Molecular epidemiology of hepatitis C virus transmission networks among men who have sex with men in Hong Kong

TH Kwan, DPC Chan, NS Wong, SS Lee *

KEY MESSAGES

1. Sexual transmission of hepatitis C virus (HCV) was common among men who have sex with men (MSM).
2. HCV infections among MSM living with HIV in Hong Kong formed a distinct closely knit cluster.
3. MSM had a shorter infection history at HCV diagnosis, compared with non-MSM.
4. The major driving force of the HCV/HIV syndemic was confined to local transmission clusters.

Hong Kong Med J 2024;30(Suppl 3):S20-2
HMRF project number: 18170282

[1,2] **TH Kwan**, [1] **DPC Chan**, [1,2] **NS Wong**, [1] **SS Lee**

The Chinese University of Hong Kong, Hong Kong SAR, China
[1] *Stanley Ho Centre for Emerging Infectious Diseases*
[2] *Jockey Club School of Public Health and Primary Care*

* Principal applicant and corresponding author: sslee@cuhk.edu.hk

Introduction

The major route of hepatitis C virus (HCV) transmission is blood contact including transfusion of contaminated blood products and needle-sharing among people who inject drugs. An increasing number of HCV infections has been reported among men who have sex with men (MSM) living with HIV in the Asia-Pacific region.[1] HCV infection and transmission are predominately associated with high-risk sexual activities involving mucosal trauma and recreational drug use for sex (chemsex) among both HIV-positive and -negative MSM because they share similar risk factors.[2]

In Hong Kong, HIV prevalence has been low among people who inject drugs, owing to the low-threshold methadone harm reduction programme. The disease burden of HIV/HCV co-infections is small among people who inject drugs, compared with MSM. This study investigated the molecular epidemiology of HCV infection among MSM living with HIV in Hong Kong to (1) delineate the transmission paths of HCV among MSM living with HIV, (2) evaluate factors associated with HCV transmission in the community, and (3) compare infection histories of intra-host variants with non-MSM.

Methods

We recruited MSM who had a diagnosis of HCV infection at the time of HIV diagnosis or experienced seroconversion during follow-up. We aimed to enrol 40 co-infected individuals, based on the estimated HCV infection prevalence of 4% among approximately 1000 HIV patients diagnosed between 2016 and 2019. A blood sample was collected from each MSM, and a survey about sexual behaviours was administered.

Two control groups were set up for comparative analysis. The first control group comprised MSM living with HIV without HCV infection at HIV diagnosis. The second control group included archived RNA specimens from non-MSM without HIV who had HCV infection and were blood donors and people who inject drugs. The total sample size was estimated to be 160.

Viral RNA was extracted. To construct near-complete genomes, 5 to 8 μL of viral RNA were reverse-transcribed and amplified. Primers were used to amplify near full-length genomes for both HIV-1 and HCV; the polymerase chain reaction products were purified.

Viral DNA libraries were prepared from amplicons to enable multiplex whole genome sequencing with MinION. MinKNOW was used to manage the sequencing experiments and to perform basecalling and demultiplexing. A custom bash command script was written to process the Nanopore reads for whole genome sequence reconstruction. To remove low-quality reads, all sequence reads were filtered. After quality checks, all reads were mapped to the reference sequence (HIV-1:HXB2 HCV: NC_004102). Variant and consensus calling were performed. The resultant consensus sequences were converted to the FASTA format.

For both HIV and HCV sequences, pairwise TN93+G+I distances were calculated to construct the respective networks with a 4% distance threshold. Undirected networks were analysed at the network level by measuring the average degree, diameter, clustering coefficient, and density. The networks were then joined to identify bridging nodes that

connected both the HIV and HCV networks. Node-level network parameters included degree, closeness centrality, and average shortest path length.

Whole genome sequences of HCV were subtyped. All sequences identified as subtype 3a were selected for analyses. The best-fit model was selected by likelihood scores, and priors were estimated. Bayesian coalescent skyline analysis was conducted to determine the time to the most recent ancestor (tMRCA) and its population dynamics. The 95% highest probability density interval of the age of the tree was calculated.

A custom bash command script was written for the bioinformatics pipeline to generate within-host variants. Quality-filtered reads were sampled to a size of 10000-read file to ensure computational feasibility while preserving the number of major variants identified. Sampled reads were mapped to the consensus sequence. The SAM files were converted to binary format, sorted, and indexed. After identifying minor variant sequences, clusters were defined as having at least 30 sequences.[3] To identify the infection history of an individual, individuals exhibiting at least 10 within-host variants were selected for phylodynamic analysis. Factors associated with the diversity of within-host variants and the infection history estimated by the tMRCA were determined using the Mann-Whitney *U* test and generalised linear models.

