	8/60 (13)	0.99 (0.33–3.04)	1.30 (0.38–4.82)
			(Trend P = 0.98)	(Trend P = 0.78)
CI, confidence interval; OR, odds ratio.				
* According to baseline information collected in 2005.				
† Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI at 2005, smoking and alcohol drinking history.				

When examined for the association between frequency of intake of foods containing high levels of vitamin B12 and liver disease progression (Table 4), higher consumption of pork or beef (Trend P = 0.096) and liver intake (OR = 0.29, 95%CI = 0.07–1.16, P = 0.08) seemed to have protective effects on liver disease progression with marginal significance.

Table 4

Association between frequency intake of foods containing high levels of vitamin B12* and progression of liver disease (cirrhosis, hepatocellular carcinoma and/or liver disease-related death).

Variables	Daily intake (Tertile)	Incidence	Age-, sex-adjusted model	Multivariate model†
		n/N (%)	OR (95%CI)	OR (95%CI)
Chicken (times per week)	< 1	9/49 (18)	1.00	1.00
	1	12/69 (17)	0.80 (0.29–2.20)	1.03 (0.33–3.22)
	≥ 2	6/62 (10)	0.47 (0.15–1.50)	0.75 (0.20–2.81)
			(Trend P = 0.20)	(Trend P = 0.68)
Pork, Beef (times per week)	< 2	14/76 (18)	1.00	1.00
	2–3	13/85 (15)	0.84 (0.35–2.01)	0.87 (0.32–2.37)
	≥ 4	0/19 (0)	Not applicable	Not applicable
			(Trend P = 0.15)	(Trend P = 0.096)
Processed meat (ham, sausage, bacon, luncheon meat) (times per week)	< 1	12/88 (14)	1.00	1.00
	1	5/41 (12)	0.85 (0.27–2.71)	0.90 (0.25–3.24)
	≥ 2	10/51 (20)	1.71 (0.64–4.54)	1.57 (0.53–4.61)

CI, confidence interval; OR, odds ratio.

* According to baseline information collected in 2005.

† Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI in 2005, smoking and alcohol drinking history.

Variables	Daily intake (Tertile)	Incidence	Age-, sex-adjusted model	Multivariate model†
		n/N (%)	OR (95%CI)	OR (95%CI)
			(Trend P = 0.32)	(Trend P = 0.44)
Liver	None	24/128 (19)	1.00	1.00
	Ate	3/52 (6)	0.28 (0.08–1.01)	0.29 (0.07–1.16)
Cuttlefish, octopus, shrimp, shellfish (times per week)	< 1	12/65 (18)	1.00	1.00
	1	7/57 (12)	0.69 (0.23–2.07)	0.77 (0.22–2.61)
	≥ 2	8/58 (14)	0.79 (0.28–2.24)	0.46 (0.14–1.51)
			(Trend P = 0.65)	(Trend P = 0.20)
Small fish with bones (times per week)	< 1	16/92 (17)	1.00	1.00
	1	5/40 (13)	0.52 (0.17–1.61)	0.44 (0.12–1.61)
	≥ 2	6/48 (13)	0.51 (0.17–1.49)	0.42 (0.12–1.44)
			(Trend P = 0.18)	(Trend P = 0.13)
Dried fish (times per week)	< 1	12/70 (17)	1.00	1.00

CI, confidence interval; OR, odds ratio.

* According to baseline information collected in 2005.

† Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI in 2005, smoking and alcohol drinking history.

Variables	Daily intake (Tertile)	Incidence	Age-, sex-adjusted model	Multivariate model[†]
		n/N (%)	OR (95%CI)	OR (95%CI)
	1	10/66 (15)	0.63 (0.24–1.70)	0.59 (0.18–1.90)
	≥ 2	5/44 (11)	0.63 (0.20–2.07)	0.59 (0.16–2.13)
			(Trend P = 0.38)	(Trend P = 0.38)
Oily fish (sardine, mackerel, Pacific saury, amberjack [yellow tail], Pacific herring, eel, tuna, etc.) (times per week)	< 1	9/49 (18)	1.00	1.00
	1	13/78 (17)	0.82 (0.30–2.23)	0.75 (0.23–2.46)
	≥ 2	5/53 (9)	0.36 (0.10–1.22)	0.34 (0.10–1.51)
			(Trend P = 0.10)	(Trend P = 0.17)
Other fish (salmon, trout, whitefish, freshwater fish, skipjack, etc.) (times per week)	< 1	11/65 (17)	1.00	1.00
	1	11/82 (13)	0.87 (0.33–2.25)	0.52 (0.17–1.58)
	≥ 2	5/32 (16)	0.87 (0.26–2.91)	0.57 (0.14–2.27)
			(Trend P = 0.78)	(Trend P = 0.33)

CI, confidence interval; OR, odds ratio.

