Hepatitis C virus infection among people who inject drugs in Bangkok, Thailand, 2005–2010

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Abstract

Background Approximately 1% of adults in Thailand are infected with hepatitis C virus (HCV). New direct-acting antiviral agents achieve sustained virologic responses in >95% of HCV-infected patients and are becoming available in countries around the world. To prepare for new HCV treatment options in Thailand, this study characterized HCV infections among people who inject drugs (PWID) in Bangkok.

Methods The Bangkok Tenofovir Study (BTS) was a pre-exposure prophylaxis trial conducted among PWID, 2005–2013. Blood specimens were randomly selected from PWID screened for the BTS, to test for anti-HCV antibody and HCV RNA. The HVR1 region was amplified by polymerase chain reaction, using multiplex primer sets with unique identifier sequences; amplification products were pooled in sets of 25; and consensus sequencing was performed to characterize individual HCV genotypes.

Results The median age of 3679 participants tested for anti-HCV antibody was 31 years, 3016 (82.0%) were male and 447 (12.2%) were HIV infected. The prevalence of anti-HCV antibody was 44.3%. The adjusted odds of testing positive for anti-HCV antibody were higher in men (adjusted odds ratio [aOR] 3.2, 95% confidence interval [CI] 2.4–4.3), those aged 40 years or older (aOR 2.7, 95% CI 2.1–3.5), those who had more than a primary school education (aOR 1.7, 95% CI 1.4–2.1), and those who tested HIV positive (aOR 5.2, 95% CI 3.7–7.4). HCV RNA was detected in 644 (81.3%) of the 792 anti-HCV antibody-positive specimens, yielding an HCV RNA-positive prevalence of 36.0% (95% CI 33.8–38.2). Among a random sample of 249 of the 644 specimens, 218 could be characterized, and the most common HCV subtypes were 1a (30.3%), 1b (12.8%), 3a (35.8%), 3b (6.9%) and 6n (8.7%).

Conclusion The prevalence of anti-HCV antibody among PWID was 44.3% and more than one third (36.0%) were HCV RNA positive. Genotypes 1, 3 and 6 accounted for all typable infections. As the government of Thailand considers introduction of direct-acting antiviral medications for people with hepatitis C, it will be important to ensure that the medications target these subtypes.

Keywords: direct-acting antivirals, hepatitis C, people who inject drugs, Thailand, viral hepatitis

Background

Hepatitis C virus (HCV) infection is a leading cause of liver disease, cirrhosis and cancer. The World Health Organization estimates that, in 2015, 1.0% of the world’s population was infected with HCV, corresponding to 71 million people chronically infected with HCV. In Thailand in 2015, an estimated 0.7% of the population was infected with HCV. Injection drug use is a common mode of HCV transmission. A study in Bangkok among people who inject drugs (PWID) during the 1990s found an HCV prevalence greater than 90%. Since 2012, the Thai government has funded the use of pegylated interferon and ribavirin to treat HCV. Clinical trials...
have shown sustained virologic responses, a result that is indicative of virologic cure, in 50–60% of chronically HCV-infected participants using pegylated interferon and ribavirin, but therapy is complicated by the length of treatment (24–48 weeks) and side-effects that cause 10–14% of participants to stop treatment. New, once-daily direct-acting oral antiviral agents, which achieve sustained virologic responses with limited adverse events in >95% of chronically infected HCV patients, are becoming available in low- and middle-income countries as the cost of generic formulations declines. Some of these agents are more effective against specific HCV genotypes, but combinations of agents can effectively treat most or all HCV genotypes.

To prepare for new HCV treatment options, this study characterized HCV infections among the population of PWID attending drug-treatment clinics, from whom participants in an HIV pre-exposure prophylaxis trial in Bangkok, Thailand, were drawn.

**Methods**

The study population was PWID attending the 17 drug-treatment clinics serving the Bangkok Metropolitan Administration area during 2005–2010, who provided a blood sample for eligibility screening for the Bangkok Tenofovir Study (BTS) after signing informed consent. BTS was a randomized, double-blind, placebo-controlled HIV pre-exposure prophylaxis trial of daily oral tenofovir disoproxil fumarate. Of 4094 PWID screened for the BTS, blood specimens were not available for 415 (10.1%) (see Fig. 1). Thus, there were 3679 blood samples from PWID available for analysis.

