Community-based molecular epidemiology study of hepatitis C virus infection in injection drug users

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KEY MESSAGES

1. The seroprevalence of hepatitis C virus (HCV) was 76.4%. HCV-1b and HCV-6a were the two most prevalent genotypes detected in injection drug users (IDU) in Hong Kong.
2. Independent risk factors associated with HCV seropositivity were needle sharing (adjusted odds ratio [OR]=3.17), midazolam injection (adjusted OR=2.53), long duration of injection behaviour of >20 years (adjusted OR=2.45), and higher education level (adjusted OR=0.61).
3. Acute HCV infection was detected in 21 IDU, of whom 85.7% had HCV-1b infection.
4. The most recent common ancestor of the predominant HCV-6a was estimated to be around 1932, with an exponential growth of infection during 1960 to 1980.

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Introduction

Injection drug users (IDU) are at risk of hepatitis C virus (HCV) infection and account for the core of the HCV epidemic in many countries. Worldwide, >185 million individuals are estimated to be chronically infected with HCV, with Africa and Asia having the highest prevalence.[1] Persistent HCV infection is associated with cirrhosis, hepatocellular carcinoma, and HCV-related advanced liver disease. As most new cases of HCV occur among current IDU, HCV infection is a major public health problem and a growing disease burden.

The pattern of HCV epidemiology is highly variable across different countries. Egypt has the highest prevalence and incidence, with 500 000 HCV infections per year.[2] In China, approximately >40 million people are infected with HCV, and the number is increasing due to changes in the mode of HCV transmission, with a decrease in iatrogenic transmission through blood transfusion and an increase in HCV transmission through injection drug use in Southern China.[3] Globally, the distribution of HCV genotypes varies considerably between countries. This reflects differences in HCV epidemics. HCV genotypes 1, 2, and 3 exhibit broad geographical distribution and are prevalent worldwide, whereas HCV genotypes 4, 5, and 6 are generally confined to distinct geographical regions. Genotype 4 is primarily in Africa and the Middle East, genotype 5 in Southern Africa, and genotype 6 in South East Asia. HCV genotypes 1b and 2 are highly prevalent in Europe, with an increase in genotype 3a and a decrease in genotypes 1b and 2 over time. The increasing prevalence of HCV-3a is attributed to immigration and injection drug use in Eastern and Southern Europe. In China, similar changing patterns of HCV genotype distribution are also observed. The most prevalent HCV-1b has declined significantly among young individuals, whereas HCV-3b and HCV-6a have become the most common among IDU. These changing patterns of HCV infection have a significant impact on control measures and raise concern about the increasing burden of HCV infection among IDU.

The burden and transmission patterns of HCV infection in the hidden at-risk IDU population in Hong Kong remain unclear. This study aimed to assess the seroprevalence of HCV in Hong Kong IDU and to track the molecular epidemiology and transmission patterns of HCV infection over time.

Methods

This cross-sectional sero-surveillance study of HCV infection in IDU was conducted between January 2013 and June 2014. Participants were recruited through community-based outreach settings across 21 identified IDU gathering sites in Hong Kong. Participants were eligible if they were aged >18 years and reported a history of injection drug use.

A dried blood spot sample was collected

for anti-HCV and HCV RNA screening. For serological assay, anti-HCV was determined using a commercially available Murex anti-HCV version 4.0 enzyme-linked immunosorbent assay (ELISA) [DiaSorin, Italy]. Weakly reactive and positive ELISA results were confirmed by repeat screening using a Ortho HCV 3.0 Enhanced SAVe ELISA (Ortho Clinical Diagnostics, UK). For HCV RNA detection, elution from dried blood spot was performed on two 6-mm spots and was used for RNA extraction using a QIAamp viral RNA mini kit (Qiagen, USA). Reverse transcription polymerase chain reaction (PCR) with HCV primers targeting the E1 and NS5B regions was performed, followed by direct sequencing of the purified PCR amplicons. For HCV genotyping, the nucleotide sequences of the PCR products were determined directly for phylogenetic analysis using reference HCV strains retrieved from GenBank. To assess the geographic origin of HCV infection, Bayesian coalescent analysis was performed using the Bayesian Evolutionary Analysis Sampling Tree programme to reconstruct the epidemic history of HCV-6a infection in Hong Kong.

