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Title Page

Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2015 in Nanjing, China: A consecutive cross sectional survey

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Abstract:

Objective: To examine the trends of HIV prevalence, risk behaviors and HIV testing among MSM in Nanjing, and to analyze the risk factors associated HIV infection.

Design: Three consecutive cross sectional surveys

Setting: MSM were recruited through snow ball sampling method and data were collected by interview surveys from 2013 to 2015 in Nanjing, Jiangsu province. Blood samples were collected to perform HIV and syphilis tests.

Results: 649, 669, 577 MSM were recruited in consecutive years. HIV prevalence among MSM were 9.9% (95%CI: 7.5-12.1) in 2013, 12.3% (95%CI: 9.8-14.7) in 2014 and 12.5% (95%CI: 9.7-15.2) in 2015, the change in trend was not significant (P=0.145). Prevalence of anal sex, unprotected anal intercourse (UAI) and having multiple sex partners in the past 6 months significantly decreased (P<0.05). Tested for HIV in the previous year remained nearly 60.0%, the changing trend was not significant (P=0.235). Rush poppers use rose from 12.9% in 2014 to 20.8% in 2015. Multivariable analyses indicated that the factors associated with HIV infection were had UAI in the past 6 months (aOR=1.764, 95%CI: 1.308~2.378), having multiple partners in the past 6 months (aOR=1.353, 95%CI: 1.001~1.829), sex role as receptive (aOR=1.774, 95%CI: 1.199~2.623) and both insertive and receptive intercourse sex (aOR=1.867, 95%CI: 1.305~2.671) and currently have syphilis infection (aOR=2.293, 95%CI: 1.487~3.537).

Conclusions: We observed decreased risk behaviors, increase HIV testing, and stable HIV prevalence. However, new challenges were identified like increasing rush poppers use. The HIV intervention for MSM must include an expansion of HIV testing, spouse intervention, drug use reduction and scale-up of ART services.

Strengths and limitations of this study

- The present study is designed to assess the HIV prevalence trend among Nanjing MSM through consecutive three cross-sectional surveys; in addition, we examined the risk factors associated with HIV infection using the pooled three rounds data.
- We firstly report that rush poppers use rate among Nanjing MSM had a rising trend.
- Convenience sampling may not be considered as representative sample of the broader MSM population, these data should be interpreted with caution.
- It is possible that face to face investigation may under evaluated risk behaviors due to the issue of social desirability.
- Our cross sectional studies are inherently observational and descriptive; thus, we cannot infer causality.

Introduction

In recent years, a fast-spreading HIV epidemic among men who have sex with men (MSM) presented a new challenge world-wide [1, 2]. In China, MSM transmission has surpassed both injection drug use and blood donors and has become the major HIV transmission route[3,4]. The overall HIV prevalence from national MSM sentinel surveillance data demonstrated a rising trend from 0.9% in 2003 to 6.3% in 2011[5]. The reported prevalence among MSM ranged from an estimated 3.5% (north-west and south-central China) to 21.21% (south-west China) [6, 7, 8].

Nanjing is the capital city of Jiangsu Province, eastern China, with a total population of 8.6 million. Consistent with many other metropolitan cities of China, MSM transmission has become the major route of HIV transmission [9]. According to the national web-based HIV/AIDS Case Reporting and Management System, , the annual proportion of newly reported HIV cases attributed to MSM transmitted infection rose from 44.3% in 2008 to 63.5% in2012 [9] and to 71.3% in 2015 in Nanjing. Consecutive surveys conducted in an STD clinic site and showed that the prevalence rates of HIV infection among MSM in Nanjing dramatically increased from 1.29% in 2008 to 10.43% in 2013[10].

