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Review article

Long-term health outcomes of chronic hepatitis C patients: A review of findings from REVEAL-HCV cohort study

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ABSTRACT

Chronic hepatitis C affects more than 180 million people worldwide. As one of the most important infectious diseases, it causes around 250,000 deaths per year. A long-term follow-up cohort study is essential for evaluating health outcomes associated with virus infection, and for exploring potential seromarkers that have high predictability for risk of developing various diseases. However, the prospective cohorts consisted of individuals with chronic hepatitis C virus (HCV) infection are still rare. The Risk Elevation of Viral Load Elevation and Associated Liver Disease/Cancer in HCV (REVEAL-HCV) study has followed a cohort of 1095 residents seropositive for anti-HCV antibodies lived in seven townships in Taiwan for 15 years. These anti-HCV seropositives were asymptomatic and relatively more healthy than chronic hepatitis C patients cared in clinics and hospitals. Most of them acquired HCV infection through iatrogenic transmission routes in study townships. The epidemiological characteristics of HCV infection were very similar to those in countries with high prevalence such as Japan, Korea, Italy, and India. As the participants in the REVEAL-HCV study rarely received antiviral therapies, it provided an exceptional opportunity to study the natural history of chronic HCV infection. In this review article, we describe the details of participant enrollment, laboratory tests, follow-up procedures, and major recent findings. Anti-HCV seropositives with elevated serum HCV RNA levels were found to have an increasing risk of developing hepatocellular carcinoma in a dose-response relationship. In addition to the serum HCV RNA level, serum alanine aminotransferase levels and HCV genotype also had long-term predictability for the risk of hepatocellular carcinoma. Moreover, anti-HCV seropositives with detectable serum HCV RNA levels had an increased mortality from extrahepatic diseases such as cerebrovascular and renal diseases. Our study revealed that anti-HCV seropositives with detectable serum HCV RNA levels had an increased risk of hepatic and extrahepatic diseases.

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† Other members of the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis C Virus (REVEAL-HCV) study are listed in the Appendix.

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1. Introduction

Hepatitis C virus (HCV) is recognized as a major cause of chronic liver disease. Liver cirrhosis eventuates in 20% to 30% patients with chronic HCV infection, generally after 2 to 3 decades [1]. Once cirrhosis occurs, hepatocellular carcinoma develops in 1% to 4% of these patients per year [2]. HCV was estimated to be attributable to one third of hepatocellular carcinoma cases globally [3]. Due to successful hepatitis B virus vaccination programs, HCV related health burdens are emerging quickly in Asian countries and represent a great public health burden [4]. Because a vaccine is not available and treatment options are still limited and expensive, the efforts of infection controls should be focused on primary prevention. A long-term follow-up cohort may help evaluate the incidence and mortality of various diseases associated with chronic HCV infection. In this review article, we describe the study population, enrollment and follow-up procedures, recent findings and future perspectives of Risk Elevation of Viral Load Elevation and Associated Liver Disease/Cancer in HCV (REVEAL-HCV) study.

2. REVEAL-HCV study cohort

The REVEAL-HCV study cohort was recruited from a community-based cancer screening program conducted in Taiwan during 1991 to 1992. There were seven townships selected as the study areas, including two northern townships (Sanchi and Chutung) and two southern townships (Potzu and Kaohsu) on main Taiwan Island, and three townships (Makung, Huhsi, and Paihsa) on Penghu Islets.

There were 89,293 inhabitants aged 30 to 65 years in the seven study townships invited to participate in the study, and 23,820 (11,973 males and 11,847 females) were enrolled after giving written informed consent. The vital status of the study participants were followed by the computerized linkage with the national cancer registration and death certification profiles. The national identification number, date of birth, and sex were used as the linking variables to double-check the vital status and causes of death of study participants. At enrollment, the participants were personally interviewed using structured questionnaires by well-trained public health nurses. The information collected included the sociodemographic characteristics (age, sex, educational levels, occupation, etc.), habits of life styles (cigarette smoking, alcohol consumption, betel nut chewing), and personal and family history of major diseases. Anthropometric measurements including weight and height were also performed.