To examine the risk factors associated with HCV infection, an interviewer-administered questionnaire was completed to collect sociodemographics, duration and frequency of drug use, current injection status, injection drug use behaviours, and awareness of HCV infection. The seroprevalence of HCV infection was calculated. Categorical variables were compared using Pearson's Chi squared test or Fisher's exact test. Predictors associated with anti-HCV positivity were assessed using univariate analysis. Variables with a P value of <0.2 were included in a multivariate logistic regression model using a stepwise backward method. A P value of <0.05 was considered statistically significant.

Results

A total of 664 participants were recruited. The IDU were mostly male (87.8%), aged >30 years (96.5%), and of Chinese ethnicity (97.3%) [Table 1]. The overall HCV seropositivity among IDU in Hong Kong was 76.4% (95% confidence interval [CI]=73.1-79.6%). Acute HCV infection was observed in 21 IDU, of whom 85.7% had HCV-1b infection. Among those with acute HCV-1b infection, 11 (61.1%) were identified in a common IDU gathering site in the New Territories. HCV RNA was detected in 260 dried blood spot samples, and sequencing of the NS5B fragment was successful in 256 (98.5%) samples. Phylogenetic analysis revealed six HCV genotypes among IDU populations, with HCV-1b and HCV-6a being the most common, followed by HCV-3a, HCV-1a, HCV-2b, and HCV-3b. No co-infection was found. Based on the Bayesian coalescent analysis of E1 sequences, the epidemic history of the divergence time of the most recent common ancestor of HCV-6a infection was estimated to be around 1932, with a transition of constant growth to exponential growth of HCV-6a during 1960 to 1980.

In univariate analysis, anti-HCV positivity was associated with age >30 years (odds ratio [OR]=2.59, 95% CI=1.03-6.44, P=0.023), male gender (OR=2.11, 95% CI=1.25-3.55, P=0.002), long duration of drug use of >20 years (OR=1.57, 95% CI=1.06-2.34, P=0.018), injection drug use of >20 years (OR=2.62, 95% CI=1.60-4.31, P<0.001), methadone treatment of at least 20 years (OR=1.55, 95% CI=1.00-2.42, P=0.040), midazolam injection (OR=2.57, 95% CI=1.72-3.83, P<0.001), needle sharing (OR=2.66, 95% CI=1.59-4.48, P<0.001), and current injection (OR=1.80, 95% CI=1.19-2.73, P=0.004). Predictor associated with decreased OR of HCV infection was observed in IDU with a higher education level (OR=0.61, 95% CI=0.42-0.89, P=0.008).

In multivariate analysis, long duration of injection behaviour of >20 years (adjusted OR=2.45, 95% CI=1.39-4.33, P=0.002), midazolam injection (adjusted OR=2.53, 95% CI=1.59-4.02, P<0.001), needle sharing (adjusted OR=3.17, 95% CI=1.73-5.81, P<0.001), and higher education level (adjusted OR=0.61, 95% CI=0.39-0.95, P=0.029) were independently associated with HCV seropositivity (Table 2).

Discussion

HCV is a major cause of chronic liver disease with most new infections attributed to injection drug use. Globally, the estimated prevalence of HCV infection among IDU is >60%. The epidemiology of HCV in Hong Kong has changed slightly over the past years. IDU remain susceptible to HCV infection as a cluster of acute HCV-1b infection has been identified in a common IDU gathering place, and possibly associated with shared social networks. In 2011, the seroprevalence of anti-HCV positivity among IDU in Hong Kong was 81.7%.[4] The decreased prevalence of HCV infection (76.4%) observed in our study could be attributed to changes in IDU behaviour, particularly a marked decline in needle sharing (6.5%) in the last 3 months.

The HCV genotype distribution is consistent with previous molecular study of HCV infection in our local IDU population.[5] In Hong Kong, genotypes 1b and 6a are more prevalent than genotype 3a. In China, there is a decreasing trend for genotype 1b and an increasing prevalence of genotype 6a. In Iran, there is a gradual decrease in the prevalence of genotype 1a and an increase in genotype 3a. The sensitivity of HCV RNA detection is markedly lower when using stored dried blood spot samples compared with plasma.[5] Due to the limitation of a relatively lower detection rate of HCV RNA,

TABLE 1. Univariate analysis of association between injection drug use behaviours and hepatitis C virus (HCV) infection