In response to the escalating HIV epidemic among MSM, Nanjing implemented comprehensive prevention strategies over the decade. Condom and lubricant promotion and distribution were implemented in MSM sites. Over 40 voluntary HIV testing and counseling (VCT) sites were established across the city to provide friendly HIV testing service [11]. From 2008 to 2012, The China-Bill Melinda Gates Foundation on HIV/AIDS program and the Global Fund to Fight AIDS, Tuberculosis and Malaria HIV program in China were implemented in Nanjing to strengthen local HIV/AIDS prevention effort. A growing number of MSM Community Based Organizations (CBOs) provided peer-led HIV rapid testing and referrals [12-14]. In 2013, inspired by the milestone strategy of “treatment as prevention” [15, 16], the Chinese national center for AIDS/STIs control and prevention launched Expanding HIV Testing and Scale-up Antiretroviral Therapy (ART) Program, to reduce HIV new infection among the MSM population in eight cities, including Nanjing. The program centered on expending health education HIV testing services, CBOs-centered behaviors intervention and HIV rapid test. Moreover, the program supported one-stop treatment service [17, 18] at the designate hospital to identify and treat all HIV positive MSM as soon as possible. Under the implementation of the program, There were 7 rapid HIV testing sites set up at MSM gathering places like bars, saunas and CBOs offices. The total number of HIV rapid tests was over 9000 every year, which is close to half of the estimated MSM population in Nanjing [19].The ART coverage rate among new reported MSM infections rose from 53.8% in 2013 to 68.8% in 2015. Moreover, the treatment surveillance system showed that the viral suppression rate among treated MSM was 95.1% in 2015. (Data from the AIDS comprehensive prevention and control information system, unpublished before)

However, it is unknown whether these strategies reduced HIV epidemic among MSM in Nanjing, and to what extent these risk behaviors were changed. Therefore, we designed and conducted three consecutive cross-sectional surveys from 2013 to 2015, aimed to identify the trends of HIV prevalence, risk behaviors and HIV testing among MSM. We also analyzed the risk factors associated with HIV infection.

Methods:

Participants

Three consecutive cross sectional surveys were conducted in Nanjing from 2013 to 2015. Participants were those who met the following criteria: 18 years of age or older, male, had oral or anal sex with a male in the previous one year, could provide written informed consent and were willing to complete questionnaires. This study was approved by the Ethics Committee of the National Center for AIDS and STD Control, China CDC.

Study procedures

MSM were recruited through the internet websites catering to MSM or recommended by local MSM CBOs. We designated two VCT clinics as survey sites in Nanjing, one at Nanjing municipal CDC and the other at Qinhuai district CDC. All interviewers and counselors were CDC staff members who were well trained. We looked for duplicate the reported telephone numbers at the end of each survey, and excluded the repeated participants. The survey sites, main interviewers, and recruit methods were consistent during the three years surveys.

After the qualification screening and consent (on internet?), participants were invited for a face to face interview to complete a questionnaire. The survey questionnaire questions on demographic characteristics, including age, marital status, ethnic identity, educational levels and sexual orientation, knowledge about HIV transmission and prevention, sexual behaviors in the past 6 months (with males and females), illegal drugs use, STIs diagnosis, and previous HIV testing history. No name or identifying information was collected. In this study, UAI is used to indicate unprotected anal intercourse with a male and UVI unprotected vagina intercourse with a female. We defined those who reported two and more sexual partners in the past 6 months as “having multiple sexual partners”. The question “Have you used rush poppers in the past 6 months” was not included in the 2013 survey.

Laboratory testing

5 ml of whole blood was collected for HIV and syphilis testing. Samples were screened for HIV-1 antibody with a rapid test (Determine HIV1/2, Alere Medical Co., Ltd. Chiba Prefecture, Japan). Positive samples were retested by an enzyme-linked immunoassay (HIV Ag/Ab ELISA KIT 96T, Zhuhai Livzon Diagnostics Ins, China), ELISA positive cases were confirmed with a Western blot assay (HIV BLOT 2.2, MP, Singapore 627885). Pre and post HIV testing counseling was provided by CDC staff. Those with confirmed HIV were subsequently referred to the Nanjing secondary hospital, the designated ART center for treatment. Syphilis screening was performed by treponema pallidum particle assay (TPPA) (Alere Medical Co, Ltd, Japan), and confirmed by the rapid plasma regain test (RPR) (Diagnosis; Shanghai, Kehua, China). Both TPPA and RPR positive participants were determined to be currently syphilis infected and were referred to standardized STD clinics for Treatment.