In addition to the questionnaire interview, 10 mL blood samples were collected from each participant at study entry. The blood samples were obtained using disposable needles and heparinized vacuum syringes. They were fractioned on the day of collection and stored at −70°C until assayed. Serum samples of all participants were tested for hepatitis B surface antigen (HBsAg) by radioimmunoassay (Abbott Laboratories, North Chicago, IL, USA), anti-HCV by enzyme immunoassay (Abbott Laboratories), serum levels of aspirate aminotransferase (AST) and alanine aminotransferase (ALT) by serum chemistry autoanalyzer (Model 736, Hitachi, Tokyo, Japan) using commercial reagents (Biomerieux, Marcy L'Etoile, France).

Participants who were seropositive for anti-HCV were further examined for serum HCV RNA levels by polymerase chain reaction using the COBAS TaqMan HCV test, v2.0 (Roche Diagnostics, Indianapolis, NJ, USA), and an *in vitro* nucleic acid amplification test for the quantification of HCV RNA. The quantification method used the high pure system viral nucleic acid kit for manual specimen preparation and the COBAS TaqMan 48 Analyzer for automated amplification and detection. The manufacturer's procedures for sample preparation to extract HCV RNA, automated reverse transcription of the target RNA to generate complementary DNA, and amplification of target cDNA were followed. In any test procedure, a replicate of negative, low-positive, and high-positive controls were included in each run for HCV RNA quantification. The HCV RNA titer was expressed in international units (IU)/mL according to the WHO International Standard for HCV RNA NAT assays, and the linear range for the COBAS TaqMan HCV test was from 25 IU/mL to 3.9×10^8 IU/mL. Moreover, those with positive serum HCV RNA levels were examined for HCV genotypes by melting curve analysis, which could effectively differentiate different HCV genotypes by showing different melting temperatures [5,6]. In the REVEAL-HCV study, HCV genotype-1 and HCV genotype non-1 were differentiated.

Participants seropositive for HBsAg or anti-HCV were invited to receive regular health examinations. The health examinations included abdominal ultrasonography examinations and blood tests. The certified hepatologists performed the high-resolution real-time abdominal ultrasonography and interpreted according to a standardized protocol. Liver cirrhosis was determined based on a quantitative scoring system, which was derived from the appearance of liver surface (normal, irregular, undulated), liver parenchymal texture (normal, heterogeneous, coarse), intrahepatic blood vessel size (normal, obscure, narrowing), and splenic size (normal, enlarged) [7–9]. The serological tests included serum levels of AST, ALT, and α-fetoprotein. To ensure all study participants received standard care, those who had abnormal serum levels of ALT and/or α-fetoprotein levels and ultrasonographic findings were referred to hepatologists in medical centers for further clinical managements.

There were 1095 participants seropositive for anti-HCV but seronegative for HBsAg. Among them, 975 (89%) had adequate retrievable serum samples for HCV RNA test. Comparing those who had adequate serum samples ($n = 975$) and those without adequate serum samples for HCV RNA test ($n = 120$), there were no significant differences in the distributions of baseline characteristics except for gender. However, for the 975 anti-HCV seropositives with adequate samples for HCV RNA test, the proportion of gender was similar to that of all 1095 anti-HCV seropositives.

3. Seroprevalence of anti-HCV by age and gender

There were 1313 participants seropositive for anti-HCV, giving seroprevalence of 5.5% in our study population. The seroprevalence increased with age. For females, the seroprevalence of HCV was 3.0%, 3.6%. 4.2%, 6.8%, 7.3%, 9.7%, and 9.8%, respectively, for the 30 to 34, 35 to 39, 40 to 44, 45 to 49, 50 to 54, 55 to 59, and 60 to 65 year age groups. The corresponding seroprevalence for males was 2.7%, 3.7%, 3.2%, 5.2%, 5.6%, 6.4%, and 6.1%, respectively. As shown in Fig. 1, females had higher age-specific anti-HCV seroprevalence than males with the overall seroprevalence of 6.2% versus 4.8%, respectively.

