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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (2): Hepatitis C virus

Epidemiology and natural history of hepatitis C virus infection

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Abstract

Hepatitis C virus (HCV) affects 130-210 million people worldwide and is one of the major risk factors for hepatocellular carcinoma. Globally, at least one third of hepatocellular carcinoma cases are attributed to HCV infection, and 350000 people died from HCV related diseases per year. There is a great geographical variation of HCV infection globally, with risk factors for the HCV infection differing in various countries. The progression of chronic hepatitis C to end-stage liver disease also varies in different study populations. A long-term follow-up cohort enrolling participants with asymptomatic HCV infection is essential for elucidating the natural history of HCV-caused hepatocellular carcinoma, and for exploring potential seromarkers that have high predictability for risk of hepatocellular carcinoma. However, prospective cohorts comprising individuals with HCV infection are still uncommon. The risk evaluation of viral load elevation and associated liver disease/cancer in HCV (REVEAL-HCV) study has followed a cohort of 1095 residents seropositive for antibodies against hepatitis C virus living in seven townships in Taiwan for more than fifteen years. Most of them have acquired HCV infection through iatrogenic transmission routes. As the participants in the REVEAL-HCV study rarely receive antiviral therapies, it provides a unique opportunity to study the natural history of chronic HCV infection. In this review, the prevalence, risk factors and natural history of HCV infection are comprehensively reviewed. The study cohort, data collection, and findings on liver disease progression of the REVEAL-HCV study are described.

Key words: Hepatitis C virus; Epidemiology; Risk evaluation of viral load elevation and associated liver disease/cancer; Long-term liver progression

Core tip: This review includes summary tables describing the epidemiology of hepatitis C virus (HCV) infection in previous studies and recent findings from the risk evaluation of viral load elevation and associated liver disease/cancer in HCV study.

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INTRODUCTION

Hepatitis C virus (HCV) is recognized as a major cause of chronic liver disease. Liver cirrhosis generally occurs in 20%-30% patients with chronic infection after 2 to 3 decades[1]. Once cirrhosis occurs, hepatocellular carcinoma develops in 1%-4% of these patients per year[2]. It was estimated that HCV was attributable to one thirds of hepatocellular carcinoma cases globally[3], representing a significant public health burden. In this review article, we summarize the prevalence, risk factors and the natural history of hepatitis C virus infection. In addition, we describe the study population, data collection, and findings of liver disease progression of the Risk Evaluation of Viral Load Elevation and Associated Liver Diseases/Cancers in HCV (REVEAL-HCV) study.

PREVALENCE OF HCV INFECTION

According to WHO reports, 3% of the world's population has been infected with HCV, representing 170 million people at risk of developing chronic liver diseases[4,5]. The HCV-estimated prevalence in economically developed countries is relatively low with 1%-2% of the adult population whereas 5%-10% in less developed countries[4-6]. The countries with higher reported prevalence were located in Africa, Eastern Mediterranean, South-East Asia and the West Pacific[5,6]; areas with lower prevalence included North America, northern and western Europe and Australia.

The HCV seroprevalence studies provide useful descriptive data to understand global HCV epidemiology. A large number surveys were conducted to estimate the distributions of HCV. However, most studies mainly enrolled specific populations, such as blood donors and clinical patients, which are not representative of the population of the regions in which they reside. Moreover, the estimated prevalence might be underestimated or overestimated because blood donors are healthier than the general population and clinical patients already had symptoms.

The community-based studies were relatively limited and not available in most countries. The seroprevalence of HCV has a considerable geographical variation, which may be explained by different distributions and different contributions of risk factors in different study regions. The community-based HCV seroprevalence in different countries is displayed in Table 1. It has a striking geographical variation in anti-HCV seroprevalence, ranging from 0.5%-24.3%[7-18].