**Fig. 1. Flow of people who inject drugs assessed for hepatitis C infection, Bangkok, Thailand, 2005–2010**

HCV: hepatitis C virus; PWID: people who inject drugs.

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**a** The random selection of 1600 specimens should have been done among all 3679 samples. However, after randomization, it was discovered that 385 specimens had not been included in the randomization, because the specimens had been identified as anti-HCV antibody negative in a prior evaluation and mistakenly excluded from the randomization. The 1600 specimens had therefore been randomly selected from 3294 PWID samples. To estimate HCV prevalence and determine predictors of HCV infection, a weighted analysis was carried out, with a sampling probability of 1 for the 385 known anti-HCV antibody-negative specimens, and a sampling probability of 0.49 (i.e. 1600/3294) for the 1600 anti-HCV antibody-unknown specimens.

**b** Other reasons include: non-Thai nationals; those younger than 20 years or older than 60 years; people who left the clinic before blood was drawn; and people who provided a blood specimen but the specimen was insufficient or could not be used.
HCV antibody testing was only possible for 1600 blood samples and genotyping of 249 samples, owing to limited resources. To maximize the accuracy of the prevalence estimates, samples were randomly selected for antibody testing and genotyping, using a computer-generated random number sequence. The random selection of samples for antibody testing should have been applied to all 3679 samples. However, after randomization, it was discovered that 385 specimens had not been included in the randomization, because the specimens had been identified as anti-HCV antibody negative in a prior evaluation and mistakenly excluded from the randomization (see Fig. 1). The 1600 blood samples had therefore been randomly selected from 3294 PWID samples. To estimate HCV prevalence and determine predictors of HCV infection, a weighted analysis was carried out, with a sampling probability of 1 for the 385 known anti-HCV antibody-negative specimens, and a sampling probability of 0.49 (i.e. 1600/3294) for the 1600 anti-HCV antibody-unknown specimens. Weighted multivariable logistic regression was used to determine factors associated with HCV infection, and SAS version 9.3 (SAS Institute, Cary, NC, United States of America [USA]) was used for analyses.

To provide context for the study findings, PubMed was searched for reports of HCV genotyping in Thailand from 2000 to 2016 and the search included reports that examined HCV RNA-positive specimens from 100 or more people (see Table 1). The search terms used were “genotyping”, “HCV”, “hepatitis C” and “Thailand”.

Specimens were tested for HIV (Bio-Rad Laboratories, Redmond, WA, USA) and anti-HCV antibody, using an enzyme immunoassay (Diasorin-Murex anti-HCV version 4.0, Dartford, United Kingdom of Great Britain and Northern Ireland), and anti-HCV-positive specimens were examined for HCV RNA, using the Aptima HCV RNA qualitative assay (Hologic Inc., San Diego, CA, USA). HCV RNA was sequenced using COBAS TaqMan HCV, version 2.0 (Roche Molecular Systems, Branchburg, NJ, USA) and the GS Junior System (454 Life Science Corp., Branford, CT, USA), and RNA was extracted using a QiAamp Viral RNA mini kit (Qiagen, Germantown, MD, USA). The HVR1 region was amplified using HCV-specific primers, with multiplex identifier sequences (Roche Molecular Systems). In each sequencing run, polymerase chain reaction products from 25 samples with unique multiplex identifiers were pooled and pyrosequenced on the GS Junior System. Consensus sequences of each sample were used to characterize the HCV genotype. Multiple alignments of sample and reference sequences were made using Clustal W (Geneious 9.1.5, Biomatter Ltd., Auckland, New Zealand), and edited using MacClade 4.08a. Phylogenetic trees were constructed using the neighbour-joining algorithm within Geneious 9.1.5, and bootstrap resampling was performed with 500 replicates and a bootstrap value of ≥70%.

The Thailand Ministry of Public Health and the Ethical Review Board of the US Centers for Disease Control and Prevention approved the study, which was consistent with the principles of the Declaration of Helsinki. The BTS was registered at ClinicalTrials.gov, identifier NCT00119106.

