Original Article

The Epidemic of Human Immunodeficiency Virus, Hepatitis C Virus, and Syphilis Infection, and the Correlates of Sexually Transmitted Infections among Men Who Have Sex with Men in Zhenjiang, Jiangsu, China

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SUMMARY: A cross sectional survey was performed to investigate the epidemic of human immunodeficiency virus (HIV), hepatitis C virus (HCV), syphilis, and HIV-1 subtype infections among men who have sex with men (MSM) and to evaluate factors that were correlated with sexually transmitted infections (STIs) among 657 MSM in Zhenjiang, Jiangsu, during 2012–2013. Data on socio-demographic parameters and risky behaviors were collected, and blood samples were obtained. In this survey, the prevalence of STIs due to HIV (5.3%), HCV (0.6%), and syphilis (19.2%) were measured. Of 35 HIV-infected MSM, 1 (0.2%) and 17 (2.6%) were co-infected with HCV and syphilis. Among 33 samples with available HIV-1 genomic fragment sequences, 6.1% B, 72.7% CRF01_AE, and 21.2% CRF07_BC were identified. Multivariate logistic regression analysis showed that age > 30 years (odds ratio [OR] 2.002; 95% confidence interval [CI], 1.144–3.502) and having had STI symptoms in the past year (OR, 11.673; 95% CI, 3.092–44.078) were positively correlated with having an STI, while consistent use of a condom when having sex with male partners (OR, 0.621; 95% CI, 0.408–0.945) was significantly associated with a low STI rate. The worsening epidemic of HIV, HCV, and syphilis infections, and the prevalence of complex subtypes of HIV-1 and other risk factors suggest that effective intervention strategies should be strengthened for MSM in Zhenjiang, Jiangsu.

INTRODUCTION

Since human immunodeficiency virus (HIV)/AIDS were first identified among men who have sex with men (MSM) in the early 1980s (1) and continue to be a major public health problem worldwide (2). Available data indicate that the HIV epidemic among MSM has been rapidly expanding in recent years in China (3,4). Nationally, HIV prevalence among MSM increased from 0.6% in 2003 to 7.4% in 2009 (3). Furthermore, the prevalence of syphilis, a sexually transmitted infection (STI), has also been increasing over the past 2 decades in China (5). Syphilis infection may facilitate HIV transmission, which has exacerbated the HIV epidemic among MSM (6,7).

Jiangsu, as one of the most developed economic regions of China, has also experienced a similar increase in the epidemic of HIV and other sexually transmitted diseases (STDs) (8). Zhenjiang is a medium-sized city of Jiangsu, located at China's most developed and promising Yangtze River Delta. By using HIV sentinel

surveillance data, we found that the prevalence of sexually transmitted HIV was 24.8% (27/109) during 2004–2007, and the predominant mode of transmission was drug use (48.6%, 53/109). Over time, the epidemic of sexually transmitted HIV in Zhenjiang, especially through male homosexuals has worsened, similar to other cities in Jiangsu. However, despite the current trend of the STIs (HIV, hepatitis C virus [HCV], and syphilis) epidemic, correlates of STIs and molecular epidemiological data for HIV among MSM in Zhenjiang remain unclear.

To address this question, we investigated the HIV, HCV, and syphilis infection epidemic among MSM from 2012 to 2013 in Zhenjiang. We also elucidated the correlates of STIs and found complex subtypes of HIV-1 circulating in Zhenjiang; this information may be useful for development of effective strategies to control these epidemics among MSM.

MATERIALS AND METHODS

Study participants and data collection: This study was designed as a cross-sectional survey of MSM in Zhenjiang during 2012–2013, after obtaining approval from the Medical Ethics Committee of the Zhenjiang Center of Disease Control and Prevention, and the agreement of participants. The inclusion criteria were: (i) age 18 years or older; (ii) having sex with men within the past 6 months, and (iii) willingness to cooperate with the study protocol. Participants were anonymized by assignment of unique identification numbers. The

Received September 17, 2015. Accepted June 13, 2016.
J-STAGE Advance Publication June 30, 2016.