RISK FACTORS FOR HCV INFECTION

The most important transmission modes of HCV are through blood or blood-related products. It was noticed that the supply of blood in the world was contaminated with an unidentified agent causing post-transfusion non-A, non-B hepatitis[19]. In developed countries post-transfusion hepatitis C has become relatively rare. Incidence of transfusion associated hepatitis, traced from 1970 to 1998, demonstrated a decrease from 33% to nearly eliminated HCV transmission caused by the effectiveness of a series of donor screening intervention[20]. In developing countries where HCV testing in blood donation has not been feasible, receiving blood products remains a dominant source of HCV infection. Most of these countries are located in Africa and Asia, where blood safety is threatened by poverty, insufficient instruments and laboratory reagents, limited supply of trained professionals, traditional cultural barriers, and difficulties in mobilizing volunteer donors[21,22].

In developed countries, HCV is mainly transmitted by drug abusers sharing injection equipment[23]. The prevalence of anti-HCV among intravenous drug users ranged from 31% to 98%[24]. It has been reported that injection drug use accounts for 60% and 80% of HCV infection in United States[7] and Australia[25], respectively. In developing countries, HCV transmission is mainly by unsafe therapeutic injections[12,17]. Unsafe injections, defined as reuse of syringes or needles from patient to patient without sterilization, resulted in 2.3-4.7 million HCV infections every year approximately[26]. Transmission of HCV through contaminated injection equipment has been recognized in most developing countries[8,27-29]. The results indicated that the injection equipment were contaminated or non-disposable, which resulted in the spread of HCV. People with increased frequencies of injections for therapeutic purposes had elevated cumulative risks of HCV infection. The evidence suggested that it is important to reduce injection reuse and overuse in the prevention aspect of HCV control, especially in areas with limited disposable injection equipment and health professionals.

Other sources of HCV transmission include activities involving the potential for percutaneous exposure to blood or blood-derived body fluids, such as tattooing[15], acupuncture[17,30], sharing cottons[31], and other biologically plausible modes of transmission, like body-piercing, cosmetic procedures, and commercial barbering[32]. Vertical transmission from mother to neonate is rare[33] and intra-household and within-couples spread of HCV infections is possible[34,35]. It was also indicated that lower education, poverty[7,23], and residing in highly deprived area[36] are risk factors for positive anti-HCV.

HCV INFECTION AND LIVER-RELATED DISEASES

HCV infection is infrequently diagnosed during the acute phase because majority of persons have either no symptoms or only mild symptoms. The asymptomatic infection becomes chronic in most cases, and people are unaware of the infections until end-stage liver diseases occur.

Several follow-up studies[37-44] were conducted to evaluate the clinical outcomes related to HCV infection, which are summarized in Table 2. These studies mainly enrolled specific populations such as patients in liver clin-

Table 1 Seroprevalence of antibodies against hepatitis C virus in community-based studies

Study site	Study period	Study population	Population size	% with HCV antibody	% with positive HCV RNA among anti-HCV seropositives
America					
United States[7]	1988-1994	National Health and Nutrition Examination Survey, subjects aged ≥ 6 yr	21241	1.8	73.9
Puerto Rico[15]	2001-2002	Individuals aged 21-64 yr residing in the municipality of San Juan	964	6.3	Not done
Europe					
Greece[11]	1997-1998	Individuals over 15 yr in south western Greece	1500	0.5	Not done
Norway[9]	2000-2001	A subset of Oslo Health Study, subjects older than 30 yr	11456	0.7	79.5
France[10]	1994	Individuals aged 20-59 undergoing routine medical checkup in social security medical centers	6283	1.2	81.0
Spain[16]	1997-1998	Random sample of all ages in northern Spain	1170	1.6	63.0
Italy[12]	1996	Individuals of all ages which representative to southern Italy	1352	12.6	84.7
Asia					
India[8]	1999	Individuals of all ages living in rural area and engaged in agriculture-related occupations	2973	0.9	80.8
China[18]	1992	Individuals aged 1-59 yr in 30 provinces	68000	3.2	Not done
Taiwan[17]	1991-1992	Males aged 30-65 yr participated a cancer screening project	11904	4.9	Not done
Japan[14]	1984-1995	Residents aged 20-89 yr in southern Miyazaki Prefecture	973	23	Not done
Africa					
Egypt[13]	1997	Adults and children aged older than 10 yr residing in Nile Delta	3999	24.3	Non done

HCV: Hepatitis C virus.