Results

In total, 132 and 115 HCV and HIV sequences were analysed, respectively. Of the HCV sequences, 67 (51%) were from MSM living with HIV and HCV, 44 (33%) were from people who inject drugs, and the remaining 21 (16%) were from blood donors. The most common HCV subtype was 3a (35%, n=46), followed by 6a (23%, n=31), 1b (20%, n=26), and 1a (16%, n=21). Five (4%) and three (2%) subtype 2a and 3b sequences were also identified. Most participants were men (89%, 116/131); the median age at sample collection was 39 years (interquartile range, 30-48 years). Most HIV sequences included were from men (96%, 107/112); the median age at data collection was 31 years (interquartile range, 27-43 years). The three major HIV subtypes were CRF01_AE (44%), CRF07_BC (24%), and B (24%). Other minority subtypes included C, CRF02_AG, CRF08_BC, CRF55_01B, and G.

Most HCV sequences (72%, 96/132) were connected in the network, with four major clusters: subtype 3a (n=42), 6a (n=24), 1a (n=6), and 1b (n=5) [Fig 1]. There were eight smaller components comprising two or three nodes. The subtype 3a cluster consisted of 44% connected nodes, forming a single dense cluster (density=0.997) with a network diameter of 2 and a clustering coefficient of 0.996. In contrast, the subtype 6a cluster was sparse, with a density of 0.279, a longer network diameter of 4, and a clustering coefficient of 0.67. Subtypes 1a and 1b clusters formed a clique-like structure with six and five nodes, respectively; there were three triads and five dyads. Almost all sequences in the subtype 3a network were from MSM living with HIV who formed a clique. Separately, the subtype 6a cluster was primarily composed of people who inject drugs.

Phylodynamic analysis of HCV subtype 3a in MSM was performed. Considering the high genetic similarity among the sequences predominated by MSM, the effective population size during the data collection period did not significantly differ over time, and the history of the tree was short (Fig 2).

Of the 132 HCV sequences, 85 (64%) had at least one within-host variant; 55 of these had ≥10 within-host variants and were analysed. The median of the mean tMRCA across all trees was 6.67 years (95% highest probability density=5.58-7.88 years). MSM had a significantly shorter infection history than non-MSM (1.77 vs 10.85 years, P=0.001). Although HCV subtype was not associated with infection history (P=0.51), subtype 6a had a more diverse within-host variant population (4 vs 15, P=0.0006). After adjustment according to subtype, MSM had a less diverse within-host variant population (P<0.001) and a shorter tMRCA (P=0.017).

Discussion

In Hong Kong, HCV transmission was phylogenetically densely connected among HIV co-infected MSM. At the 4% distance threshold, the HCV sequences in people living with HIV showed unique clustering patterns, which varied according

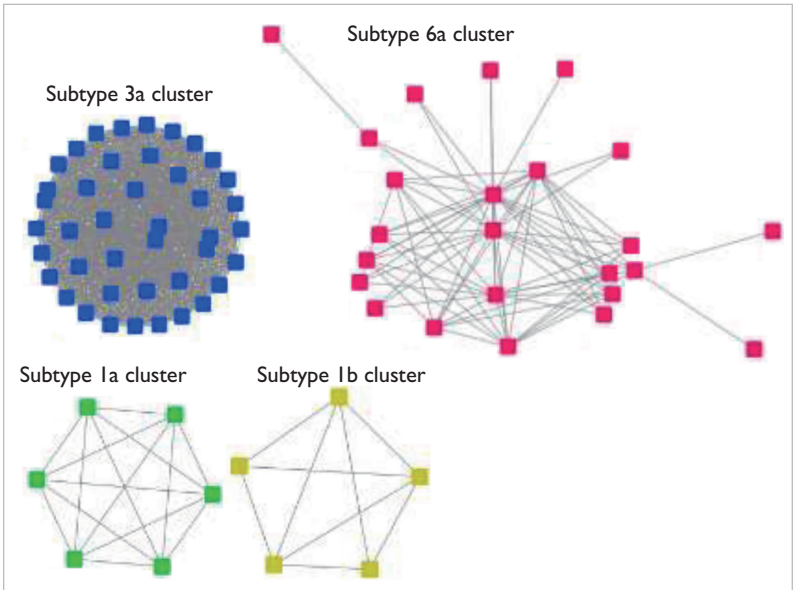

FIG 1. Transmission network of hepatitis C virus: each node represents an HCV sequence, and an edge connecting two nodes indicates a genetic distance of <4%.