Statistical analysis

Data were double entered and checked for accuracy using Epi Data software (Version 3.0: Epi Data Association, Odense, Denmark). HIV and syphilis prevalence was calculated by dividing the sum of all confirmed cases by the total number of subjects who participated each year. Confidence intervals (CIs) of prevalence were calculated by approximating the binomial distribution with a normal distribution. Descriptive statistics were used to describe participants demographic and prevalence data. Trend tests were performed using χ^2 tests with linear-by-linear association. Bivariate logistic regression and Multivariate logistic regression were conducted to adjust ORs for potential confounding. Only variables that were significant in bivariate analyses at P<0.1 were included in the multivariate logistic regression models. *P* value <0.05 (two tailed) was considered to be statistically significant. All statistical analyses were conducted using SPSS software (version 20; IBM, Armonk, NY, USA).

Results

Trends in demographic characteristics among MSM

Trends in the demographic characteristics of the participants in the three surveys are presented in table 1. The number of qualified MSM was 649, 669 and 577 in each subsequent survey. The composition of the samples and their corresponding characteristics were comparable in the three surveys. The majority of participants were 20~49 years old, single, Han ethnic, Jiangsu residents and highly educated. Over 90% of participants were either homosexual or bisexual. Significantly more MSM were single (rising from 73.3% to 81.3%) and highly educated (the proportion of college and higher level graduates rising from 57.6% to 67.8%).

Trends in HIV prevalence, risk behaviors and HIV testing among MSM

The trend of HIV prevalence is presented in table 2. According to the serological testing results, the HIV prevalence among MSM was 9.9% (95%CI: 7.5-12.1) in 2013, 12.3% (95%CI: 9.8-14.7) in 2014 and 12.5% (95%CI: 9.7-15.2) in 2015 respectively, the difference was not significant (P=0.196). Table 2 also described the trends of risk sexual behaviors, STD diagnosis and HIV testing rates. Over the three years, the rates of having had anal sex, had UAI and had multiple sexual partners in the past 6 months all showed a decreasing trend (all P trend <0.05). The trends of other behaviors including had commercial sex, had sex with a female and had UVI in the past 6 months, as well as the STD diagnosis rate and HIV testing rate in the past 12 months were not significant (all P trend>0.05). However, the rate of rush poppers use in the last 6 months increased significantly from 12.9% in 2014 to 20.8% in 2015 (P =0.000).

Risk factors associated with HIV infection

We used the pooled three years' surveys data to analysis the risk factors correlated with HIV infection. A total of 1895 participants were analyzed. Table 3 presentsthe results of bivariate and multivariate logistic analyses of variables in relation to HIV infection among participating MSM. Factors with p-values less then 0.10 in bivariate analysis were included in the multivariate model, including sex role, had anal sex, had UAI, had multiple sex partners, had injected drugs in the past 6 months and currently with syphilis infected. Because the variables of "had anal sex" and "had UAI" in the past 6 months were correlated each other (r=0.400, p=0.000), "had anal sex" was not included in multivariate logistic analysis. In the final multivariate model, factors independently associated with HIV infection were sex role as receptive (aOR=1.774, 95%CI: 1.199~2.623) and dual role (aOR=1.867, 95%CI: 1.305~2.671), had UAI in the past 6 months (aOR=1.764, 95%CI: 1.308~2.378), had multiple partners in the past 6 months (aOR=1.353, 95%CI: 1.001~1.829) and currently syphilis infected (aOR=2.293, 95%CI: 1.487~3.537)

Discussion

Through the consecutive cross sectional surveys, we found that the HIV prevalence among MSM in Nanjing had level off at a high level from 2013 to 2015. This trend is different from the period of 2008 to 2012 when a rapid increase from 6.6% to 10.1% was reported [20, 21]. Decreased trends of risk sexual behaviors including had anal sex, UAI and multiple sexual partners were declined by the survey years. Compared to other cities, HIV prevalence in Nanjing was much lower than Chongqing (21.21% in 2014) [8] and Kunming (17.0% in 2014) [22]. The trend was concurrent with the implementation of comprehensive prevention strategies including "treat as prevention", targeting MSM in Nanjing. Despite the decline in the HIV epidemic among MSM, the rate remains high and we haven't seen a turning point. Persistent interventions, linkage to care and quality of treatment service provision should be strengthened to push the documented trend further.

