Serial changes of hepatitis C core antigen levels in people who inject drugs in Haiphong, Northern Vietnam

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Abstract

Hepatitis C virus (HCV) infection is one of the major global health problems, particularly for people who inject drugs (PWID). This study aimed to investigate the spontaneous clearance rate of HCV and associated factors with the clearance among PWID using an HCV core antigen (HCV-cAg) test. Blood samples were collected from 311 male PWID concentrated in a rehabilitation center in Haiphong, Vietnam, every 6 months for up to 18 months from 2007, and analyzed serologically and genetically. Of the 311, 240 (77.2%) were positive for anti-HCV antibody (HCV-Ab), and 219 (70.4%) for HCV-cAg, though three HCV-cAg positives were negative for HCV-Ab. Of the 219, 52.5% were co-infected with human immunodeficiency virus (HIV). The most prevalent HCV genotype was genotype 1 (45.7%), followed by genotypes 6 (40.6%), and 3 (7.6%). Spontaneous clearance of HCV-cAg was observed in 12 (5.5%) of 219 PWID during the observation period, and the clearance rate was 6.5 per 100 person-years (95% confidence interval, 3.5–11.3). HCV-cAg level was marginally higher in PWID co-infected with HIV than those without HIV ($p = 0.054$) and lower in PWID with HCV genotype 3 than those with other genotypes ($p < 0.001$). Only low HCV-cAg level at baseline was associated with HCV clearance ($p < 0.001$). HCV-cAg level correlated with the HCV-RNA load in plasma at baseline (Rho = 0.55, $p < 0.001$). Low HCV-cAg level at baseline could be a predictor of HCV clearance. HCV-cAg test could be applicable for HCV screening and used for monitoring plasma HCV load.

Key word: people who inject drugs, spontaneous clearance, hepatitis C virus core antigen

Introduction

Chronic hepatitis C (CHC) due to hepatitis C virus (HCV) is one of the major global public health problems. Approximately 71 million individuals had CHC, with 399,000 related deaths in 2015[1]. Among the people with CHC, around 20% progress to cirrhosis after 20 years of infection, and approximately 1–3% of the people with cirrhosis progress to hepatocellular carcinoma annually[2)–6)].

The elimination of viral hepatitis as a public health threat by 2030: reductions in new infections by 90% and mortality by 65% from 2015 to 2030, was endorsed as a global target by the World Health Assembly in 2016[1]. The main intervention to achieve this goal is to increase the number of individuals who are tested and treated for CHC. The guideline of the World Health Organization (WHO) has recommended diagnosing HCV infection by screening with anti-HCV antibody (Ab) test and confirmation with a qualitative or quantitative HCV-RNA[7]. The high costs of HCV confirmation tests using molecular techniques and new direct-acting antivirals treatment are the key barriers to

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Abbreviations: CHC, Chronic hepatitis C; IQR, Interquartile range; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HCV-Ab, HCV antibody; HCV-cAg, HCV core antigen; HIV, Human immunodeficiency virus; HR, Hazard ratio; NS5B, Nonstructural protein 5B; PWID, People who inject drugs; 5'UTR, 5' untranslated region

expand the access to test and treatment for HCV. Therefore, HCV core Antigen (HCV-cAg) tests with cost-effectiveness and simple technique requirement might be practically applicable worldwide, especially in resource-limited settings[8)9)].

There is a high prevalence of HCV infection among PWID, particularly in low-income and middle-income countries[10)11)]. We previously reported that among the 760 male PWID who participated in the epidemiological survey on HCV, HIV, and HBV in Haiphong, Northern Vietnam[12)–14)], HCV prevalence in PWID was significantly decreased from 2007 (62.1%) to 2008 (42.7%) and rebounded to 58.4% in 2012[15)]. Thus, PWID have a high risk of ongoing HCV transmission, however, they are limited to approach HCV testing services due to stigma and finance in resource-limited settings[16)17)].

HCV clearance occurs in 27.9% to 36.1% of infected people within 6 to 12 months after initial infection in general population[18)]. HCV genotype 1 infection, female sex, host genetics, broad and strong immune response, and less HCV viral diversity are reportedly the predictors of HCV clearance[2)18)19)]. In studies mainly conducted in Australia and the United States, the clearance rate among PWID was about 25% by 12 months[19)].