Table 2 Follow-up studies to evaluate liver-related morbidities and mortality associated with hepatitis C virus infection

Ref.	Study population	Mean follow-up (yr)	Identification of infection	Liver-related disease		Mortality		Comments
				LC	HCC	All-cause	Liver-related	
Tong *et al*[42], 1995 United States	213 patients	3.9	Patients recalled the time of transfusion	51.1%	5.3%	15.3%	14.5%	Most participants had symptoms Patients from tertiary care center with a history of transfusion Recall bias
Seeff *et al*[38], 2001 United States	222 patients	25	Time of transfusion			67.1%	4.1%	70% were males
Wiese *et al*[44], 2005 Germany	683 CHC women	25	Vaccinated in 1978-1979	1.3%	0.1%			Relatively young and healthy CHC defined as HCV RNA (+)
Kenny-Walsh[37], 1999 Ireland	376 CHC women	17	Vaccinated in 1977-1978	2%				Relatively young and healthy CHC defined as HCV RNA (+)
Thomas *et al*[41], 2000 United States	1667 drug abusers	8.8	First injection use	ESLD incidence: 310[1]				1/3 with HIV (+) Recall bias
Tanaka *et al*[40], 2004 Japan	1927 blood donors	8.3	Unknown		334[1]			Participants from Osaka Red Cross Blood Center relatively healthy
Suruki *et al*[39], 2006 Japan	667 CHC adults	7.9	Unknown		983[1]			Community-based CHC defined by at least 1 HCV RNA/core antigen result 70% participants of age older than 60 yr Serial tests for serum ALT
Uto *et al*[43], 2009 Japan	1125 adults	8.2	Unknown			2500[1]		Community-based Tested for HCV RNA/core antigen

[1]Per 100000 person-year. LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; CHC: Chronic hepatitis C.

ics[42], patients with post-transfusion hepatitis[38], blood donors[40], drug abusers[41] and women vaccinated with contaminated immunoglobulin[37,44]. The Japanese studies recruited residents in a community where HCV is endemic (prevalence approximately 25%), and most of the participants were of advanced age[39,43]. Most of the studies had difficulties in defining the source of HCV infection[38-43], particularly for the studies that enrolled the general population[39,43]. Although the time of HCV infection was reported in some studies, it might be biased by asking participants if they recalled their transfusion time or first injection use[38,41,42].

Table 3 Serum levels of hepatitis C virus RNA and liver-related diseases

Ref.	Study population	Study design	Serum RNA measurements	Findings	Comments
Naito *et al*[50]	22 HCV carriers with detectable RNA and persistently normal serum ALT levels in Japan	Cross-sectional	Competitive RT-PCR	Serum viral load were correlated with HAI score (r = 0.68, P < 0.01)	Limited number of study participants Temporality No control for confounders
De Moliner *et al*[45]	96 patients without antiviral treatments in Italy	Cross-sectional	First-generation bDNA assay (QuantiplexTM HCV RNA 1.0)	Serum viral load was not correlated with liver histological diagnosis (r = 0.58)	Temporality No control for confounders
Fanning *et al*[47]	77 women infected HCV genotype 1b through vaccination in Ireland	Cross-sectional	RT-PCR	Serum viral load was weakly (r = 0.26, P < 0.05) correlated with HAI score Not correlated with the degree of fibrosis (r = 0.22, P > 0.05)	Temporality Homogeneous participants with defined source of infection and the same duration of infection
Lagging *et al*[48]	98 patients without antiviral treatments in Sweden	Cross-sectional	RT-PCR with Cobas Amplicor HCV monitor test	Serum viral load was not associated with the degree of inflammation or fibrosis	Temporality
Hisada *et al*[51]	385 drug users with detectable HCV RNA in United States	Case-cohort	Third-generation of bDNA assay (QuantiplexTM HCV RNA 3.0)	Elevated serum levels of HCV RNA increased the risk of ESLD death (relative hazard = 2.3 per log10 IU/mL, 95%CI: 1.5-5.9)	Coinfected with HIV or HTLV-Ⅱ Large population with eight yr of follow-up

RT-PCR: Reverse-transcription polymerase chain reaction; HAI: Histological activity index; HCV: Hepatitis C virus; ESLD: End-stage liver disease; HTLV: Human T lymphotropic virus; HIV: Human immunodeficiency virus.