[*] According to baseline information collected in 2005.

[†] Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI in 2005, smoking and alcohol drinking history.

Table 5 shows the results of sensitivity analysis after excluding patients with a history of HCC and/or liver cirrhosis before SVR. Among 153 patients, 14 (9%) showed liver disease progression after SVR, including 13 (9%) who developed liver cirrhosis and 2 (1%) with HCC. In multivariate analysis, broadly similar results were obtained, although the width of confidence intervals for each variable became wider because of the decreased number of patients. Older age at SVR showed an OR of 17 (P = 0.01), whereas APRI ≥ 1.0 before SVR (OR = 12.6, P < 0.01) and an alcohol drinking habit before SVR (OR = 5.59, P = 0.06) were also associated with liver disease progression after SVR. The protective association with higher consumption of vitamin B12 also remained with marginal significance (OR = 0.20, P = 0.09).

Table 5

Association between selected characteristics and development of liver disease (cirrhosis, hepatocellular carcinoma and/or liver disease-related death): Sensitivity analysis among patients without cirrhosis or hepatocellular carcinoma before SVR.

Variables	Category	Incidence	Age-, sex-adjusted model	Multivariate model*
		n/N (%)	OR (95%CI)	OR (95%CI)
Total subjects		14/153 (9)		
Age at SVR (years)	< 59.5	2/57 (4)	1.00	1.00
	59.5–71.7	5/55 (9)	3.00 (0.55–16.4)	5.32 (0.65–43.6)
	≥ 71.8	7/41 (17)	6.21 (1.20–32.2)	17.1 (1.79–164.1)
			(Trend P = 0.02)	(Trend P = 0.01)
Sex	Male	6/52 (12)	1.00	1.00
	Female	8/101 (8)	0.55 (0.17–1.74)	0.87 (0.20–3.82)
Clinical characteristics before SVR				
APRI	< 1.0	7/125 (6)	1.00	1.00
	≥ 1.0	7/26 (27)	7.12 (2.07–24.4)	12.6 (2.83–56.4)
Lifestyle habits before SVR†				
Smoking	Never	9/102 (9)	1.00	1.00
	Former	3/31 (10)	0.81 (0.17–3.92)	0.33 (0.05–2.11)
	Current	2/20 (10)	1.32 (0.21–8.20)	1.45 (0.20–10.8)
			(Trend P = 0.87)	(Trend P = 0.89)
Alcohol drinking	Never	3/68 (4)	1.00	1.00

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI at 2005, smoking and alcohol drinking history.

† According to baseline information collected in 2005.

Variables	Category	Incidence	Age-, sex-adjusted model	Multivariate model*
		n/N (%)	OR (95%CI)	OR (95%CI)
	Former	5/40 (13)	2.68 (0.55–13.1)	2.57 (0.45–14.8)
	Current	6/45 (13)	3.94 (0.81–19.3)	5.59 (0.95–33.1)
			(Trend P = 0.09)	(Trend P = 0.06)
Dietary habits before SVR†				
Vitamin B12 (μg/4184 kJ)	< 3.86	7/48 (15)	1.00	1.00
	3.86–5.84	5/54 (9)	0.53 (0.15–1.85)	0.57 (0.13–2.50)
	≥ 5.85	2/51 (4)	0.25 (0.05–1.30)	0.20 (0.03–1.28)
			(Trend P = 0.08)	(Trend P = 0.09)
Liver	None	14/108 (13)		
	Ate	0/45 (0)	Not applicable	Not applicable

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DAA, direct-acting antivirals; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SVR, sustained virological response.

* Model includes age at SVR, sex, history of hepatocellular carcinoma, APRI at 2005, smoking and alcohol drinking history.

† According to baseline information collected in 2005.

In addition, to consider the possibility that patients had already suffered undiagnosed outcomes at the time of SVR, patients with follow-up period less than 1.0 year were excluded from analysis. However, the results did not change markedly, and similar associations were obtained with each variable (data not shown).

Discussion

The present study revealed that even in patients who obtained SVR, some patients showed progression of liver disease after SVR, and that the proportion was nearly 10%. Moreover, 1% of patients were diagnosed with HCC after SVR, and the incidence was broadly similar to results from other studies in Japan [12–14]. The incidence of HCC among SVR patients is significantly reduced compared to patients who do not achieve SVR [8–9], but is still higher than that in general population without viral infection (0.1%) [21]. It might be difficult for hepatitis C patients, even after SVR, to reduce the HCC incidence to the level of

general population, because of the liver damage sustained in the period between HCV infection and SVR. However, now that antiviral treatments achieving a high rate of SVR have been developed, early detection of HCV infection and early treatment for viral eradication are important to minimize the period of liver damage as much as possible.