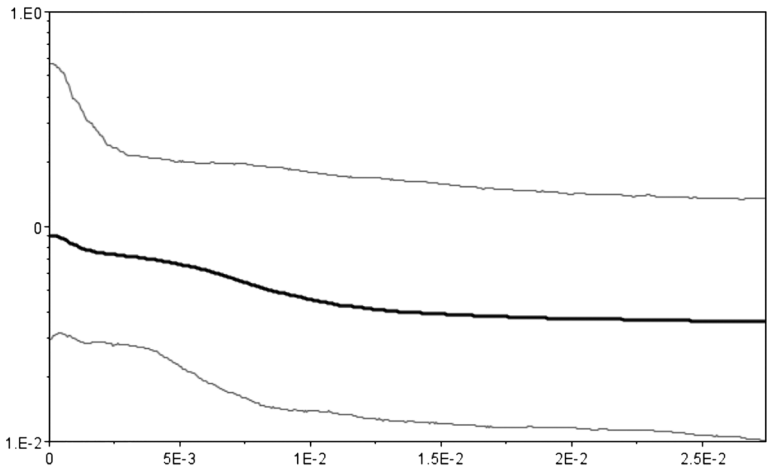

FIG 2. Temporal change in the effective population size of hepatitis C virus subtype 3a sequences: black line represents the median effective population size, and grey lines represent the 95% highest probability density. The x-axis represents time, and the y-axis represents the effective population size using a log10 scale.

to subtype. Most clusters were clique-like, reflecting a pattern of continuous local transmission of HCV in the community within a short period. Notably, the subtype 6a cluster was loosely connected with a low density and a low clustering coefficient. The transmission history of the entire cluster was assumed to be longer such that sufficient time was allowed for in vivo mutations before the virus was transmitted to another individual. Some non-local cases may have originated from neighbouring cities where HCV strains were similar, but less similar than those in the local core transmission cluster; these peripheral nodes formed a star network topology. This observation was confirmed by phylodynamic analysis, in which the estimated duration of infection history was <1 year.

The diversity of within-host variants can infer the infection history and the undiagnosed period; MSM had a shorter within-host variants tMRCA and a less diverse within-host variants population, compared with non-MSM. This indicates that non-MSM had a longer undiagnosed period, whereas MSM were diagnosed soon after HCV infection.[4] The lack of HCV screening, suboptimal access to treatment, and structural barriers may have contributed to the prolonged periods without diagnosis or treatment among non-MSM.[5] The HIV/HCV co-infected MSM were recruited from HIV specialist clinics; clinical management procedures could have contributed to the earlier diagnosis and treatment.

There were some limitations in this study. The transmission networks generated were based on pairwise genetic distances and did not imply direct transmission relationships. The sequences can never be complete because there could be undiagnosed individuals in the population as well as missing nodes owing to refusal to join the study; these missing nodes could have affected the network configuration. As most clusters formed a clique-like structure, the addition or removal of a node would not affect the overall configuration, except in rare instances where an entire cluster was not sampled.

Conclusion

The major driving force of the HCV/HIV syndemic was confined to local transmission clusters. Targeted intervention is warranted to prevent outbreaks in the MSM community. As routine HCV testing in HIV specialist clinics can shorten the diagnosis period, similar testing strategies should be offered to the wider MSM community, particularly individuals involve in chemsex and slamming, to pre-empt HCV transmission.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#18170282). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Kwan TH, Wong BCK, Wong KH, Lee SS. Hepatitis C co-infection in people living with HIV-epidemiologic differences between men who have sex with men MSM and non-MSM. Front Public Health 2022;10:925600.
2. Kwan TH, Wong BCK, Chan DPC, Wong NS, Lee SS. Phylodynamics of sexually acquired HCV-3a in HIV co-infected patients in Hong Kong. J Int AIDS Soc 2020;23(S7):P094.

References

1. Chan DP, Sun HY, Wong HT, Lee SS, Hung CC. Sexually acquired hepatitis C virus infection: a review. Int J Infect Dis 2016;49:47-58.
2. Charre C, Cotte L, Kramer R, et al. Hepatitis C virus spread from HIV-positive to HIV-negative men who have sex with men. PLoS One 2018;13:e0190340.
3. Riaz N, Leung P, Barton K, et al. Adaptation of Oxford Nanopore technology for hepatitis C whole genome sequencing and identification of within-host viral variants. BMC Genomics 2021;22:148.
4. Kwan TH, Wong BCK, Wong KH, Lee SS. Hepatitis C co-infection in people living with HIV-epidemiologic differences between men who have sex with men MSM and non-MSM. Front Public Health 2022;10:925600.
5. Wong NS, Chan DP, Chan CP, et al. Point-of-care hepatitis C reflex testing and treatment referral in methadone clinic settings in Hong Kong: a pilot study. IJID Reg 2022;5:8-12.