The present study aimed to investigate the spontaneous clearance of HCV infection and its associated factors among PWID, who were concentrated in a rehabilitation center with limited risks of acquiring HCV in Haiphong, Vietnam, and usefulness of the HCV core antigen test in diagnosis and monitoring of HCV infection.

Materials and Methods

Ⅰ. Subjects and sampling period

Previously, we conducted epidemiological surveys on HCV, HIV, and HBV infections among 760 male PWID who were concentrated in a rehabilitation center in Haiphong, northern Vietnam from 2007 to 2012[12)–14)]. Of the 760, 311 agreed to join this prospective observational study. Their blood samples were collected every 6 months from 2007 for up to 18 months: at baseline (M0), at month 6th (M6), at M12, and at M18. No participants in this study had been treated for HIV and HCV infection.

Ⅱ. Serological tests

At M0, plasma samples were tested for anti-HCV-Ab by HCV PHA (Abbott Japan, Tokyo, Japan), HCV-cAg by ARCHITECT HCV Ag (Abbott Japan, Tokyo, Japan) or Lumispot Eiken HCV Antigen (Eiken Chemical, Tokyo, Japan), hepatitis B virus (HBV) surface antigen (HBsAg) by DAINA SCREEN HBsAg II (Abbott Japan, Tokyo, Japan), and anti-HIV-Ab by DAINA SCREEN HIV 1/2 (Alere Medical Co., Ltd., Tokyo, Japan), according to the manufacturers' instructions. The HCV-cAg was quantified at M0, M6, M12, and M18. The detection limit of HCV-cAg in plasma is 0.48 log fmol/L.

Ⅲ. HCV Genotyping

HCV genotyping was done as previously described[14)]. Briefly, HCV-RNA was extracted from 100 μl of HCV-cAg positive plasma using SMITEST EX-R&D (Genome Science Laboratories, Fukushima, Japan), and reverse-transcribed (RT) with random primers to cDNA synthesis using the First-Strand cDNA synthesis kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The NS5B gene was amplified by nested polymerase chain reaction (PCR) with the primers hep31b/32 and hep33b/34b in the first and second rounds, respectively[14)20)]. The 5'UTR-Core gene was amplified by nested PCR with the primers KY80/C0751R and hep21b/C0727R in the first and second rounds, respectively[14)21)]. PCR products were visualized by ethidium bromide staining of samples electrophoresed on an agarose gel. Population sequencing and/or clonal sequencing, if necessary, of the PCR products was done. The primers used for the sequencing were hep33/hep34 and hep21b/C0727R for the NS5B and the 5'UTR-Core regions, respectively. The PCR products were cloned into a vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) for clonal sequence determination. The sequences were aligned with the sequences retrieved from the HCV sequences database (GenBank/EMBL/DDBJ) using ClustalW, followed by subsequent visual inspection and manual modification. Phylogenetic trees for the NS5B and

5'UTR-Core regions were constructed and visualized to determine their genotypes. Dual infection was defined when the sequences of different genotypes were detected in one sample.

Ⅳ. Measurement of HCV-RNA load

HCV-RNA load was measured by Amplicor HCV Monitor version 2.0 (Roche Diagnostics, Tokyo, Japan). Of the 219 HCV-cAg positive samples collected at M0, 87 were randomly selected to measure the plasma HCV-RNA load.

Ⅴ. Statistical analysis

Statistical analyses were performed by SPSS version 25. Mann-Whitney *U* test and Kruskal-Wallis test with Dunn-Bonferroni post hoc methods were used to compare two and more than two different groups, respectively. The Wilcoxon signed-ranks test was used to evaluate the changes of HCV-cAg levels. Spearman's rank correlation coefficient was calculated to assess the correlation between the levels of HCV-cAg and HCV-RNA load. To identify the factors associated with the HCV-cAg clearance including age, HIV and/or HBV co-infection, HCV genotypes, and HCV-cAg level at M0, we performed Cox proportional hazards analyses with a backward stepwise model. Variables with $P < 0.20$ in univariable analysis were considered as potential independent factors in the multivariable analysis. *P* values <0.05 were considered statistically significant.