DOI: 10.7883/yoken.JJID.2015.464

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questionnaire survey information was based on the national sentinel surveillance program and included age, marital status, educational level, sexual behavior relevant to STIs, drug use, and STI status. Information was obtained via one-on-one interviews by qualified investigators.

Laboratory testing for STIs: Whole blood samples were collected from MSM for HIV, HCV, and syphilis testing. All assays were performed in the HIV Confirmatory Laboratory at the Zhenjiang Center for Disease Control and Prevention. HIV testing was performed by enzyme-linked immunosorbent assay (ELISA) kits (Bio-Rad, Hercules, CA, USA), and positive results were further confirmed by western blot assay (Genelabs Diagnostics, Singapore Science Park, Singapore). The presence of anti-HCV and syphilis-specific antibody was detected by ELISA kits (Beijing Wantai Biotechnology, Zhuhai Lizhu Biotechnology; Beijing, China). Samples positive for syphilis-specific antibody then underwent rapid plasma reagin testing (RPR, Shanghai Kehua Biotechnology, Shanghai, China). Double seropositive samples determined by ELISA and RPR were considered syphilis infection in this study. Participants were assigned to an STI group and a control group according to their HIV, HCV, and syphilis infection status.

RNA extraction and amplification of HIV-1 gene fragments: All confirmed HIV-seropositive plasma samples were prepared and stored in a -70°C freezer until use. HIV RNA was extracted from $140\ \mu\text{l}$ of plasma with QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and then subjected to amplification of HIV-1 genomic fragments (*p17* and *C2V3*). Reverse transcription PCR reactions were performed using the One-step RT-PCR kit (Takara Bio, Shiga, Japan) with outer primer pairs PBS-172A/p24-173B (*p17*) and ED-5/ED-12 (*C2V3*). Then, the PCR products were subjected to second PCR (nested PCR) using Premix Taq PCR kit (Takara Bio) with inner primer pairs PSI-174A/p17-175B (*p17*) and ED-31/ED-33 (*C2V3*). The primer pair and the amplification fragment information used in this study were described in previous literature (9). The procedures for reverse transcription and PCR testing were performed according to the instruction manual. Amplified PCR products were sent to Sangon Biotech (Shanghai, China) for sequencing.

Phylogenetic analysis of HIV-1 sequences: HIV reference sequences were downloaded from Los Alamos HIV Sequence Database <www.hiv.lanl.gov>. To avoid potential laboratory errors, all nucleotide sequences obtained in this study were aligned together with reference sequences using the Clustal W program implemented in MEGA 6.0 and were further adjusted manually. The phylogenetic trees of *p17* and *C2V3* segments were constructed with MEGA 6.0 by using neighbor-joining method with a bootstrap evaluation of 1,000 replications.

Nucleotide sequence accession numbers: The GenBank accession nos. of the HIV-1 sequences reported in this article are KM484699-KM484730 and KM879896-KM879928.

Statistical analysis: All statistical analysis in this study was conducted by using IBM SPSS Statistics 20 (Chicago, IL, USA). For all analyses, we set the signifi-

cance level for *P* values at 0.05. Logistic regression analysis was performed with STIs as a dependent variable.

RESULTS

Prevalence of HIV, HCV, and syphilis: A total of 657 MSM were recruited for this study. The prevalence rates of HIV, HCV, and syphilis infections were 5.3%, 0.6%, and 19.2%, respectively. Of 35 HIV-infected MSM, 1 (0.2%) and 17 (2.6%) were co-infected with HCV and syphilis respectively (Table 1).