The major risk factors of HCV infection in the REVEAL-HCV study population were iatrogenic risk factors including blood transfusion, hemodialysis, medical injections, and dental procedures. In our previous reports, >80% HCV infection could be attributable to iatrogenic factors [10,11]. Older people had an increased chance to receive multiple medical injections and had an increased cumulative risk of HCV infection in their lifetime. The gender difference in the seroprevalence of HCV infection might be explained by: 1) females being more concerned about their minor illness and more likely to receive glucose-based nutrient or vitamin injections than males, which were frequently prescribed to sick people; or 2) males infected with HCV having a higher mortality rate than infected females, thus the anti-HCV prevalence in males would more likely to be lower than females due to a faster attrition of the HCV-infected [12].

4. HCV RNA seropositive rate and its associated baseline characteristics

Serum HCV RNA was detectable in 676 (69.3%) anti-HCV seropositives in the REVEAL-HCV study cohort. Table 1 shows the HCV RNA seropositive rate by baseline characteristics. The HCV RNA seropositive rate was 78.8% in males and 62.0% in females, suggesting that females were more likely to have spontaneous seroclearance of HCV RNA. Participants with the habit of cigarette smoking or alcohol consumption had a higher HCV RNA seropositive rate than those without these habits. However, the associations might result from the higher proportions of cigarette smokers and alcohol drinkers in males than females. After adjustment for gender, there was no association between the HCV RNA seropositivity and habits of cigarette smoking and alcohol drinking. There was no significant association with HCV RNA seropositivity for body mass index (BMI) and history of diabetes.

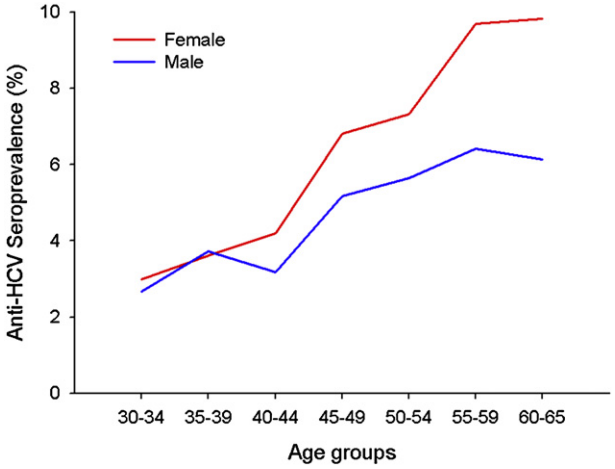

Fig. 1 – Seroprevalence of anti-HCV by age and gender.

Participants with increasing serum ALT levels had elevated HCV RNA seropositive rates. In comparison to those with serum ALT levels ≤15 U/L as the referent, the sex-adjusted odds ratio [95% confidence interval (CI)] of having detectable serum HCV RNA levels was 3.69 (2.69–5.06) and 10.18 (5.58–18.60), respectively, for serum ALT levels of 15 to 45 U/L and higher than 45 U/L. Males had a 2.26-fold (95% CI 1.66–3.07) higher risk of having detectable serum HCV RNA after adjustment for serum ALT levels. It is interesting to note that females had a higher anti-HCV seroprevalence, as shown in Fig. 1, but a lower HCV RNA seropositive rate among anti-HCV seropositives than males, as shown in Fig. 2. It suggests that the serum HCV RNA level might be a seromarker to be considered in management of anti-HCV seropositives. However, the importance and significance of this seromarker should be further evaluated by comparing the health outcomes between HCV RNA seronegative and seropositive participants who were seropositive for anti-HCV.

5. Incidence of hepatocellular carcinoma by baseline characteristics

There were 101 newly developed hepatocellular carcinoma (HCC) cases that occurred after 17,944 person-years of follow-up, giving an incidence rate of 562.9 per 100,000 person-years. Table 2 shows the number of participants, person-years of follow-up, number of HCC cases, and the incidence rate of HCC by baseline characteristics. Older individuals, or those with habits of cigarette smoking or alcohol consumption, increased BMI (≥25 kg/m^2), elevated serum ALT levels, or detectable serum HCV RNA levels had an increased incidence of HCC among the 1095 anti-HCV seropositives who were seronegative for HBsAg. The baseline characteristics that were significantly associated with increased HCC risk in univariate analyses were included in the subsequent multivariate analyses. Participants with older age, a habit of cigarette smoking or alcohol consumption, or increased BMI still had a significantly increased HCC risk after adjustment for each other, but no significant association was observed for habits of cigarette smoking or alcohol consumption after further adjustment for serum levels of ALT and HCV RNA.