Ⅵ. Ethical consideration

The study protocol was reviewed and approved by the ethical committees of Hanoi Medical University (No. IRB00003121) in Vietnam and Kanazawa University in Japan. All subjects provided oral or written informed consents.

Results

I. Characteristics of the study subjects at M0

Three hundred and eleven PWID [all male; median age, 34 years; interquartile range (IQR), 30–40] were followed every 6 months for up to 18 months. The median observation period was 6 months (IQR, 6–12). Of the 311 PWID, 240 (77.2%) were positive for HCV-Ab and 219 (70.4%) were positive for HCV-cAg (Figure 1). Among the 240 HCV-Ab-positive people, 216 (90%) were positive for HCV-cAg. Three cases whose HCV-Ab was negative were positive for HCV-cAg at M0.

Among the 219 HCV-cAg positive people, 16 (7.3%) were co-infected with HBV, and 115 (52.5%) with HIV, though 10 (4.6%) were co-infected with both HBV and HIV.

HCV genotypes based on 5'UTR-Core and NS5B regions were successfully analyzed for 197 samples. Most prevalent genotypes were genotype 1 (45.7%; 1a, n = 49; 1b, n = 41) followed by genotype 6 (40.6%; 6a, n = 59; 6e, n = 19; 6h, n = 2), genotype 3 (7.6%; 3a, n = 2; 3b, n =13), and genotype 2 (1.0%; n = 2). 5.1% were found to be dual genotype infections (n = 10; 1a/6a, n = 6; 6a/6e, n = 2; 1a/6e, n = 1; 6d/6e, n = 1).

II. Level of HCV-cAg and its related factors at M0

The median plasma HCV-cAg level of the 219 PWID at M0 was 3.7 log fmol/L (IQR 2.9–4.0). The HCV-cAg level was marginally higher in the PWID infected with HIV (median, 3.7; IQR, 3.1–4.1) than those without HIV (3.6; IQR 2.5–4.0; $p = 0.054$). The HCV-cAg level was significantly lower in the PWID infected with genotype 3 (2.9; IQR, 2.0–3.3) than those with genotype 1 (3.7; IQR 3.1–4.0; $p = 0.01$), genotype 6 (3.8; IQR 3.0–4.1; $p = 0.006$), and unknown genotypes (3.8; IQR 2.9–4.2; $p = 0.03$) (Table 1).

III. Spontaneous clearance of HCV and its related factors

Comparing with the plasma HCV-cAg level at M0, the level was significantly lower at M6 (3.6; IQR, 3.0–4.0, vs. M0: 3.7; IQR; 3.1–4.1; n = 131, $p < 0.001$), at M12 (3.2; IQR, 2.4–3.7, vs. M0: 3.4; IQR, 2.6–3.9; n = 76, $p < 0.001$), and at M18 (3.4; IQR 2.5–3.7, vs. M0: 3.8; IQR, 3.2–4.1; n = 38, $p < 0.001$) (Figure 2).