Characterization of HIV-1 subtypes: A total of 35 HIV-1 positive samples were detected, and 32 *p17* fragments (about 572 bp) and 33 *C2V3* fragments (about 515 bp) were amplified from these samples. However, 1 HIV-1 positive sample (13-ZJ-061) failed to amplify regardless of *p17* or *C2V3*, likely due to low viral load. Phylogenetic trees of *p17* and *C2V3* sequences are shown in Fig. 1. Table 2 shows that among 32 amplified *p17* sequences, 3.1% (1/32) belonged to HIV-1 subtype B, 18.8% (6/32) to CRF07_BC, and the remaining 78.1% (25/32) to CRF01_AE. Among 33 *C2V3* sequences, 6.1% (2/33) belonged to subtype B, 24.2% (8/33) to CRF07_BC, and the remaining 69.7% (23/33) to CRF01_AE. Among these amplified sequences, 1 sample (12-ZJ-532) showed inconsistent results for *p17* and *C2V3*. The phylogenetic analyses of *p17* and *C2V3* sequences showed that these belonged to CRF01_AE and CRF07_BC, respectively.

Socio-demographic characterization and risky behaviors: This study included 657 participants. Of 146 MSM with STIs, 57.5% were unmarried. Most were <30 years old (56.2%), and 56.8% were junior and senior high school graduates. Socio-demographic characteristics such as age and marital status were statistically significant ($P < 0.05$) in a comparison between the STI group and control group (Table 3).

Among the 146 MSM with STIs, 126 (86.3%) engaged in sex with men in the past 6 months, and 55.6% used condoms every time. Among the 11 participants who had sex with commercial male partners, 72.7% used condoms consistently. Meanwhile, 40.9% of the 44 participants who had sex with women used condoms every time. In the control group, among the 437 (85.5%) who had sex with men, 68.2% used condoms consistently. 61.1% of the 18 participants who had sex

Table 1. Prevalence of HIV, HCV, syphilis and their co-infection among MSM in different regions (%)

Region	HIV-positive	HCV-positive	syphilis-positive	HIV and HCV co-infection	HIV and syphilis co-infection
Zhenjiang	5.3	0.6	19.2	0.2	2.6
Jiangsu ¹⁾	5.8	0.7	27.7	0.0	3.7
China	4.9 ²⁾	na	11.8 ²⁾	1.9 ³⁾	2.7 ⁴⁾

na, not available.

¹⁾: The data were adapted from reference (10).

²⁾: The data including 47,231 MSM from 61 cities in China were from reference (11).

³⁾: The data including 513 HIV-infected MSM were from reference (12).

⁴⁾: The data were adapted from reference (13).

Fig. 1. The phylogenetic tree of *p17* and *C2V3* fragments of HIV-1 strains isolated from Zhenjiang was constructed with MEGA 6.0 using the neighbor-joining method. The subtype references were downloaded from Los Alamos HIV Sequence Database. The stability of the nodes was assessed by bootstrap analyses with 1,000 replications.

with commercial male partners used condoms every time. Among the 132 participants who had sex with women, 43.9% used condoms consistently. Compared with the control group, the STI group had a significantly higher rate of unsafe sexual behaviors with non-commercial male partners and more engaged in sex with commercial male partners ($P < 0.05$), but there was no significant difference in sexual behaviors with women between the 2 groups.

Injected drug use (IDU) and needle-sharing was also a common factor associated with STIs (10,11). However, IDU was unusual in our survey. Only 3 MSM reported drug use. We also found that 7.5% of the STI group and 0.6% of the control group already had STI

symptoms in the past year, and this was statistically significant ($P < 0.05$).

Multivariate logistic regression analysis of factors associated with STIs: Multivariate logistic regression analysis showed that age > 30 years (odds ratio [OR], 2.002; 95% confidence interval [CI] 1.144–3.502) and having had STI symptoms in the past year (OR, 11.673; 95% CI, 3.092–44.078) were positively correlated with having an STI, while consistent use of a condom when having sex with male partners (OR, 0.621; 95% CI, 0.408–0.945) was significantly associated with a low STD infection rate (Table 4).