### Table 1. Hepatitis C genotypes and subtypes of people who inject drugs in Bangkok, Thailand, 2005–2010, and other cohorts reported in Thailand, 2000–2016

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Population</th>
<th>Year</th>
<th>Samples with HCV RNA sequenced, n</th>
<th>Hepatitis C genotypes and subtypes, n (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Non-typable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bangkok (findings of this study)</td>
<td>PWID</td>
<td>2005–2010</td>
<td>249</td>
<td>1a: 66 (26.5)</td>
<td>1b: 28 (11.2)</td>
</tr>
<tr>
<td>Chiang Mai&lt;sup&gt;10&lt;/sup&gt;</td>
<td>PWID</td>
<td>1999–2000</td>
<td>168</td>
<td>1a: 37 (22.0)</td>
<td>1b: 15 (8.9)</td>
</tr>
<tr>
<td>Chiang Mai&lt;sup&gt;11&lt;/sup&gt;</td>
<td>HCV-infected</td>
<td>2003–2010</td>
<td>158</td>
<td>1a: 20 (12.7)</td>
<td>1b: 29 (18.4)</td>
</tr>
<tr>
<td>Chiang Mai, Chiang Rai, Lamphun, Lamphun, Mae Hong Son&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Blood donors</td>
<td>1998–2000</td>
<td>126</td>
<td>1a: 18 (14.3)</td>
<td>1b: 16 (12.7)</td>
</tr>
<tr>
<td>Pattani, Songkla&lt;sup&gt;13&lt;/sup&gt;</td>
<td>PWID</td>
<td>2000</td>
<td>290</td>
<td>1a: 3 (1.0)</td>
<td>1b: 52 (17.9)</td>
</tr>
<tr>
<td>Bangkok, Phetchabun&lt;sup&gt;14&lt;/sup&gt;</td>
<td>HCV-infected</td>
<td>2003–2009</td>
<td>356</td>
<td>1a: 75 (21.1)</td>
<td>1b: 49 (13.8)</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus; PWID: people who inject drugs.

<sup>a</sup> Percentages rounded to one decimal place; thus, totals may not exactly equal 100.

<sup>b</sup> HCV RNA was detected by qualitative methods, but amplicons for sequence analysis could not be generated using HCV-specific primers previously described.
Results

From June 2005 to July 2010, 4094 people were assessed for enrolment; 415 (10.1%) individuals were excluded before blood was collected. Among the 415 excluded, 241 (58.1%) completed the demographic questionnaire and did not differ significantly in age from the 3679 people assessed who provided specimens (median age of those excluded, 32 years; median age of those included, 31 years; Wilcoxon rank sum \( P = 0.54 \)), sex distribution (200 [83.0%] males in the excluded group, 3016 [82.0%] males in the included group; Chi-squared \( P = 0.79 \)), or education level (45 [18.7%] of those excluded and 699 [19.0%] of those included had more than a primary school [grade 6] education; Chi-squared \( P = 0.36 \)).

The median age of the 3679 individuals tested was 31 years (interquartile range, 27–38 years); 3016 (82.0%) were male and 447 (12.2%) were HIV infected. The weighted prevalence of anti-HCV antibody was 44.3% (95% confidence interval [CI] 42.1–46.6%). In multivariable logistic regression including sex, age, education level and HIV status, the odds of testing anti-HCV antibody positive were higher in men (adjusted odds ratio [aOR] 3.2, 95% CI 2.4–4.3, \( P < 0.0001 \)), those aged 40 years or older (aOR 2.7, 95% CI 2.1–3.5, \( P < 0.0001 \)), those who had more than a primary school (grade 6) education (aOR 1.7, 95% CI 1.4–2.1, \( P < 0.0001 \)), and those who tested HIV positive (aOR 5.2, 95% CI 3.7–7.4, \( P < 0.0001 \)) (see Table 2).

Among the 1600 randomly selected specimens, anti-HCV antibody was detected in 158 (76.0%) of the 208 HIV antibody-positive specimens and 634 (45.5%) of the 1392 HIV antibody-negative specimens (not shown in Fig. 1). HCV RNA was detected in 644 (81.3%) of the 792 anti-HCV antibody-positive specimens, yielding an HCV RNA-positive prevalence of 36.0% (95% CI 33.8–38.2%). Among the 249 randomly selected HCV RNA-positive specimens, 29 (11.6%) could not be amplified and two (0.8%) yielded short sequences that could not be characterized (see Fig. 1). Among the 218 specimens that could be characterized, the most common subtypes were 1a \( (n = 66 [30.3%]) \), 1b \( (n = 28 [12.8%]) \), 3a \( (n = 78 [35.8%]) \), 3b \( (n = 15 [6.9%]) \) and 6n \( (n = 19 [8.7%]) \) (see Fig. 1 and Table 2).