	No. (%) of subjects		OR (95% CI)	P value
	Total (n=664)	HCV seropositivity (n=507)		
Age (years)				0.023
≤30	23 (3.5)	13 (56.5)	1.00	
>30	641 (96.5)	494 (77.1)	2.59 (1.03-6.44)	
Gender				0.002
Female	81 (12.2)	51 (63.0)	1.00	
Male	583 (87.8)	456 (78.2)	2.11 (1.25-3.55)	
Ethnicity				0.778
Chinese	646 (97.3)	494 (76.5)	1.00	
Asian	18 (2.7)	13 (72.2)	0.80 (0.26-2.61)	
Education				0.008
Primary school graduate or below	382 (57.5)	306 (80.1)	1.00	
Secondary school graduate or above	281 (42.3)	200 (71.2)	0.61 (0.42-0.89)	
Duration of drug use (years)				0.018
≤20	221 (33.3)	156 (70.6)	1.00	
>20	379 (57.1)	300 (79.2)	1.57 (1.06-2.34)	
Duration of injecting (years)				<0.001
≤20	435 (65.5)	318 (73.1)	1.00	
>20	203 (30.6)	178 (87.7)	2.62 (1.60-4.31)	
Methadone treatment				0.040
≤20	433 (65.2)	322 (74.4)	1.00	
>20	198 (29.8)	162 (81.8)	1.55 (1.00-2.42)	
Midazolam injection				<0.001
No	347 (52.3)	238 (68.6)	1.00	
Yes	317 (47.7)	269 (84.9)	2.57 (1.72-3.83)	
Ever needle sharing				<0.001
No	480 (72.3)	346 (72.1)	1.00	
Yes	173 (26.1)	151 (87.3)	2.66 (1.59-4.48)	
Current injection (past 3 months)				0.004
No	161 (24.2)	109 (67.7)	1.00	
Yes	502 (75.6)	397 (79.1)	1.80 (1.19-2.73)	
Needle sharing (past 3 months)				0.143
No	597 (89.9)	447 (74.9)	1.00	
Yes	43 (6.5)	37 (86.0)	1.15 (1.01-1.31)	
Ever drug use outside Hong Kong				0.879
No	492 (74.1)	377 (76.6)	1.00	
Yes	167 (25.2)	127 (76.0)	0.97 (0.63-1.49)	
Ever injection drug use outside Hong Kong				0.333
No	525 (79.1)	397 (75.6)	1.00	
Yes	137 (20.6)	109 (79.6)	1.26 (0.77-2.04)	
Ever heard of HCV				0.936
No	117 (17.6)	89 (76.1)	1.00	
Yes	547 (82.4)	418 (76.4)	1.02 (0.62-1.67)	
Ever anti-HCV testing				0.647
No	481 (72.4)	355 (73.8)	1.00	
Yes	183 (27.6)	152 (83.1)	0.87 (0.53-1.43)	

TABLE 2. Multivariate logistic regression analysis of risk factors associated with hepatitis C virus infection

Risk factor	Adjusted OR (95% CI)*	P value
Age (reference, ≤30 years)	3.25 (0.97-10.86)	0.056
Male (reference, female)	1.23 (0.65-2.33)	0.532
Education (reference, primary school graduate or below)	0.61 (0.39-0.95)	0.029
Duration of drug use (reference, ≤20 years)	0.72 (0.43-1.20)	0.210
Duration of injecting (reference, ≤20 years)	2.45 (1.39-4.33)	0.002
Methadone treatment (reference, ≤20 years)	1.17 (0.68-2.02)	0.580
Midazolam injection (reference, never)	2.53 (1.59-4.02)	<0.001
Ever needle sharing (reference, never)	3.17 (1.73-5.81)	<0.001
Injection in past 3 months (reference, no)	1.60 (0.96-2.66)	0.071
Needle sharing in past 3 months (reference, no)	1.39 (0.35-5.50)	0.638

* Adjusted for age, gender, education level, duration of drug use, injecting behaviours, methadone treatment, midazolam injection, needle sharing, and injection and needle sharing in the past 3 months

variation in the frequency of HCV genotype over time among IDU could not be explored. Further studies of HCV RNA–based testing using freshly prepared dried blood spot are needed to track the dynamics of HCV genotype distribution and the impact on HCV transmission among IDU in Hong Kong.

In this study, phylogenetic analysis did not reveal the clustering of specific HCV genotypes, suggesting no outbreaks of HCV infection among local IDU. The association of high connectivity and risk behaviour within the HCV social networks in common IDU gathering places would play a major role in increasing HCV transmission and could impact on the rates of HCV infection and the emergence of HCV clusters.

Conclusion

Among IDU in Hong Kong, the relatively high prevalence of acute HCV-1b infection detected in a common IDU gathering site highlights the need to strengthen preventive measures and education to reduce the disease burden and improve public awareness of HCV infection.

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