Table 2. Subtype characterizations of 2 genomic fragments from HIV-infected MSM in Zhenjiang, Jiangsu (2012–2013)

Specimen	<i>p17</i>	<i>C2V3</i>	Subtype
12-ZJ-008	07_BC	07_BC	07_BC
12-ZJ-019	01_AE	01_AE	01_AE
12-ZJ-020	B	B	B
12-ZJ-027	01_AE	na	01_AE
12-ZJ-040	01_AE	01_AE	01_AE
12-ZJ-098	01_AE	01_AE	01_AE
12-ZJ-116	01_AE	01_AE	01_AE
12-ZJ-144	01_AE	01_AE	01_AE
12-ZJ-149	07_BC	07_BC	07_BC
12-ZJ-184	01_AE	01_AE	01_AE
12-ZJ-253	01_AE	01_AE	01_AE
12-ZJ-272	01_AE	01_AE	01_AE
12-ZJ-273	01_AE	01_AE	01_AE
12-ZJ-401	01_AE	01_AE	01_AE
12-ZJ-457	01_AE	01_AE	01_AE
12-ZJ-510	01_AE	01_AE	01_AE
12-ZJ-522	07_BC	07_BC	07_BC
12-ZJ-532 ¹⁾	01_AE	07_BC	na
13-ZJ-007	01_AE	01_AE	01_AE
13-ZJ-016	07_BC	07_BC	07_BC
13-ZJ-054	01_AE	01_AE	01_AE
13-ZJ-061 ²⁾	na	na	na
13-ZJ-063	01_AE	01_AE	01_AE
13-ZJ-068	01_AE	01_AE	01_AE
13-ZJ-069	01_AE	01_AE	01_AE
13-ZJ-082	01_AE	01_AE	01_AE
13-ZJ-094	01_AE	01_AE	01_AE
13-ZJ-098	na	07_BC	07_BC
13-ZJ-115	01_AE	01_AE	01_AE
13-ZJ-121	07_BC	07_BC	07_BC
13-ZJ-122	07_BC	07_BC	07_BC
13-ZJ-123	01_AE	01_AE	01_AE
13-ZJ-130	01_AE	01_AE	01_AE
13-ZJ-133	01_AE	01_AE	01_AE
13-ZJ-138	na	B	B
Total	32	33	33

na, not available.

¹⁾: *p17* showed that it was CRF01_AE, but *C2V3* showed it was CRF07_BC.

²⁾: Not available for *p17* and *C2V3*.

DISCUSSION

We demonstrated that the MSM population in Zhenjiang had relatively high rates of STIs and circulating complex HIV-1 subtypes. Furthermore, being older and having had STI symptoms in the past year were associated with increased probability of STIs. In contrast, consistent condom use was associated with a lower risk of STI.

Our study showed that the prevalence rates of HIV, HCV, and syphilis infection among MSM in Zhenjiang were 5.3%, 0.6%, and 19.2%, respectively. These results were similar to those of studies reported for other cities in Jiangsu Province. Guo et al. (12) reported that the prevalence rates of HIV, HCV, and syphilis infection among MSM were 5.8%, 0.7%, and 27.7% in a comprehensive survey involving MSM in Nanjing and Yangzhou, Jiangsu. Our results for the HIV and syphilis infection rates were higher than the

overall prevalence (HIV 4.9% and syphilis 11.8%) among MSM in a larger national survey across 61 cities (13), which suggested that the HIV and syphilis epidemic is more serious in Jiangsu than nationwide. However, the HIV-HCV co-infection rate in Zhenjiang (0.2%) was lower than the prevalence (1.94%) among HIV-infected MSM in China (14). This showed that the HCV infection rate was still at a quite low level among HIV-infected MSM in China, especially in Jiangsu (0%) (12).

In our study, 2.6% of MSM had HIV-syphilis co-infection and 7.5% of the STI group in the MSM population had other STI symptoms in the past year. Chow et al. (15) also reported that HIV-syphilis co-infection was 2.7% during 2007–2008 nationally and estimated that the co-infection rate would keep rising. Such a high proportion was a warning signal, as co-infection can facilitate the transmission and acquisition of other STIs, including HIV (6). There is also the possibility that HIV and other STIs might spread to the general population, as we found that 26.8% of the recruited MSM were married, and about 56.8% of the participants had a low rate of condom use when having sex with women. The considerably high STI prevalence in Zhenjiang may ultimately become a critical public health and social problem without effective intervention strategies. We should continue to monitor the dynamics of HIV, other STIs, and co-infections to develop a suitable management strategy for this population.