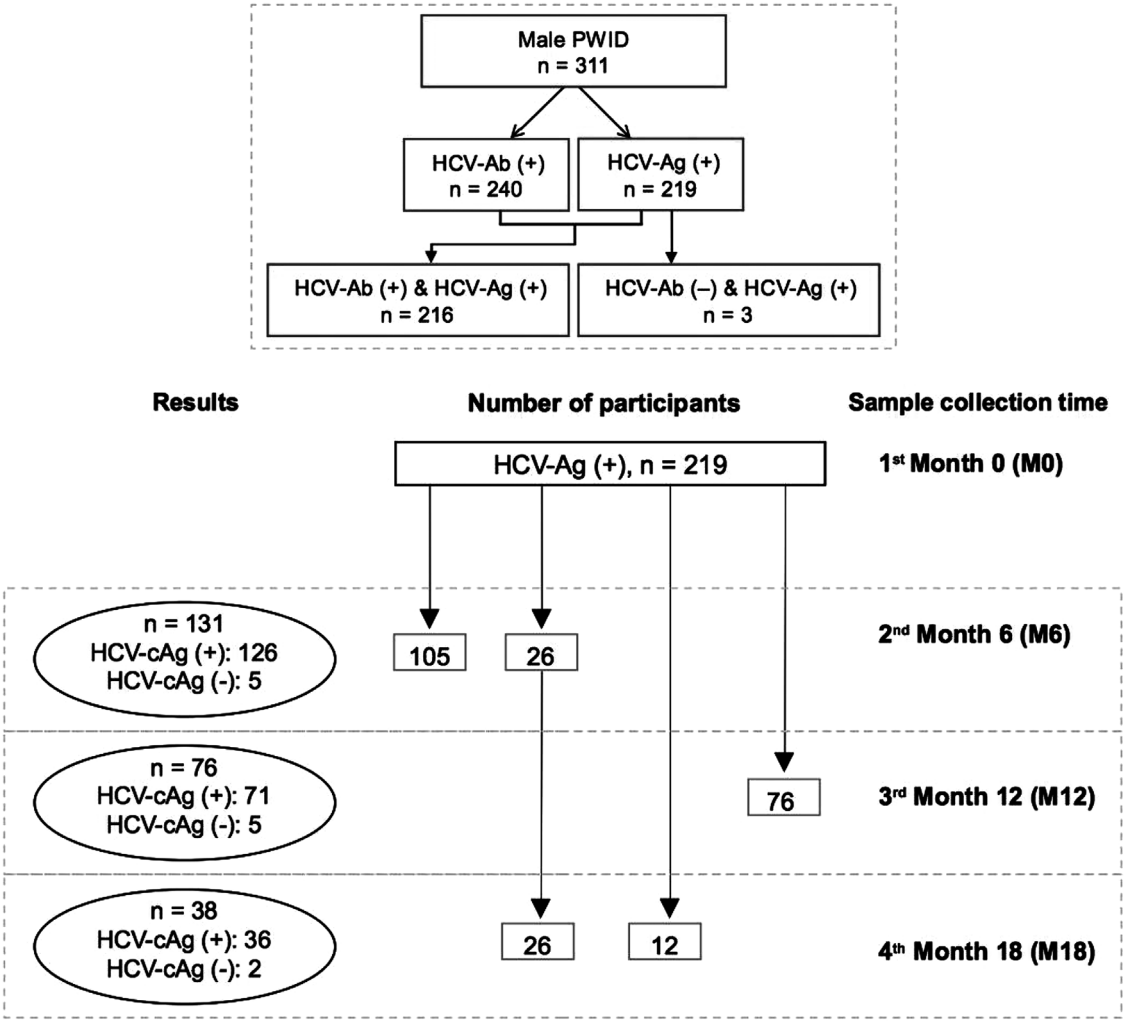

Fig.1. Flow chart of study population over the observational period.
PWID: People who inject drugs; HCV: hepatitis C virus; HCV-cAg: HCV core antigen; Ab: antibody; HCV-Ab: anti-HCV antibody.