In comparison to those with serum ALT levels ≤15 U/L as the referent group, the adjusted-hazard ratio (95% CI) was 1.78 (1.01–3.14) and 2.98 (1.65–5.40), respectively, for serum ALT levels of 16 to 44 U/L and ≥45 U/L. Individuals with detectable serum HCV RNA had 5.67 times greater risk of HCC than those with undetectable HCV RNA. In a recent study [13], it was found that serum levels of ALT and HCV RNA and HCV genotype had long-term predictability for HCC. These seromarkers had predictability 5 years or earlier than the

Table 1 – HCV RNA seropositive rates by baseline characteristics in REVEAL-HCV study.

Baseline characteristics	Total $n = 975$	HCV RNA undetectable $n = 299$ (30.7%)	HCV RNA detectable $n = 676$ (69.3%)	p value
Sex				
Females	550	209 (38.0%)	341 (62.0%)	<0.001
Males	425	90 (21.2%)	335 (78.8%)	
Age at recruitment, years				
30–39	163	55 (33.7%)	108 (66.3%)	0.49
40–49	217	72 (33.2%)	145 (66.8%)	
50–59	399	113 (28.3%)	286 (71.7%)	
60–65	196	59 (30.1%)	137 (69.9%)	
Cigarette smoking				
Never	701	245 (35.0%)	456 (65.0%)	<0.001
Yes	270	52 (19.3%)	218 (80.7%)	
Unknown	4	2	2	
Alcohol consumption				
No	893	286 (32.0%)	607 (68.0%)	0.003
Yes	80	13 (16.2%)	67 (83.8%)	
Unknown	2	0	2	
Body mass index (kg/m^2)				
<25	589	170 (28.9%)	419 (71.1%)	0.12
≥25	385	129 (33.5%)	256 (66.5%)	
Unknown	1	0	1	
History of diabetes				
No	930	285 (30.6%)	645 (69.4%)	0.78
Yes	42	12 (28.6%)	30 (71.4%)	
Unknown	3	2	1	
Serum ALT level (U/L)				
≤15	429	207 (48.3%)	222 (51.7%)	<0.001
16–45	387	79 (20.4%)	308 (79.6%)	
>45	159	13 (8.2%)	146 (91.8%)	

ALT = alanine aminotransferase.

occurrence of HCC. After 15 years of follow-up, the cumulative HCC risk was only 0.4% for participants seronegative for anti-HCV. There was an increasing cumulative HCC risk for anti-HCV-seropositive participants with undetectable, low, and high serum HCV RNA (1.1%, 6.4%, and 14.7%, respectively, $p < 0.001$ for trend). Among participants seropositive for anti-HCV, the cumulative HCC risk was 1.7%, 4.2%, and 13.8% for serum ALT levels of persistently ≤15 U/L, 15 to 45 U/L, and >45 U/L, respectively ($p < 0.001$ for trend). Among participants with detectable serum HCV RNA, the cumulative HCC incidence was 12.6% for HCV genotype 1 and 4.5% for genotype non-1 ($p < 0.001$) [13]. Moreover, the increasing HCC risk by the elevating serum levels of HCV RNA was found not only in men but also in women [14].