We examined the risk factors associated with HIV infection using the pooled three rounds data. Consistent with other reports [8, 23-25]; UAI, multiple sexual partners, anal sex role as receptive or dual, and current syphilis infection were all associated with HIV infection. Although statistically the trends of UAI and multiple sexual partners were declining, the rates of UAI (43%) and multiple sexual partners (46%) were still very high, and there was no decreasing trend in syphilis infection, which indicated that MSM were still at high risk for HIV infection via anal sex. We also noticed that about one third of MSM reported that they were bisexual. Pooled data showed that over 20% of participants (405/1895) had sex with female in the past 6 months and only one third of these (146/405) consistently use condoms. As HIV prevalence among MSM is much higher than in the general population, the high rate of having sex with female and low condom use spread HIV transmission from MSM to the general population [26, 27]. So strategies including pre-marital HIV testing, spouse testing and other interventions targeting bisexual MSM and their partners should be strengthened.

We found that 60% of participants reported being tested for HIV in the previous year, higher than the testing rate reported in Chongqing (43.5% in 2014) [8,28], Beijing (42.6% in2016) [29], and Guangzhou (53.4% in 2013) [30]. It was also higher than the national annual HIV testing rate (50.4% in 2011) [31]. The disclosure of HIV positive status can have individual health benefits and potentially reduce community spread of HIV through early diagnosis and early initiation of ART [32]. HIV testing rate was enhanced from only one in five in 2008 to nearly half in 2012 [33], and then to almost two thirds currently with collaboration of MSM NGOs. However, HIV testing rates for Nanjing are lower than the Australia (60-70% in 2013) [34, 35] and the USA (67% in 2011)[36]. We need to expand and increase the uptake of HIV testing, especially for concealed MSM.

It is alarming that participant's rush poppers use rate in the past 6 months rose from 12.6% in 2014 to 20.8% in 2015, which was similar to that reported for Beijing (26.8%)[37] and Shenyang (26.3%) [38]. Rush poppers belong to the amyl, alkyl or butyl nitrites which are prescribed to relieve angina pectoris. It has been used more and more among MSM to facilitate anal intercourse, due to the effect of relaxing the anal sphincter and dilating capillaries [39]. Although in this study we have not observed a correlation between HIV infection and rush poppers use, we found an association in a study reported previously [40]. Prior Research had documented that rush poppers use may increase HIV transmission through their engagement in high-risk sexual behaviors like group sex, multiple sex partners and UAI [41, 42]. Because there is an increasing trend of rush poppers use among Nanjing MSM, risk behaviors interventions to reduce their use as a recreational drug should be implemented..

There are several limitations in this study. First, convenience sampling of MSM websites and NGOs may not be considered as representative sample of the broader MSM population; these data should be interpreted with caution.

Second, it is possible that face to face investigation may have falsely elevated the levels of under reported risk behaviors due to the issue of social desirability. The study was however, anonymous; fake names and study serial number were used to match the records. Third, our cross sectional studies are inherently observational and descriptive; thus, we cannot infer causality. Last, for monitoring HIV prevalence and risk behaviors trend, three years is short, thus more consecutive surveillance efforts are needed.

However, our three consecutive surveys were carefully designed, implemented, and monitored. Commensurate the implementation of comprehensive interventions including expanding HIV testing and "treatment as prevention", there was decreasing risk behaviors, higher HIV testing rate and stable HIV prevalence. However, some new challenges like increasing rush poppers use were observed. In response to the high HIV prevalence and disease burden among MSM, the HIV intervention must be tailored to increase HIV testing, spouse intervention, drug use reduction, improved diagnosis and treatment of STDs and scale-up of ART service for MSM.

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Contributors

SS Wu, YY Xu, L Liu, X Li were contributed to data collection; WJ Xu contributed to laboratory testing, F Xu contributed to the study design and quality control, ZP Zhu responsible for data analysis and manuscript writing, HJ Yan and R Detels were contributed to manuscript revision.

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Conflicts

The authors declare that they have no competing interests.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Ethics approval

This study was approved by the Ethics Committee of the National Center for AIDS and STD Control, China CDC. Written informed consent was obtained from each participant prior to the survey.