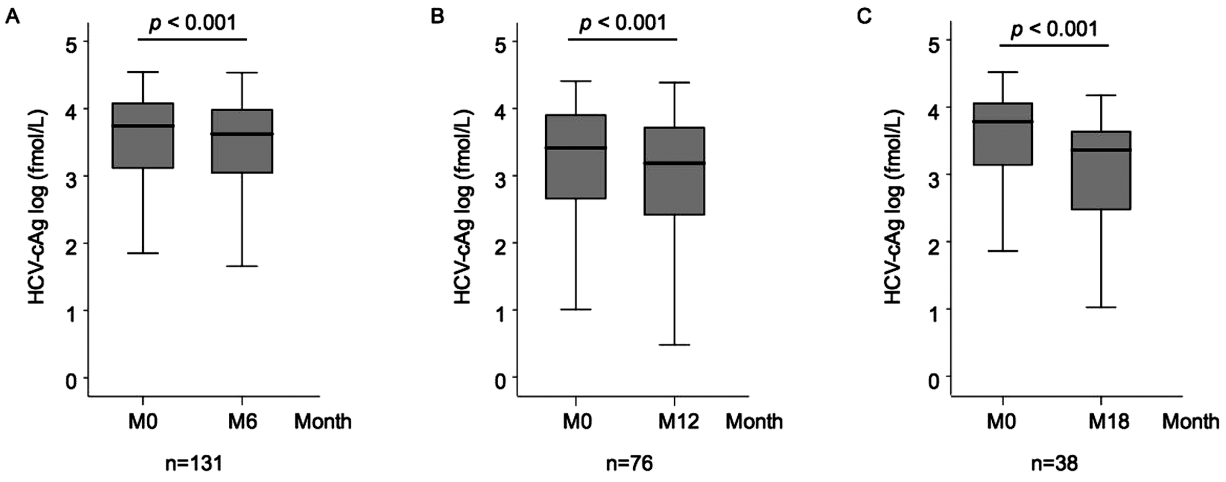

Fig.2. The change of the plasma HCV core antigen levels during the observational period. (A) Box plots of pair samples between M0 and M6, n = 131; (B) Box plots of pair samples between M0 and M12, n = 76; (C) Box plots of pair samples between M0 and M18, n = 38. *P* values < 0.05 are statistically significant, based on Wilcoxon signed-ranks test.

Table 1. Comparison of the level of HCV core Ag stratified by factors

Factors	n	Median (IQR) log fmol/L	p- value*	p- value** Post hoc
Total	219	3.7 (2.9−4.0)		
Age				
≤34 years	115	3.7 (2.9−4.1)	0.80	
>34 years	104	3.7 (2.9−4.0)		
HIV infection				
Negative	104	3.6 (2.5−4.0)	0.054	
Positive	115	3.7 (3.1−4.1)		
HBV infection				
Negative	203	3.7 (2.9−4.0)	0.52	
Positive	16	3.7 (2.8−4.1)		
HCV-Ab				
Negative	3	4.0 (0.9−)	0.81	
Positive	216	3.7 (2.9−4.0)		
HCV genotypes			**0.02**	
HCV genotype 3	15	2.9 (2.0−3.3)		
HCV genotype 1	90	3.7 (3.1−4.0)		**0.01**
HCV genotype 2	2	3.1 (2.3−)		1.00
HCV genotype 6	80	3.8 (3.0−4.1)		**0.006**
HCV dual genotypes	10	3.7 (2.7−3.9)		1.00
Unknown	22	3.8 (2.9−4.2)		**0.03**

IQR, interquartile range; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus. *p-values in bold are statistically significant, based on the Mann-Whitney U test and Kruskal-Wallis test with Dunn-Bonferroni post hoc methods. ** The pair-wise comparison of the level of HCV-cAg between genotype 3 and the genotype listed in the column of factors. The other genotype pairs showed no significant difference.

During the follow-up period, the HCV-cAg became undetected in 12 PWID (5.5%) (Table 2, Figure 1). The clearance rate was 6.5 per 100 person-years [95% confidence interval (95% CI), 3.5–11.3].

Cox proportional hazards analyses showed that the HCV-cAg level at M0 was the only factor associated with the HCV-cAg clearance (hazard ratio = 0.26; 95% CI, 0.15–0.45; $p < 0.001$), whereas age, HIV or HBV infectious status, and HCV genotype were not (all $p > 0.05$; Table 3).

VI. Correlation between the level of HCV-cAg and HCV-RNA load

Plasma HCV-cAg level in 87 PWID randomly selected from the 219 at M0 was significantly correlated with their HCV-RNA load (Rho = 0.55; $p < 0.001$) (Figure 3).

Discussion

In this study, we investigated the change in plasma HCV-cAg level, the rate of spontaneous HCV-cAg clearance, and the factors associated with the clearance among Vietnamese PWID with a maximum of 18-month follow-up. We found that the rate of HCV-cAg clearance in PWID was 6.5 per 100 person-years (95%CI, 3.5–11.3); the HCV-cAg level was marginally higher in PWID co-infected with HIV than those without HIV ($p = 0.054$) and significantly lower in PWID infected with HCV genotype 3 than those with other genotypes ($p < 0.001$); however, only low HCV-cAg level at baseline was associated with the HCV clearance. These findings suggest that the low HCV-cAg level at baseline could be a predictor of HCV clearance.