The clinical outcomes after HCV infection were highly variable. There were around 1.3%-51% of individuals with HCV infection who developed liver cirrhosis and 0.1%-5.3% developed hepatocellular carcinoma during 3.9-25 years[42,44]. The incidence of hepatocellular carcinoma was lower in blood donors than in the general population because the blood donors were relatively healthy and young[40]. Liver-related mortality ranged from 15.3%-67.1%[38,42]. Discrepancies resulted not only from the risk factors associated with progression distributed differently between these studies but also heterogeneity in study populations. Study participants from tertiary care facilities[42] might suffer from more severe conditions and the referral bias might lead to overestimations of serious liver diseases after HCV infection. Vaccinated women of childbearing age and blood donors were relatively young and healthy[37,40,44]. The estimations of progressions of liver disease were strongly influenced by study population samplings.

HCV INFECTION MARKERS AND RISK FOR LIVER DISEASES

There have been a number of studies that attempted to examine the relationship of serum concentration of HCV RNA with liver disease severity by relating it to histopathological abnormality[45-50] (Table 3). Some studies identified that the HCV RNA load correlated with hepatic inflammation[47,49,50]; however, others indicated that serum levels of HCV RNA were not associated with hepatic inflammation[45] nor fibrosis[46,48-50]. These studies were conducted to elucidate serum HCV RNA in the prediction of severity of liver diseases used cross-sectional design, resulting in the limitations of causal temporality. A report followed 6570 drug users for around eight years with a case-cohort design and showed that the HCV RNA level was a predictor of end-stage liver disease death, with adjusted hazard ratio and 95%CI 2.3 (1.5-5.9)-fold higher per $\log_{10}$ IU/mL increase in HCV load[51]. However, some of the drug users were co-infected with HIV and some had human lymphotropic virus type Ⅱ; thus, the generalizability to the population in the community was limited.

There are six major HCV genotypes[52]. Table 4 shows the associations of HCV genotype and liver related diseases. Most of the studies were limited to cross-sectional design[53] or enrolled clinical patients[53-57]. A prospective study followed 163 liver cirrhosis patients for seventeen years and indicated that HCV genotype 1b was a major risk factor with a three times higher risk of developing hepatocellular carcinoma compared with participants infected with other types[54]. However, most patients in the study had other liver-related co-morbidities and had been treated with interferon. Since patients infected with HCV genotype-1 had lower likelihood of sustained virological response and were recommended for longer duration of therapy[58], it was possible that the lower response rate resulted from the HCV genotype-1b infected patients with higher hepatocellular carcinoma incidence observed in the study. Moreover, the findings only indicated that infected HCV genotypes had an impact on the prognosis of late clinical stage. Whether HCV genotypes could predict progression of liver disease, especially for healthy carriers of chronic hepatitis C searching for clinical con-

Table 4 Hepatitis C virus genotypes and liver-related diseases

Ref.	Study population	Study design	HCV genotypes determination	Findings	Comments
Martinot-Peignoux *et al*[53]	1872 HCV infected patients from 14 tertiary referral centers in France	Cross-sectional	Reverse hybridization with line probe assay (LiPA)	LC in genotype 1b and 4 (13% and 13%) were found more frequently than in genotype 1a, 2, or 3 (8%, 9%, and 7%), $P = 0.03$	Clinical patients temporality Only proportions provided, not control for other confounders
Silini *et al*[57]	162 LC and 162 HCC cases in Italy	Case-control	Polymerase chain reaction	Genotype 1b *vs* others: OR = 1.7 (1.1-2.9)	Clinical patients Temporality Matched with age, gender, child's class
Kobayashi *et al*[56]	140 untreated CHC patients in Japan	Retrospective follow-up	Enzyme-linked immunosorbent assay	Deterioration of the stage of liver histology: Genotype 1, 63% Genotype 2, 39% $P < 0.05$	Clinical patients Only proportions calculated and time not taken into analytical consideration
Fattovich *et al*[55]	292 biopsy-proven LC patients form 7 referral centers in Europe	Prospective follow-up	Nested polymerase chain reaction	HCC risk Genotype 1b *vs* others HR = 1.0 (0.5-2.3)	Clinical patients and more than 1/2 were treated with interferon
Bruno *et al*[54]	163 liver cirrhosis patients in Italy	Prospective follow-up	INNO-LiPA HCV Ⅱ (Bayer Corp., Tarrytown, NY)	HCC risk Genotype 1b *vs* 2a/c HR = 3.0 (1.4-6.5)	Interferon treated patients Incidence of HCC calculated