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Table 1 Demographic characteristic among MSM in Nanjing, 2013 to 2015

Variable	2013 N=649	2014 N=669	2015 N=577	*Liner by liner* χ^2	P trend
Age group				0.591	0.442
<20	6.0(39)	4.3(29)	5.4(31)		
20～49	86.0(558)	84.9(568)	89.1(514)		
⩾50	8.0(52)	10.8(72)	5.5(32)		
marital status				10.322	0.001
single, divorced or widowed	73.3(476)	74.6(499)	81.3(469)		
married or live together	26.7(173)	25.4(170)	18.7(108)		
Han ethnic	97.4(632)	98.7(660)	97.9(565)	0.526	0.468
Residency (hukou) in Jiangsu	68.4(444)	71.4(478)	66.7(385)	0.333	0.564
Education				16.244	0.000
junior high school and lower	18.0(117)	13.2(88)	11.4(66)		
senior high school or skill school	24.3(158)	22.1(148)	20.8(120)		
college and higher	57.6(374)	64.7(433)	67.8(391)		
Sex Orientation				0.960	0.327
homosexual	62.4(405)	60.4(404)	60.8(351)		
heterosexual	2.6(17)	1.8(12)	2.1(12)		
Bisexual/ not sure	34.9(227)	37.8(253)	37.1(214)		

Table 2 Trends in HIV prevalence, risk behaviors and HIV testing among MSM in Nanjing, 2013 to 2015

Variable	2013 N=649	2014 N=669	2015 N=577	*Liner by liner* χ^2	*P* trend
HIV prevalence	9.9(64)	12.3 (82)	12.5 (72)	2.128	0.145
Current syphilis infection	6.2(40)	10.6(71)	5.9(34)	0.003	0.958
Anal sex, last 6 months	86.1(559)	81.0(542)	76.4(441)	19.027	0.000
UAI, last 6 months	50.7(329)	42.5(284)	43.0(248)	7.684	0.006
⩾2 sex partners, last 6 months	55.6(361)	49.6(332)	46.3(267)	10.817	0.001
commercial sex, last 6 months	2.8(18)	3.6(24)	3.1(18)	0.138	0.710
female sex, last 6 months	22.2(144)	22.4(150)	19.2(111)	1.508	0.219
UVI, last 6 months	14.0(91)	15.1(101)	11.6(67)	1.388	0.239
used rush poppers, last 6 months	-	12.9(86)	20.8(120)	14.150	0.000
Ever injected drugs	2.0(13)	0.9(6)	1.7(10)	0.195	0.658
Self reported STI, last 12 months	6.9(45)	6.6(44)	4.7(27)	2.627	0.105
tested for HIV, last 12 months	60.6(393)	64.1(429)	57.0(329)	1.407	0.235

Table 3 Bivariate and multivariate logistic analysis of HIV infection among MSM in Nanjing

Various	HIV infection	Bivariate analysis		Multivariate analysis	
	% (n)	P	OR(95% CI)	P	OR(95% CI)
age group					
<20	6.1(6)		1.000		
20～49	12.0(196)	0.167	1.277(0.903-1.805)		
≧50	10.3(16)	0.146	1.303(0.912-1.863)		
Residency (hukou)					
Jiangsu	11.7(153)		1.000		
Others	11.1(65)	0.681	0.937(0.689,1.276)		
education					
Junior middle school and lower	15.1(41)		1.000		
Senior middle school/skill school	12.0(51)	0.231	0.763(0.490-1.188)		
college and higher	10.5(126)	0.032	0.659(0.451-0.964)		
sex role					
insertive	7.9(51)		1.000		1.000
receptive	13.3(65)	0.003	1.798(1.220-2.647)	0.004	1.774(1.199-2.623)
dual	13.5(102)	0.001	1.826(1.282-2.601)	0.001	1.867(1.305-2.671)
Anal sex, last 6 months					
No	7.9(28)		1.000		
Yes	12.3(190)	0.021	1.631(1.077-2.470)		
sex partners,last 6 months					
0 or 1	9.1(85)		1.000		1.000
≥2	13.9(133)	0.001	1.608(1.205-2.146)	0.049	1.353(1.001-1.829)
UAI					
No	8.5(88)		1.000		1.000
Yes	15.1(130)	0.000	1.912(1.434-2.548)	0.000	1.764(1.308-2.378)
Commercial sex, last 6 months					
No	11.4(210)		1.000		
Yes	13.3(8)	0.652	1.190(0.558-2.541)		
female sex, last 6 months					
No	11.9(177)		1.000		
Yes	10.1(41)	0.327	0.836(0.584-1.196)		
UVI, last 6 months					
No	11.8(193)		1.000		
Yes	9.7(25)	0.316	0.799(0.515-1.239)		
Ever used “rush poppers”					
No	11.7(122)		1.000		
Yes	15.5(32)	0.131	1.384(0.908-2.110)		
Ever injected drugs					
No	11.3(211)		1.000		
Yes	24.1(7)	0.038	2.496(1.053-5.913)		
Self reported STI, last 12 months					
No	11.3(201)		1.000		
Yes	14.7(17)	0.274	1.348(0.789-2.302)		
Current syphilis infection					