The phylogenetic analyses of HIV-1 *p17* and *C2V3* segments showed that 3 major subtypes including subtype B (6.1%), CRF01_AE (72.7%), and CRF07_BC (21.2%) were circulating among MSM in Zhenjiang. Our results were similar to those in the surrounding provinces (e.g., Shanghai, Anhui, and Shandong) (16–18), in which CRF01_AE appeared to be the most predominant HIV-1 genotype. However, the subtype distribution of HIV-1 among MSM in Zhenjiang was significantly different from that reported by Guo et al. (12) in Jiangsu. They reported that 3 major subtypes including subtype B, CRF01, and B/C inter-subtype recombinants (especially CRF07) were circulating among MSM in Nanjing and Yangzhou, Jiangsu. While it is reported (19) that CRF01_AE was the most predominant strain circulating in the sexual transmission group (including heterosexuals and homosexuals), CRF07_BC and CRF08_BC were the major circulating subtypes among IDU in China. Guo et al. recruited MSM from 2 bathhouses in Nanjing and Yangzhou, Jiangsu and surmised that the emergence of a high proportion of B/C recombinants (especially CRF07) among MSM was possibly introduced through IDU by phylogenetic analysis (12). In our survey, most participants were recruited from gay bars. We found that they preferred to use recreational drugs rather than injected drugs to feel relaxed and pleasant, and this may lead to multiple sexual encounters and unprotected sex, thus providing an interpretation for why CRF01_AE (72.7%) was the most predominant HIV-1 genotype among MSM in Zhenjiang. Furthermore, these different results also enabled us to estimate the HIV-1 genotype distribution by using multiple fragments (*p17*, *pol*, *vif-env*, and *C2V3*). As HIV-1 recombination often

Table 3. Chi-square test on socio-demographic characterization and risky behaviors of participants

Variable	STI group <i>n</i> = 146 (%)	Control group <i>n</i> = 511 (%)	Total <i>n</i> = 657 (%)	<i>P</i> value
Age (yr)				< 0.001
18–30	82 (56.2)	374 (73.2)	456 (69.4)	
> 30	64 (43.8)	137 (26.8)	201 (30.6)	
Marital status				0.004
Unmarried	84 (57.5)	364 (71.2)	448 (68.2)	
Married	50 (34.2)	126 (24.7)	176 (26.8)	
Divorced/widow	12 (8.2)	21 (4.1)	33 (5.0)	
Education level				0.070
Junior and senior high school	83 (56.8)	247 (48.3)	330 (50.2)	
Above high school	63 (43.2)	264 (51.7)	327 (49.8)	
Sexual behaviors				
Have sex with male partner in the past 6 months				0.812
Yes	126 (86.3)	437 (85.5)	563 (85.7)	
No	20 (13.7)	74 (14.5)	94 (14.3)	
Condom use for sex with male partner				0.009
Never and inconsistent	56 (44.4)	139 (31.8)	195 (34.6)	
Consistent	70 (55.6)	298 (68.2)	368 (65.4)	
Have Sex with commercial male partner in the past 6 months				0.039
Yes	11 (8.7)	18 (4.1)	29 (5.2)	
No	115 (91.3)	419 (95.9)	534 (94.8)	
Condom use for sex with commercial male partner				0.523
Never and inconsistent	3 (27.3)	7 (38.9)	10 (34.5)	
Consistent	8 (72.7)	11 (61.1)	19 (65.5)	
Have sex with female partner in the past 6 months				0.300
Yes	44 (30.1)	132 (25.8)	176 (26.8)	
No	102 (69.9)	379 (74.2)	481 (73.2)	
Condom use for sex with female partner				0.725
Never and inconsistent	26 (59.1)	74 (56.1)	100 (56.8)	
Consistent	18 (40.9)	58 (43.9)	76 (43.2)	
Drug use				0.063
Yes	2 (1.4)	1 (0.2)	3 (0.5)	
No	144 (98.6)	510 (99.8)	654 (99.5)	
Had STI symptoms in the past year				< 0.001
Yes	11 (7.5)	3 (0.6)	14 (2.1)	
No	135 (92.5)	508 (99.4)	643 (97.9)	

STI group, infected with HIV/HCV/syphilis; Control group, HIV/HCV/syphilis were not infected.