In the present study, older age at SVR and APRI ≥ 1 were suggested as risk factors for progression of liver disease after SVR. These characteristics have been reported as important risk factors for HCC in previous studies [12–13]. APRI has commonly been used as proxy marker of HCV-related fibrosis [22]. Previous studies have indicated that fibrosis-4 index offers an independent predictor associated with HCC [12, 14]. When we conducted additional analysis in which fibrosis-4 index was used instead of APRI, fibrosis-4 index was also associated with liver disease progression (OR = 1.76, 95%CI = 1.28–2.42). The present results thus support the previous finding that APRI and fibrosis-4 index represent important predictors of liver disease progression, even among patients with SVR.

Regarding alcohol drinking habit, we suggested that a drinking habit before SVR was associated with liver disease progression after SVR. However, the present study did not consider potential changes in alcohol drinking habits after achieving SVR, since we did not collect such information. In general, however, alcohol drinkers before SVR may tend to continue that habit after achieving SVR. In addition, alcohol drinking is a known risk factor for HCC in countries with low infection rates of HCV or HBV [23]. Thus, alcohol drinking could conceivably represent a risk factor for liver disease progression among patients with SVR.

On the other hand, the present study suggested that higher consumption of vitamin B12 might have preventive effects on the risk of liver disease progression, representing an unexpected finding. In light of previous studies, serum vitamin B12 level or vitamin B12 binding capacity increased in patients with some kinds of HCC; increased vitamin B12 binding capacity has been suggested to be useful in diagnosis, prognosis, and monitoring treatment for HCC patients [24–26]. Other studies have indicated a relationship between vitamin B12 level and severity of liver cirrhosis [27, 28]. Since liver cirrhosis patients and HCC patients present with nutritional alterations and metabolic disorders, patients with higher serum vitamin B12 might have severe disease resulting in lower consumption of vitamin B12. However, we had no information on serum vitamin B12 level among subjects in the present study. One animal experimental study showed that a lower frequency of liver cirrhosis was observed among rats that received vitamin B12 in addition to a low-protein diet, compared with rats who only received the low-protein diet [29]. This finding might be comparable to the present study result. Further studies are warranted to examine the association between consumption of vitamin B12 or serum vitamin B12 level on risk of liver disease progression.

The present study had the following strengths. Since we used a database of chronic HCV patients established in 2005, liver disease progression was able to be assessed for a mean of 4.1 years after SVR. In addition, we could examine lifestyle factors for the risk of subsequent liver disease progression, since we had collected lifestyle factors in detail at the time of recruitment to the database.

However, the present study also showed a variety of limitations. First, some patients might have changed lifestyle habits after achieving SVR. However, we had no information on whether lifestyle habits had changed after SVR, and thus could not consider such effects in the present study. Second, among patients who had registered to the database in 2005, those patients who had died before SVR or had transferred to another hospital before SVR were not included in the present study. Therefore, the number of study subjects was relatively small, making the statistical power too low to detect several risk factors for HCC. To overcome this lower power, we included not only HCC, but also liver cirrhosis as study outcomes, combined as the present study outcome of “liver disease progression”. However, the present study detected several risk factors, suggesting confirmation of risk factors for HCC according to previous studies. This seems to suggest that the present study results are reliable.

Conclusions

The present prospective cohort study of SVR patients showed that some patients develop liver cirrhosis, HCC, and/or liver disease-related death even after SVR. Among lifestyle factors, alcohol drinking habits or vitamin B12 intake might influence disease prognosis after eradication of HCV. Further studies are warranted to confirm these findings.

List Of Abbreviations

HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; DAAs, direct-acting antivirals; SVR, sustained virological response; BDHQ, brief self-administered diet history questionnaire; APRI, aspartate aminotransferase-to-platelet ratio index; OR, odds ratio; CI, confidence interval.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of Osaka City University Graduate School of Medicine, and was performed in accordance with the Declaration of Helsinki. All study patients provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

S.O contributed to study design, overall management, statistical analysis, data interpretation, and drafting of the work or revising it critically for important intellectual content. T.M contributed to study design, and data management and statistical analysis. A.T and S.K contributed to data acquisition, and data interpretation. S.S, K.K, K.I, and WF contributed to study design and data interpretation. SO, S.S, and W.F developed the study concept. All authors provided comments on the drafts and have read and approved the final manuscript.

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