No	10.7(187)		1.000	1.000
Yes	21.4(31)	0.000	2.273(1.486-3.477)	0.000 2.293(1.487-3.537)
tested for HIV, last 12 months				
No	12.6(94)		1.000	
Yes	10.8(124)	0.215	0.835(0.628-1.111)	

Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2015 in Nanjing, China: A consecutive cross sectional survey

The STROBE-Vet statement checklist.

	Item	*STROBE-Vet recommendation*	*Page #*
Title and Abstract	1	(a) Indicate that the study was an observational study and, if applicable, use a common study design term	2
		(b) Indicate why the study was conducted, the design, the results, the limitations, and the relevance of the findings	2
Background / rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	(a) State specific objectives, including any primary or secondary prespecified hypotheses or their absence	3
Study design	4	Present key elements of study design early in the paper	4
Setting	5	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Variables	7	(a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. If applicable, give diagnostic criteria	4
Data sources / measurement	8*	(a) For each variable of interest, give sources of data and details of methods of assessment (measurement). If applicable, describe comparability of assessment methods among groups and over time	6
Bias	9	Describe any efforts to address potential sources of bias due to confounding, selection, or information bias	7-8
Study size	10	(a) Describe how the study size was arrived at for each relevant level of organization	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	4
Statistical methods	12	(a) Describe all statistical methods for each objective, at a level of detail sufficient for a knowledgeable reader to replicate the methods. Include a description of the approaches to variable selection, control of confounding, and methods used to control for non-	4

		independence of observations	
Descriptive data on exposures and potential confounders	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders by group and level of organization, if applicable	4
Outcome data	15*	(a) Report outcomes as appropriate for the study design and summarize at all relevant levels of organization	6
		(b) For proportions and rates, report the numerator and denominator	6
		(c) For continuous outcomes, report the number of observations and a measure of variability	
Main results	16	(a) Give unadjusted estimates and, if applicable, adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders and interactions were adjusted. Report all relevant parameters that were part of the model	6,12-15
Key results	18	Summarize key results with reference to study objectives	7
Strengths and Limitations	19	Discuss strengths and limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
Generalizability	21	Discuss the generalizability (external validity) of the study results	8
~~Funding~~ Transparency	22	(a) Funding- Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (b) Conflicts of interest-Describe any conflicts of interest, or lack thereof, for each author (c) Describe the authors' roles- Provision of an authors' declaration of transparency is recommended (d) Ethical approval- Include information on ethical approval for use of animal and human subjects (e) Quality standards-Describe any quality standards used in the conduct of the research	8

[a] Level of organization recognizes that observational studies in veterinary research often deal with repeated measures (within an animal or herd) or animals that are maintained in groups (such as pens and herds); thus, the observations are not statistically independent. This non-independence has profound implications for the design, analysis, and results of these studies.
[b] The word "participant" is used in the STROBE statement. However, for the veterinary version, it is understood that "participant" should be addressed for both the animal owner/manager and for the animals themselves.
*Give such information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey

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Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey

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Key words: HIV, MSM, Prevalence, Risk behaviors

Title and Abstract Page

Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey

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Abstract:

Objective: To examine the trends of HIV prevalence, risk behaviors and HIV testing among MSM in Nanjing.