Table 2. The 12 cases whose HCV core Ag became undetectable (spontaneous viral clearance)

ID	M0	M6	M12	M18	M0	
	HCV-cAg	HCV-cAg	HCV-cAg	HCV-cAg	HCV-Ab	HIV-Ab
PWID 1	0.9	< 0.48			+	−
PWID 2	1.51	< 0.48			+	+
PWID 3	1.56	< 0.48			+	−
PWID 4	0.9	< 0.48			−	−
PWID 5	1.25	< 0.48			+	−
PWID 6	1.01		< 0.48		+	−
PWID 7	1.49		< 0.48		+	+
PWID 8	1.16		< 0.48		+	−
PWID 9	1.17		< 0.48		+	−
PWID 10	2.7		< 0.48		+	+
PWID 11	4.31	4.19		< 0.48	+	+
PWID 12	3.98	4.16		< 0.48	+	−

HCV, hepatitis C virus; HCV-cAg, HCV core antigen (log fmol/L); HCV-Ab, anti-HCV antibody; HIV-Ab, anti-human immunodeficiency virus antibody; M0, the first time of blood samples collected; M6, after 6 months from M0; M12, after 12 months from M0; M18, after 18 months from M0.

Table 3. Potential predictors of HCV-cAg clearance

Predictors (n)	Clearance n (%)	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age					
≤34 years (115)	5 (4.3)				
>34 years (104)	7 (6.7)	1.60 (0.51−5.05)	0.42		
HIV infection					
Negative (104)	8 (7.7)				
Positive (115)	4 (3.5)	0.30 (0.09−1.03)	0.056	−	−
HBV infection					
Negative (203)	10 (4.9)				
Positive (16)	2 (12.5)	2.01 (0.45−9.46)	0.35		
HCV genotypes			0.74		
HCV genotype 3 (15)	2 (13.3)				
HCV genotype 1 (90)	4 (4.4)	0.36 (0.07−1.96)	0.24		
HCV genotype 2 (2)	0 (0)	0.00 (0.00−)	0.99		
HCV genotype 6 (80)	3 (3.8)	0.28 (0.05−1.69)	0.17		
HCV dual genotype (10)	1 (10)	0.84 (0.08−9.29)	0.89		
Unknown (22)	2 (9.1)	0.65 (0.09−4.69)	0.67		
HCV-cAg level at M0 (log fmol/L) (219)	12 (5.5)	0.26 (0.15−0.45)	**< 0.001**	0.26 (0.15−0.45)	**< 0.001**

HR, hazard ratio, HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HCV-cAg, HCV core antigen. The predictors with p < 0.2 in the univariable analysis were included in the multivariable analysis of the Cox proportional hazards model with a backward stepwise method. p-values in bold are statistically significant.

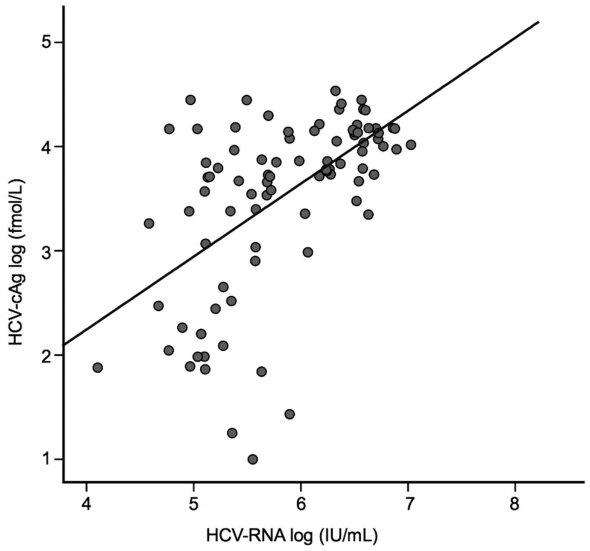

Fig.3. Correlation between the level of plasma HCV core antigen and the HCV-RNA load at M0, n = 87. The coefficient (Rho) was 0.55, $p < 0.001$, based on Spearman's rank correlation analysis.