CHC: Chronic hepatitis C; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; OR: Odds ratio; HR: Hazard ratio.

sultation, still lacked sufficient evidence.

REVEAL-HCV STUDY COHORT

The REVEAL-HCV study cohort[59,60] is a community-based study that recruited subjects from seven townships in Taiwan during 1991-1992. The study areas included two northern townships (Sanchi and Chutung) and two southern townships (Potzu and Kaohsu) on the main Taiwan Island, and three townships (Makung, Huhsi, and Paihsa) on the Penghu Islets.

At the beginning, 89293 inhabitants aged 30-65 years old in the seven study townships were invited to participate in the study. Among them, 23820 (11973 males and 11847 females) were enrolled after giving written informed consent. At enrollment, well-trained public health nurses personally interviewed the participants using structured questionnaires. The collected information included sociodemographic characteristics (age, sex, educational levels, occupation, *etc.*), lifestyle (cigarette smoking, alcohol consumption, and betel nut chewing), and personal and family history of major diseases. Anthropometric measurements including weight and height were also performed. The vital statuses of the study participants were followed by the computerized linkage with the national cancer registration and death certification profiles. The national identification number, date at birth, and sex were used as the linking variables to double-check the vital status and causes of death of study participants.

In addition to the questionnaire interview, 10 mL blood samples were collected from each participant at study entry and during follow-up. We invited the participants to undergo health examinations every six to twelve months. The blood samples were obtained using disposable needles and heparinized vacuum syringes. They were fractioned on the day of collection and stored at -70 ℃ until assayed. Serum samples of all participants were tested for hepatitis B surface antigen (HBsAg) by radioimmunoassay (Abbott Laboratories, North Chicago, IL, United States), anti-HCV by enzyme immunoassay (Abbott Laboratories), and serum levels of aspirate aminotransferase and alanine aminotransferase by a 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, United States), 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 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

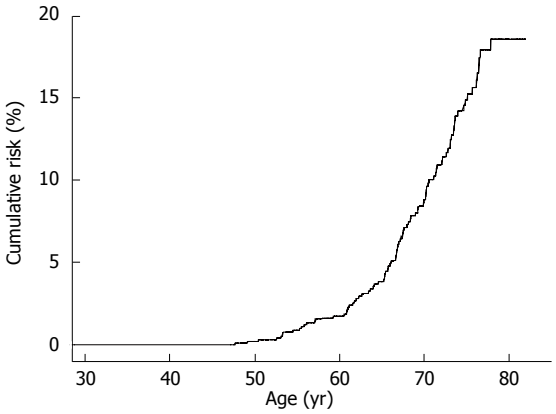

Figure 1 Cumulative life-time risk (30-80 years old) of hepatocellular carcinoma.

by showing different melting temperatures[61,62].

AGE AND SEX SEROPREVALENCE OF ANTI-HCV IN REVEAL-HCV STUDY COHORT

Among the 23820 participants who agreed to be enrolled in this study, there were 1,313 seropositive for anti-HCV. The overall anti-HCV seroprevalence in the community was 5.5%. Seroprevalence increased with advancing age in the population. For females, the seroprevalences of HCV were 3.0%, 3.6%. 4.2%, 6.8%, 7.3%, 9.7% and 9.8%, respectively, for the age groups 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, and 60-65 years old. The corresponding seroprevalences for males were 2.7%, 3.7%, 3.2%, 5.2%, 5.6%, 6.4%, and 6.1%, respectively. Females had higher age-specific anti-HCV seroprevalences than males, with an overall seroprevalence of 6.2% *vs* 4.8%, respectively[63].