Design: Five consecutive cross sectional surveys.

Setting: Nanjing, China

Primary and secondary outcome measures: HIV and syphilis prevalence, HIV testing rate, and factors associated with HIV infection; demographic characteristics and behaviors

Results: 649, 669, 577, 633, 503 MSM were recruited from 2013 to 2017. HIV prevalence was 9.9%, 12.3%, 12.5%, 9.8% and 10.1%, respectively. Syphilis prevalence decreased with a range from 10.6% to 5.6%. risk behaviors like unprotected anal intercourse (UAI) and unprotected virginal sex (UVI) in the past 6 months decreased, but multiple sex partners and ever used rush popper rose significantly. MSM tested for HIV in the previous remained stable from 57.0% to 64.1%, t (P=0.633). Multivariate analysis showed that tested for HIV in the past year was protective against HIV infection. MSM who had UAI in the past 6 months, sex role as receptive and dual, diagnosed with STDs in the past year and currently syphilis infected were risk factors for HIV infection.

Conclusions: We observed stable high HIV prevalence, a steady HIV testing rate, decreasing syphilis prevalence and UAI among MSM in Nanjing. However, rush popper use rose dramatically. The HIV preventive strategies for MSM including condom promotion, HIV testing expansion, and reduction of rush popper use, STDs screening and standardized treatment should be strengthened.

Strengths and limitations of this study

- The present study is designed to assess the recent HIV prevalence, risk behaviors and HIV testing trends among MSM in Nanjing.
- We analyzed and confirmed several factors associated with HIV infection using pooled five years' data.
- Rush popper use among Nanjing MSM had a rising trend.
- Snow ball sampling and internet convenience sampling may not yield a representative sample of the general MSM population; these data should be interpreted with caution.
- Face to face investigation may under evaluated risk behaviors due to the issue of social desirability.

Introduction

In recent years, a fast-spreading HIV epidemic among men who have sex with men (MSM) presented a new challenge world-wide [1, 2]. In China, MSM transmission has surpassed both injection drug use and blood donors and has become the major HIV transmission route [3, 4]. The overall HIV prevalence, from national MSM sentinel surveillance data, demonstrated a rising trend from 0.9% in 2003 to 6.3% in 2011 [5].

Nanjing is the capital city of Jiangsu Province, eastern China, with a total population of 8.6 million. Consistent with many other metropolitan cities of China, homosexual transmission has become the major route of HIV transmission in Nanjing [6]. According to the national direct network HIV/AIDS Case Reporting System, the annual proportion of newly reported HIV cases attributed to homosexual transmission rose from 44.3% in 2008 to 63.5% in 2012 [7] and to 71.3% in 2015 in Nanjing. Recent studies have highlighted a higher prevalence of club drugs use among MSM [8], and rush popper (inhalant nitrites) is the most popular. Research has documented that rush popper use may increase HIV transmission by increasing engagement in high risk sex behaviors among MSM [9].

In response to the escalating HIV epidemic among MSM, Nanjing implemented comprehensive prevention strategies over the past decade. Condom promotion and lubricant distribution were free in MSM popular venues. Almost 40 voluntary HIV testing and counseling sites were established across the city to provide friendly HIV testing service. From 2008 to 2012, under the implementing of China-Bill Melinda Gates Foundation on HIV/AIDS program and the Global Fund to Fight AIDS program in Nanjing, a growing number of MSM Community Based Organizations (CBOs) established and provided peer-led HIV rapid testing and referrals to treatment.

In 2013, the Chinese national center for AIDS/STDs control and prevention launched Expanding HIV Testing and Scale-up Antiretroviral Therapy (ART) Program in eight cities over the country, Nanjing was one of them. The program implemented various measures. On one hand, CDC led health education programs, HIV testing days,

referrals for ART and standardized STDs treatment. On the other hand, CBOs carried out condom and lubricant distribution, behavioral interventions and provision of HIV rapid testing kits. After each test, staff of CDC or CBO provided risk-reduction counseling.