In the present study, we recruited the PWID who stayed in a rehabilitation center in northern Vietnam. They were supposed to have no chance of injecting drug and therefore had the least risk for newly acquiring HCV infection. Thus, we truly observed a "spontaneous" change of plasma HCV-cAg in the Vietnamese PWID in this study.

Spontaneous HCV clearance was reportedly observed in 27.9% to 36.1% of the general population within 6 to 12 months after primary infection[18], and in 25% of PWID in Australia and the United States within 12 months post-infection[19]. In our study, however, of the 240 HCV-Ab positive PWID at baseline, only 24 (10.0%) had cleared HCV-cAg by the recruitment, and the clearance rate among the 219 HCV-cAg positives was 6.5 per 100 person-years (95%CI, 3.5–11.3) with a maximum of 18-month follow-up. Thus, in the PWID in Vietnam, the spontaneous HCV clearance rate was much lower than those previously reported. HCV infection was more likely to persist, though we did not know when they got infected with HCV, except three who were negative for HCV-Ab and positive for HCV-cAg were presumed to have been recently infected with HCV.

The rate of HCV clearance in HIV co-infected individuals has been reported to be lower than that of HCV mono-infected individuals[18)22]. In this study, 52.5% of the PWID with HCV (n = 219) were co-infected with HIV and had marginally higher plasma HCV-cAg level ($p = 0.054$) and marginally lower HCV clearance rate ($p = 0.056$) than those without HIV. Thus, the lower clearance rate of HCV in the Vietnamese PWID might be partially due to the high prevalence of co-infection with HIV.

It has been reported that the people dually infected with HCV and HIV had twice and 6-times higher risks of development of cirrhosis and end-stage liver disease, respectively, than those with HCV mono-infection[2)23)24]. In addition, we previously reported that current approaches to control and prevent HIV infection, such as expansion of harm reduction programs for PWID, may be insufficient to reduce the prevalence of HCV in Vietnam[15]. Considering these, it is recommended that PWID start HCV treatment without delay to eliminate HCV infection in Vietnam.

Screening for current HCV infection is important to eliminate HCV infection. The HCV-Ab test is commonly used as a screening test for HCV infection, then confirmation is done by an HCV-RNA test[7]. In this study, three cases were negative for HCV-Ab but positive for HCV-cAg at baseline. Thus, using HCV-cAg test could prevent missing of the cases in the early stage of HCV infection. These findings indicate the usefulness of the HCV-cAg test as a screening for HCV infection. Additionally, the level of HCV-cAg was significantly correlated with HCV-RNA load (Rho = 0.55, $p < 0.001$) as previously reported[25)–27]. The application of a cost-effective, easy to perform, and reproducible plasma HCV-cAg test could be considered as an alternative test for screening and monitoring of current HCV infection, especially in resource-limited settings.

The most prevalent HCV genotypes were genotypes 1 and 6, followed by genotype 3 among the PWID in northern Vietnam. The plasma HCV-cAg level was significantly lower in the PWID infected with genotype 3 than those infected with other genotypes. This finding is consistent with previous reports that patients infected with genotype 3 had lower levels of HCV-RNA load and HCV-Ag than those infected with other genotypes, particularly genotype 1 and 6[28)29]. However, it has also been

reported that the viral polymorphisms identified at the amino acid residues of 48 and 49 in the core protein of genotype 3 might affect HCV-cAg serological results[30]. Therefore, HCV-cAg tests should be applied with caution in the regions where genotype 3 is endemic.

This study had some limitations. First, the information of the timing when they got infected with HCV was not available in this study. Therefore, it was not possible to know the precise duration of HCV infection between the timing of acquisition and clearance of the virus in the study participants. Secondly, we could not recruit all the participants at once, nor followed them every 6 months for up to18 months. Thirdly, no clinical data of the participants, such as data on liver function and liver fibrosis tests, was available.

Conclusions

In the present study, we prospectively monitored plasma HCV-cAg among PWID in Haiphong, Vietnam for the maximum of 18 months. Although the HCV-cAg level decreased during the study periods, the spontaneous clearance in the HCV-infected PWID was relatively low. Low HCV-cAg level at baseline could be a predictor of HCV clearance. HCV-cAg test could be applicable for HCV screening and used for monitoring plasma HCV load, particularly in resource-limited settings.

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