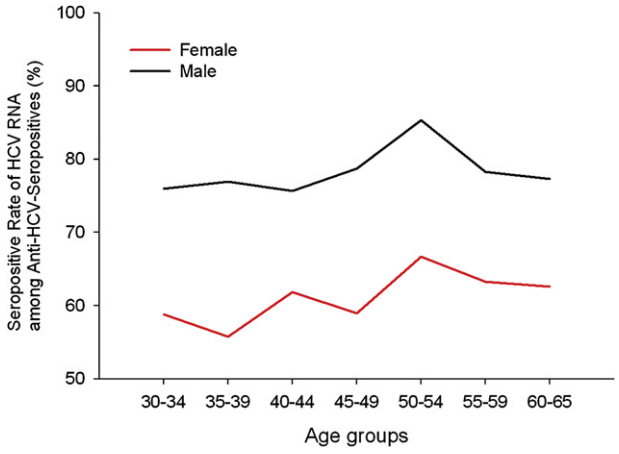

Fig. 2 – Seropositive rate of HCV RNA among anti-HCV-seropositives by age and gender.

6. All causes and liver-related mortality by baseline characteristics

Table 3 shows all-causes and liver-related mortality rates and associated hazard ratios for each baseline characteristic. The mortality rate from all causes was 1557.7 per 100,000 person-years and the liver-related mortality rate was 493.5 per 100,000 person-years, respectively. The cumulative mortality from all causes of death was 30.1% and 12.8% after 17 years of follow-up for participants with detectable and undetectable serum HCV RNA, respectively. Similarly, those with detectable serum HCV RNA had an increased cumulative mortality from liver-related diseases compared with those with undetectable

Table 2 – Numbers of participants, person-years of follow-up, hepatocellular carcinoma case numbers and incidence rates by baseline characteristics.

Baseline risk factors	No. (%) of participants	Pearson-years of follow-up	No. of hepatocellular carcinoma cases	Incidence rate per 100,000 person-years	Crude hazard ratio (95% CI)	Multivariate adjusted hazard ratio (95% CI)
Sex						
Females	630 (57.5)	10430	51	489.0	1.00	Not included
Males	465 (42.5)	7514	50	665.4	1.36 (0.92–2.01)	
Age at recruitment, years						
30–39	186 (17.0)	3148	3	95.3	1.00	1.00
40–49	243 (22.2)	4055	11	271.3	2.86 (0.80–10.26)	2.45 (0.68–8.80)
50–59	444 (40.6)	7196	56	778.2	8.32 (2.60–26.57)	5.70 (1.77–18.37)
60–65	222 (20.3)	3546	31	874.3	9.36 (2.86–30.62)	6.78 (2.05–22.39)
Cigarette smoking						
Never	793 (72.7)	13092	65	496.5	1.00	1.00
Ever	298 (27.3)	4782	36	752.8	1.52 (1.01–2.29)	1.12 (0.70–1.80)
Alcohol consumption						
No	1007 (92.1)	16540	87	526.0	1.00	1.00
Yes	86 (7.9)	1371	13	948.3	1.83 (1.02–3.28)	1.38 (0.69–2.76)
Body mass index (kg/m^2)						
<25	663 (60.7)	11003	45	409.0	1.00	1.00
≥25	430 (39.3)	6906	56	810.9	2.01 (1.36–2.97)	1.76 (1.16–2.67)
History of diabetes						
No	1043 (95.7)	17115	95	555.1	1.00	Not included
Yes	47 (4.3)	747	6	802.7	1.48 (0.65–3.37)	
Serum ALT level (U/L)						
≤15	495 (45.2)	8302	21	252.9	1.00	1.00
16–44	426 (38.9)	6950	43	618.7	2.49 (1.48–4.19)	1.78 (1.01–3.14)
≥45	174 (15.9)	2692	37	1374.4	5.66 (3.31–9.67)	2.98 (1.65–5.40)
Serum HCV RNA level (IU/mL)						
<25 (undetectable)	299 (30.7)	5040	5	99.2	1.00	1.00
≥25 (detectable)	676 (69.3)	10943	86	785.9	8.08 (3.28–19.90)	5.67 (2.25–14.31)

ALT = alanine aminotransferase.

serum HCV RNA, with the cumulative mortality of 12.8% and 1.6% [15]. Generally, male gender, older age, habits of cigarette smoking and alcohol consumption, BMI ≥25 kg/m^2, history of diabetes, elevated serum ALT levels, and detectable serum HCV RNA levels were associated with an increased mortality from all causes or liver-related deaths. After adjustment for potential risk factors, participants with detectable serum HCV RNA had an increased risk for all causes of death and hepatic-related deaths with the adjusted hazard ratio (95% CI) of 2.78 (1.56–3.33) and 6.53 (2.32–18.37), respectively. This implies that those with detectable serum HCV RNA levels might die from other extrahepatic diseases in addition to hepatic diseases. Our findings indicate that the serum HCV RNA level is an important marker for management of individuals seropositive for anti-HCV.