There were 1095 participants who were seropositive for anti-HCV but seronegative for HBsAg. Among them, 975 (89%) had adequate retrievable serum samples for the HCV RNA test. Comparing those who had adequate serum samples (n = 975) and those without adequate serum samples for an 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 gender proportion was similar to that of all 1095 anti-HCV seropositives.

CUMULATIVE LIFETIME INCIDENCE OF HEPATOCELLULAR CARCINOMA IN REVEAL-HCV STUDY COHORT

The participants were followed from 1991 to the end of 2008. One hundred and one newly developed hepatocellular carcinoma cases occurred after 17944 person-years of follow-up, giving the incidence rate of 562.9 per 100000 person-years. Figure 1 shows the cumulative lifetime incidence of hepatocellular carcinoma, using age as a follow-up scale. The cumulative lifetime incidence (from 30 to 80 years old) of HCC was 18.6% for the participants in the REVEAL-HCV cohort. In previous reports of the REVEAL-HCV study, the risk of developing hepatocellular carcinoma was significantly associated with increasing age, positive HCV RNA, elevated serum ALT levels and HCV genotype 1[59]. Figure 2 shows the cumulative lifetime incidence of hepatocellular carcinoma by sex, HCV RNA, serum levels of ALT, HCV genotype. The cumulative lifetime risk was 19.7% and 17.15% for males and females (P = 0.18), respectively. The lifetime risk was 3.63% and 24.77% for those with undetectable and detectable serum HCV RNA levels, respectively. There was a biological gradient of cumulative lifetime incidence of hepatocellular carcinoma across the serum ALT levels. For those with serum ALT levels ≤ 15 U/L, 16-45 U/L, > 45 U/L, the cumulative lifetime risks were 11.62%, 18.45% and 34.3%, respectively. In addition, the cumulative risks for HCV genotype non-1 and HCV genotype 1 were 20.1% and 25.85%, respectively. In Taiwan, the most prevalent HCV genotypes were 1b and 2a[64]. Thus, the results implied that HCV genotype 1b infection increased the risk of hepatocellular carcinoma[65].

LONG-TERM PREDICTORS OF HEPATOCELLULAR CARCINOMA IN REVEAL-HCV STUDY COHORT

In the multivariate analysis, the seromarkers, including serum levels of HCV RNA and ALT and HCV genotype, remained significantly associated with hepatocellular carcinoma after adjustment for age, sex, cigarette smoking, alcohol consumption, obesity and history of diabetes[59]. The seromarkers were mutually independent risk predictors of hepatocellular carcinoma among patients with chronic hepatitis C virus infection. It will be interesting to integrate the relevant seromarkers for the development of prediction models for hepatocellular carcinoma among chronic hepatitis C patients. Furthermore, the new predictors, such as host genetic markers, will increase the accuracy of long-term prediction of end-stage liver diseases.

ADVANTAGES AND LIMITATIONS OF REVEAL-HCV STUDY

The REVEAL-HCV can be considered as a natural history cohort. Most of the participants in this cohort had no experience of antiviral treatment. In Taiwan, chronic hepatitis C patients rarely received antiviral treatment with interferon because of 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. To ensure study participants received standard care, those who had abnormal serum levels of ALT and α-fetoprotein or abnormal