Table 4. Multivariate logistic regression analysis of explanatory variables against STIs

Variable	OR (95% CI)	<i>P</i> value
Age (yr)	2.002 (1.144–3.502)	0.015
Marital status	1.044 (0.665–1.641)	0.851
Condom use for sex with male partner	0.621 (0.408–0.945)	0.026
Have sex with commercial male partner in the past 6 months	1.838 (0.817–4.133)	0.141
Had STI symptoms in the past year	11.673 (3.092–44.078)	< 0.001

occurs in the *pol* and *vif-env* regions (9), some recombinants in Zhenjiang might not be found by the analyses of *p17* and *C2V3*. Interestingly, we found 1 sample (12-ZJ-532) with inconsistent results for *p17* and *C2V3*. The phylogenetic analyses of *p17* and *C2V3* sequences showed that those of these samples belonged to CRF01_AE and CRF07_BC, respectively. Wei et al. (20) reported a near full-length genome of a novel HIV-1 recombinant between CRF07_BC and CRF01_

AE in MSM from Sichuan. We surmised that 12-ZJ-532 might represent a co-infection or unique inter-subtype recombinant of 2 different CRF types. In future, we will amplify the near full-length genome and sequence this sample to estimate the HIV-1 genotype.

Our study findings also reinforce the fact that age > 30 years and having had STI symptoms in the past year were positively correlated with having an STI. These findings are consistent with those of other studies regarding the risk factors for STI (21,22). Li et al. (23) indicated that unprotected anal sex is common among older Chinese MSM and further puts them at high risk of acquiring and transmitting HIV. Co-infection could facilitate both disease processes, which could facilitate the transmission and acquisition of other STIs, including HIV (6).

It is obvious that consistent use of a condom when having sex with male partners was significantly associated with a low STI rate. This may be because condom use during sexual intercourse could reduce the probability of transmitting the virus through anal mucosal trauma (24). Interestingly, we found there was no sig-

nificant difference in condom use during sex with commercial male partners ($P \geq 0.05$). One possible explanation was that compared with regular male partners, casual sexual partners may represent greater danger, resulting in more frequent condom use.

In our survey, we found there was no statistically significant difference in educational level ($P \geq 0.05$) and drug use ($P \geq 0.05$). Some reports showed that less education was potentially associated with HIV infection (25). Other reports said that more HIV-infected MSM had a higher educational level (22). We also discovered that more college students in our city were engaging in risky sexual behavior with men in recent years. Most had basic knowledge of HIV/AIDS. However, some students may not use condoms when having sex with their partners in order to show loyalty. IDU was unusual in our survey, but some reports (26,27) showed that recreational drug use is increasingly popular among Chinese MSM and may be associated with sexually risky lifestyles. The relationship between recreational drug use and HIV infection is currently the subject of research in our city.

Our study had some limitations. First, selection bias may exist, due to the sampling method, which might limit generalization of this study. Second, participants may not have provided honest responses to sensitive questions. This may be ameliorated through the use of MSM volunteers to establish good relationships between data collectors and participants. We can then conduct additional research about the details of risky behaviors (such as selling or buying commercial sex, drug use, and number of sex partners).

In summary, the high prevalence of STIs (HIV, HCV, and syphilis) and co-infections, and the presence of complex HIV-1 subtypes circulating among MSM in Zhenjiang present a serious challenge for HIV prevention. The study also showed that being older and having had STI symptoms in the past year were positively correlated with having an STI. Effective intervention strategies should urgently be developed. In addition, a large-scale longitudinal study should be planned for further investigation.

Acknowledgments This work was supported by a grant from the Health Science and Technology Foundation of Zhenjiang (No. SH2013096).

Conflict of interest None to declare.

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