7. HCV infection and deaths from extrahepatic diseases

The mortality from extrahepatic diseases was 1064.2 per 100,000 person-years for the participants in the REVEAL-HCV cohort. Among participants seronegative for HBsAg, the cumulative mortality from extrahepatic diseases after 17 years of follow-up was 12.2% for anti-HCV seronegatives and 17.7% for anti-HCV seropositives. In other words, participants seropositive for anti-HCV had a 1.4-fold increased risk of dying from extrahepatic diseases after adjustment for age and sex. Anti-HCV seropositives had an increased mortality from either extrahepatic cancers or extrahepatic diseases other than cancers with age-sex-adjusted hazard ratio (95% CI) of 1.23 (0.92–1.65) and 1.43 (1.19–1.73), respectively. Fig. 3 shows the associations between extrahepatic deaths and HCV infection. The HCV infection was associated with an increased mortality from circulatory diseases. Based on the long-term follow-up design of the REVEAL-HCV study, there was a correct causal temporality for the HCV-induced atherosclerotic diseases [16]. Moreover, the increasing serum HCV RNA levels were found to be associated with mortality from cerebrovascular disease in a dose-response relationship after adjustment for conventional risk factors for cerebrovascular disease. Compared with participants seronegative for anti-HCV as the referent group, the multivariate-adjusted hazard ratio (95% CI) of dying from cerebrovascular disease was 1.43 (0.63–3.23), 2.29 (1.38–3.82), and 2.81 (1.25–6.35), respectively, for anti-HCV-seropositive participants with undetectable, low, and high serum levels of HCV RNA ($p < 0.001$ for trend). However, there was no significant association between HCV genotype and mortality from cerebrovascular disease [17]. In addition, HCV infection was associated with an increased mortality from renal disease and cancers of the esophagus, prostate, and thyroid, and the mortality was even higher for those with detectable serum of HCV RNA [15].

Table 3 – All-causes and liver-related mortality in patients with HCV infection by baseline characteristics.

Baseline risk factors	All causes of death				All liver-related deaths			
	No. of deaths	Mortality rate per 100,000 person-years	Crude HR (95% CI)	Multivariate adjusted HR (95% CI)	No. of deaths	Mortality rate per 100,000 person-years	Crude HR (95% CI)	Multivariate adjusted HR (95% CI)
Sex								
Females	118	1187.0	1.00	1.00	39	392.3	1.00	1.00
Males	144	2093.3	1.80 (1.41–2.30)	1.30 (0.90–1.88)	44	639.6	1.69 (1.10–2.60)	1.04 (0.54–1.99)
Age at recruitment, years								
30–39	18	592.1	1.00	1.00	3	98.7	1.00	1.00
40–49	28	712.9	1.21 (0.67–2.19)	1.19 (0.62–2.27)	5	127.3	1.30 (0.31–5.43)	1.10 (0.26–4.63)
50–59	129	1940.3	3.38 (2.06–5.53)	2.96 (1.72–5.10)	49	737.0	7.80 (2.43–25.01)	5.20 (1.20–16.88)
60–65	87	2715.6	4.85 (2.92–8.06)	4.29 (2.45–7.51)	26	811.5	8.96 (2.71–29.61)	6.74 (2.01–22.61)
Cigarette smoking								
Never	156	1250.9	1.00	1.00	51	409.0	1.00	1.00
Ever	106	2477.4	2.05 (1.60–2.62)	1.35 (0.92–1.98)	32	747.9	1.93 (1.24–3.00)	1.30 (0.65–2.62)
Alcohol consumption								
No	228	1460.3	1.00	1.00	71	454.8	1.00	1.00
Yes	34	2900.7	2.07 (1.44–2.96)	1.71 (1.14–2.57)	12	1023.8	2.38 (1.29–4.39)	1.77 (0.85–3.67)
Body mass index (kg/m^2)								
<25	140	1355.3	1.00	1.00	38	367.9	1.00	1.00
≥25	121	1873.6	1.40 (1.10–1.79)	1.35 (1.04–1.76)	45	696.8	1.94 (1.26–2.99)	1.84 (1.16–2.93)
History of diabetes								
No	230	1422.5	1.00	1.00	77	476.2	1.00	1.00
Yes	31	5404.8	4.26 (2.93–6.21)	3.99 (2.62–6.08)	6	1046.1	2.62 (1.14–6.03)	2.27 (0.90–5.70)
Serum ALT level (U/L)								
≤15	91	1163.8	1.00	1.00	19	243.0	1.00	1.00
16–44	108	1662.5	1.46 (1.10–1.92)	1.03 (0.76–1.41)	32	492.6	2.08 (1.18–3.68)	1.37 (0.75–2.53)
≥45	63	2515.3	2.24 (1.63–3.09)	1.24 (0.86–1.78)	32	1277.6	5.54 (3.14–9.78)	2.72 (1.46–5.05)
Level of HCV RNA (IU/mL)								
<25 (undetectable)	36	744.5	1.00	1.00	4	82.7	1.00	1.00
>25 (detectable)	194	1902.2	2.63 (1.84–3.75)	2.78 (1.56–3.33)	70	686.4	8.67 (3.16–23.73)	6.53 (2.32–18.37)