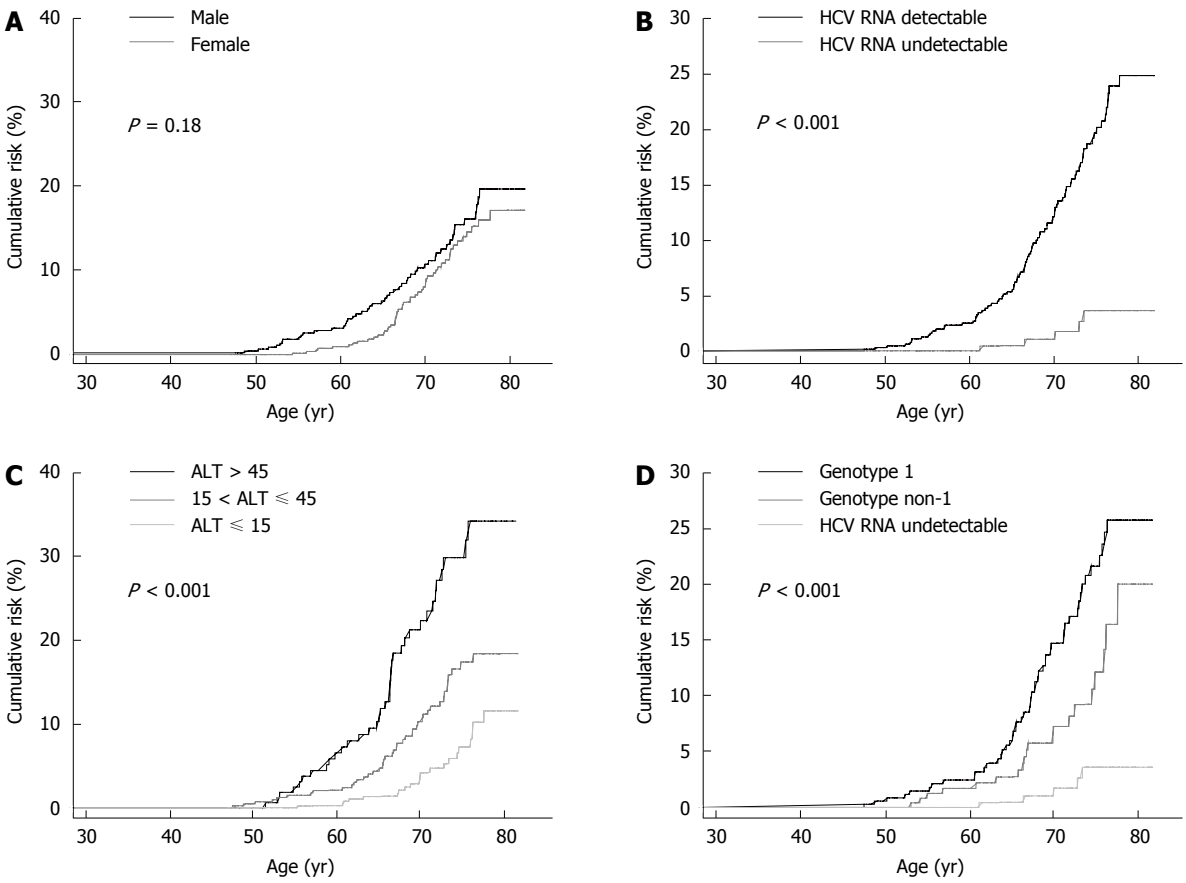

Figure 2 Cumulative life-time risk. A: Cumulative lifetime risk (30-80 years old) of hepatocellular carcinoma by gender; B: Cumulative lifetime risk (30-80 years old) of hepatocellular carcinoma by hepatitis C virus (HCV) RNA; C: Cumulative lifetime risk (30-80 years old) of hepatocellular carcinoma by serum levels of alanine aminotransferase; D: Cumulative lifetime risk (30-80 years old) of hepatocellular carcinoma by HCV genotype. ALT: Alanine aminotransferase.

ultrasound findings were referred to medical centers for further clinical managements in this study. This cohort, comprising 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.

Unlike other cohorts that enrolled patients with experiences of drug injections[51] or HCV-contaminated vaccinations[44], the REVEAL-HCV cohort enrolled participants living in the community. Thus, the exact time of HCV infection was not obtainable for our study participants. The major risk factors of HCV infection in the REVEAL-HCV cohort were iatrogenic factors[17]; therefore, it was difficult to obtain the exact time of HCV infection. In addition, it was not practical to have the asymptomatic participants examined by liver biopsy, thus the information on advanced fibrosis or mild cirrhosis was not available in this community-based cohort.