Discussion

Among PWID screened for the BTS, the prevalence of anti-HCV antibody was 44.3%. This is lower than the 95.6% prevalence reported among PWID in Bangkok in 1995–1996. Declines in HIV incidence have been noted among PWID in Bangkok, and these declines in HIV and HCV prevalence may reflect changes in drug use (e.g. increasing use of methamphetamines and midazolam that do not require injection) and decreased needle sharing. Despite the lower prevalence of HCV antibody, the present analysis found that more than one third (36.0%) of PWID screened for the study were positive for HCV RNA. Using respondent-driven sampling, investigators estimated there were 4200 PWID in Bangkok in 2009, suggesting that, at the time of samples being taken, approximately 1500 PWID in Bangkok were chronically infected with HCV and in need of treatment.

The HCV genotype distribution among PWID screened for the BTS is similar to the distributions reported for other cohorts of PWID, blood donors and HCV-infected populations in Thailand, with genotypes 1, 3 and 6 accounting for most or all infections (see Table 1). A study in Southern Thailand found a higher proportion of genotype 3, predominantly subtype 3a, and studies in Northern Thailand found higher proportions of genotype 6. As the government of Thailand considers various antiviral medications and combination therapies, it will be important to ensure that the medications target these subtypes.

Table 2. Results of weighted logistic regression analyses to evaluate characteristics associated with the presence of hepatitis C antibody in 1985 people who inject drugs, Bangkok, Thailand, 2005–2010

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>( n )</th>
<th>Bivariate analysis (weighted)</th>
<th>Multivariable analysis (weighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unadjusted odds ratio (95% CI)</td>
<td>( P ) value</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>381</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1604</td>
<td>3.3 (2.5–4.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at enrolment, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–39</td>
<td>1562</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td>423</td>
<td>2.3 (1.8–2.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school (grade 6) or less</td>
<td>984</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>More than primary school</td>
<td>1001</td>
<td>1.5 (1.3–1.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1777</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>208</td>
<td>4.7 (3.4–6.6)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\( CI \): confidence interval.
There are a number of limitations to this analysis. Only HIV-uninfected PWID were allowed to enrol in the BTS; thus, people with known HIV-positive status may have chosen not to screen for the trial. Because HCV infection is correlated with HIV infection, the prevalence of HCV among PWID in Bangkok may, therefore, have been underestimated. Complete information on the 415 people who began the screening process but did not provide blood specimens was not available. Although 241 (58.1%) of the 415 who completed the screening questionnaire did not differ by age, sex or education from those who provided a blood specimen, there may have been differences that could not be assessed. The findings of this study may not be generalizable to all PWID or all HCV-infected people in Thailand, but a large population of PWID was sampled and the results are consistent with those of other studies in Thailand, suggesting the HCV genotype and subtype distribution will be a useful guide for policy-makers. The initial sampling did not include all PWID screened for the study, so a weighted analysis was used to provide accurate prevalence estimates.

The antiviral combination sofosbuvir and velpatasvir has achieved sustained virologic responses in ≥95% of people infected with HCV genotypes 1, 3 and 6, and other combinations such as sofosbuvir and ledipasvir have shown high levels of efficacy against genotypes 1 and 6. The appropriate antiviral regimen for an individual may vary if the patient has cirrhosis or if the virus has antiviral mutations, but newer antiviral medicines have simplified and shortened treatment regimens, substantially reduced adverse events, and increased chances for sustained viral suppression and cure.

These effective and safe antiviral medicines hold much promise for people who are chronically infected with HCV in Thailand; however, these individuals will not benefit unless the medicines are accessible. More than 60% of chronically HCV-infected people live in middle-income countries like Thailand. Pursuing licensure and developing innovative pricing strategies for these new antiviral medicines would help to increase their availability to patients in need.

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Authorship: MM drafted the manuscript with input from the other authors. KC was the Principal Investigator of the Bangkok Tenofovir Study. SV, US and KC managed staff in the study clinics. WL and PAM were responsible for data management and MM and PAM for statistical analysis and interpretation. MM, RA, BR, TC, JMM and MEC were responsible for laboratory testing, analysis and interpretation. All authors contributed to the manuscript and approved the final version.

Data availability: GenBank accession – BankIt2174015: MK270399–MK270509.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.


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