ALT = alanine aminotransferase.

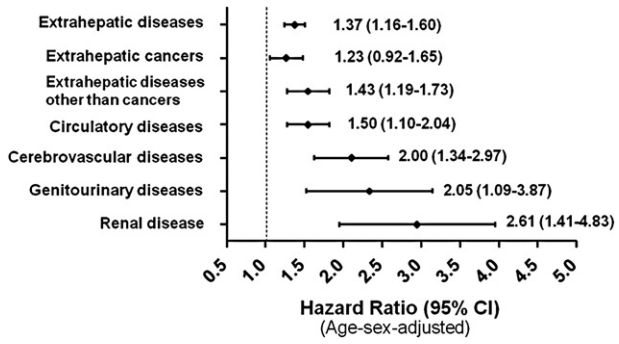

Fig. 3 – Hepatitis C virus infection and mortality from extrahepatic diseases.

8. Advantages and limitations of the REVEAL-HCV study

Chronic hepatitis C patients in Taiwan rarely received antiviral treatment with interferon due to its high cost and adverse effects until November 2003, when patients with abnormal serum ALT levels (>82 U/L) and moderate fibrosis proven by liver biopsy could be reimbursed for treatment by the National Health Insurance. Therefore this cohort study may be considered as a natural history study of chronic hepatitis C. To ensure that study participants received standard care, those who had abnormal serum levels of ALT and α-fetoprotein or abnormal ultrasonographic findings were referred to medical centers for further clinical management in this study. This cohort, consisting of 1000 anti-HCV seropositives, provided an exceptional opportunity to examine the seromarker changes and liver disease occurrence of anti-HCV seropositives during the natural course of HCV infection.

Participants enrolled in the REVEAL-HCV cohort lived in the community. Unlike other cohorts, which enrolled patients with experiences of drug injections [18] or HCV-contaminated vaccinations [19,20], the exact time of HCV infection was unavailable for our participants. As the major risk factors of HCV infection in the REVEAL-HCV cohort were iatrogenic factors, it was difficult to obtain the exact time of HCV infection; the information on advanced fibrosis or mild cirrhosis was not available in this community-based cohort because it is not practical to have asymptomatic participants examined by liver biopsy. Liver cirrhosis is an intermediate clinical outcome before the occurrence of HCC among chronic hepatitis C patients. Based on the abdominal ultrasonographic examination and serial tests of serum levels of AST and ALT, >80% of newly-developed HCC cases in participants seropositive for anti-HCV had liver cirrhosis detected by ultrasonography and/or an increased ratio between serum levels of AST and ALT.