CONCLUSION

Individuals with HCV infection are often asymptomatic and unaware of their illness until severe liver diseases present; therefore, it is necessary to understand the natural history of chronic hepatitis C virus infection from a prospective viewpoint. Residents living in the same community as clinical patients represent the general population. Based on the findings by including this population, prevention strategies could precede clinical stages. Individuals seropositive for anti-HCV should be monitored regularly and tested for their serum HCV RNA by sensitive assays. Those who have high serum HCV RNA levels and ALT levels, and HCV genotype 1 infection, should be consulted for their high risk for the liver diseases and intensive care options discussed.

In the near future, several issues could be addressed. The seromarkers, serum HCV RNA and ALT levels and HCV genotype, could be used to predict subsequent risk of HCV related hepatocellular carcinoma, indicating that the seromarkers have potential to be used as pretreatment markers in clinical decisions to classify high-risk patient who need intensive care. However, a risk assessment calculator, which incorporates several patients' characteristics, is more convenient and comprehensive for clinical consultations. It is helpful for communications between clinicians and patients to discuss treatment options based on patients' individual risk profiles. Therefore, to develop a risk calculator including the seromarkers found in our study will be informative.

In addition, it is probable that the host genetic background affects HCV infection outcomes. IL28B gene variants were found to be associated with sustained vi-

rological response among chronic hepatitis C patients receiving antiviral therapy[66-68]. Moreover, the individuals who carried the variants with favorable treatment response had increased probability of experiencing spontaneous HCV RNA clearance[69,70]. Patients without experiencing spontaneous HCV resolution, who were considered to have active HCV infection and with detectable HCV RNA, had an increased risk of hepatocellular carcinoma and liver-related mortality[59,60]. Thus, the associations between IL28B variants and the hepatocarcinogenesis deserve to be investigated. However, most individuals in Asia carry the favorable genotype[71]. In other worlds, the minor allele frequency of IL28B in the Asian population is rare. It is essential to carry out a large-scale study to elucidate the associations between IL28B variants and the risk of hepatocellular carcinoma. Recently, the development of high-throughput technology has enabled researchers to test hundreds of thousands of single nucleotide polymorphisms distributed throughout the human genome. Comparing hepatocellular carcinoma cases and controls to evaluate the differences in their genetic variants will help to identify genetic markers that could be utilized as predictive tools.

The substitution of amino acids 70 and 91 in the HCV core region was associated with hepatocarcinogenesis among clinical patients with HCV genotype 1b infection and antiviral treatment[72]. In addition, the amino acid substitution had predictability for early and sustained virological responses in treated patients[73,74]. Even considering the genetic variation of *IL28B* gene, the association between the amino acid substitutions in HCV core region and antiviral treatment response remained[73]. Interestingly, the amino acid substitution in HCV core region had impact on the risk of hepatocellular carcinoma and the survival of HCV-infected patients without treatment[75]. Both host and virus factors are important determinants of liver diseases. The elucidation of the interactive effects of host and virus factors on hepatocarcinogenesis will help prevent severe HCV-related liver diseases.

The expression of HCV core protein in the transgenic mice was directly responsible for the insulin resistance[76]. Among non-diabetic patients infected with HCV genotype 3, insulin resistance was associated with an increased risk of liver fibrosis[77]. Metabolic factors, including obesity and diabetes, were found to be predictors for the development of hepatocellular carcinoma among hepatitis C patients[78]. The relationship between diabetes and hepatocellular carcinoma were observed in a large scale community-based study conducted in the United States[79]. Therefore, it was suspected that the lipid metabolism might represent one of the pathways leading to hepatocarcinogenesis. The association between HCV infection and the development of diabetes remains controversial[80,81]. A large follow-up study will help the elucidation of the relationship between HCV infection and the incidence of diabetes. Other than diabetes, HCV infection has been reported to be responsible for extrahepatic diseases[60,82]. In addition, to investigate the associations and mechanisms of HCV infection and extrahepatic diseases, it is worth evaluating the reductions of hepatic and extrahepatic diseases after implementation of HCV antiviral therapy.

In conclusion, chronic hepatitis C patients have increased risk for hepatocellular carcinoma and need intensive care. Determining host and virus genetic variants, and their interactions, will aid the development of predictive biomarkers and therapeutic strategies.

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