9. Summary

Based on the REVEAL-HCV cohort study, we found that anti-HCV seropositives with detectable serum HCV RNA levels had an increased risk of both hepatic and extrahepatic diseases. Anti-HCV seropositives with undetectable serum HCV RNA levels had cumulative HCC risk similar to anti-HCV seronegatives (1.1 vs. 0.4%), implying that antiviral treatment to aid seroclearance of HCV RNA may benefit patients. In addition, the findings suggest that clinical patients experienced sustained virologic response after receiving antiviral therapy may have reduced HCC risk and improved survival [21–26]. Recent trials showed that use of direct-acting antiviral agents may achieve sustained virologic response among patients who had not had a response to prior therapy [27,28]. Our study provides evidence that patients with HCV infection, particular for those with active HCV infection (seropositive for HCV RNA), should be encouraged for intensive management because they had an increased risk of HCC and mortality from hepatic or extrahepatic diseases. We also found that the prevalence of anti-HCV in a community was associated with HCV RNA seropositive rate among anti-HCV seropositives in the community, suggesting that anti-HCV seropositives with detectable serum HCV RNA levels played a major role in the transmission of the virus in the community [12]. For the control of hepatic or extraheptic diseases and virus infection, anti-HCV seropositives should be tested for serum HCV RNA levels by a sensitive assay. Those with active HCV infection should be instructed to be aware of HCV-related health outcomes and HCV transmission routes as well as the need to take actions for HCV RNA seroclearance.

10. Future perspectives

Recently, human genetic variants predicting successful treatments have been identified by genome-wide association study (GWAS) from several independent study groups [29–31]. They studied different ethnic populations and found that genetic variants near the IL28B gene were associated with antiviral response in patients infected with HCV genotype 1. Two single nucleotide polymorphisms (SNPs) near the interleukin 28B gene (IL28B, also called IFNλ3), rs12979860 and rs8099917, were associated with antiviral treatment response in chronic hepatitis C patients [29–31]. The C allele of the SNP (rs12979860) was found to be associated with the spontaneous clearance of HCV in a follow-up study [32]. A recent study showed that Taiwanese patients with chronic hepatitis C receiving antiviral therapy have a lower daily viral production rate than western patients, and the rs8099917 TT genotype may contribute to the increased viral clearance rate and better virological responses [33]. These findings imply that host genetic factors may be involved in the natural course of HCV infection and the pathogenesis of liver diseases. IL28B polymorphism (T allele) seems to be involved in the development of HCV-induced HCC and the course of HCV recurrence after liver transplantation in a recent study [34]. In Taiwan, most chronic hepatitis C patients carried the favorable genotype associated with better treatment responses and the minor allele frequency (T of rs12979860 and G of rs8099917) were very rare [35–38]. To better understand the associations between the SNPs near IL28B and the risk of liver cirrhosis or HCC, a study with a large sample size is needed. In addition to the IL28B gene, a recent GWAS conducted in Japan identified SNPs associated with the occurrence of hepatocellular carcinoma among chronic hepatitis C patients [39,40]. It will be

interesting to discover these genetic variants to understand the pathogenesis of liver disease progression further or to apply them as diagnostic or risk predictive biomarkers [41]. Although high-throughput technologies to discover human genetic variants have developed rapidly to accelerate the genotyping, validation of genetic markers in other external populations is still essential and functional studies are needed. Moreover, to stratify high-risk patients who need intensive care is essential. Recently, several study groups focus on the development of prediction models for liver-related outcomes among chronic hepatitis C patients [41–47], which may aid physicians to communicate with patients and enhance patients' compliance to receive standard care. In Taiwan, pegylated-interferon plus ribavirin is the standard care for chronic hepatitis C patients [48,49]. The sustained virologic response rate for patients with genotype 1 was around 70% [25,49]. It will be important to follow the subsequent risk for liver-related outcomes among patients with sustained virologic response or with nonvirologic response as well as to compare the disease burdens occurred in patients with treatment experiences or not [50]. Collaborative studies to understand the diseases associated with HCV infection better and to promote appropriate clinical managements of chronic hepatitis C patients are in urgent need.

Appendix

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