However, it is unknown whether these strategies reduced the HIV prevalence among MSM, and to what extent their risk behaviors were changed. Therefore, we designed and conducted five consecutive cross-sectional surveys from 2013 to 2017, aimed to identify the trends of HIV and syphilis prevalence, risk behaviors, and HIV testing among MSM. We also analyzed the factors correlated with HIV infection using the pooled five years' survey data.

Methods

Participants

Participants met the following criteria: 16 years of age or older, male, had oral or anal sex with a male in the previous one year, could provide written informed consent and were willing to complete the study .

Sampling method and participant recruitment

We employed two methods to recruit participants: snow ball sampling and internet convenience sampling. For snow ball sampling, the participants were recruited from MSM venues such as bars, clubs, saunas, and public restrooms. Initial "seed" participants were recommended by volunteers of CBOs or staffs of MSM bars. Each initial seed was invited to participate in the study and then asked to invite other person with the same inclusion criteria. We also posted study advertisements on some MSM social platforms and the Nanjing CDC official website, inviting MSM to participate in our study. These recruit processes continued from April to July in each year.

Study procedures

We designated two survey sites in Nanjing. They were VCT clinics in the Nanjing municipal CDC and Qinhuai district CDC. All interviewers were CDC staff members who were well trained. We screened for duplicate reported telephone numbers and excluded those repeated participants during a same survey year. The survey sites, main interviewers and recruit methods were consistent during the five years surveys. After the qualification screening, every participant signed a consent form before the formal investigation. Face to face interviews were used to collect information including demographic characteristics, sexual behaviors in the past 6 months (with males and females), drugs use, STD diagnoses, and HIV testing history. No name or identifying information was collected. Five ml of whole blood was collected for HIV and syphilis test. After testing, we offered HIV risk-reduction counseling for each participant. Confirmed HIV cases were subsequently referred to the designated free ART clinic for treatment, and current syphilis cases were referred to standardized

STDs clinics for Treatment.

Laboratory testing

Blood samples were screened for HIV-1 antibody with a rapid test (Determine HIV1/2, Alere Medical Co., Ltd. Chiba Prefecture, Japan). Positive samples were retested by an enzyme-linked immunoassay (HIV Ag/Ab ELISA KIT 96T, Zhuhai Livzon Diagnostics Ins, China), ELISA positive cases were confirmed with a Western blot assay (HIV BLOT 2.2, MP, Singapore 627885). Syphilis screening was performed by treponema pallidum particle assay (TPPA) (Alere Medical Co, Ltd, Japan), and confirmed by the rapid plasma regain test (RPR) (Diagnosis; Shanghai, Kehua, China). TPPA and RPR positive participants were determined to be currently infected.

Variable definitions

"UAI (unprotected anal sex)" was defined as inconsistent use of condoms during anal sex with male partners in the past six months; "UVI ((unprotected vaginal sex)" was defined as inconsistent use of condoms during vaginal sex with female partners in the past six months. We defined "multiple sexual partners" as having had two or more sexual partners in the past 6 months. "Ever used drugs" as ever used heroin or opium.

Statistical analysis

Data were double entered and checked for accuracy using Epi Data software (Version 3.0: Epi Data Association, Odense, Denmark). Descriptive statistics were used to describe participant's demographic characteristics and prevalence rate. Trend tests were performed using χ^2 tests with linear-by-linear association. Univariate and multivariate logistic regression were conducted to adjust ORs for potential confounding. Only variables that were significant in univariate analyses at $P<0.1$ were included in the multivariate logistic regression models. A P value <0.05 (two tailed) was considered to be statistically significant. All statistical analyses were conducted using SPSS software (version 20; IBM, Armonk, NY, USA).

Patient involvement

Patients were not involved in setting the research question, the outcome measures, the design, or the implementation of the study. No patients were asked to advise on interpretation or writing up of results. No patients were advised on dissemination of the present study and its main results.

Results

Demographic and social characteristics

Demographic characteristics of the participants in the five-year surveys are presented in Table 1. The number of self-reported MSM was 649, 669, 577, 633 and 503 respectively. The composition of the samples and their corresponding characteristics were comparable in the three surveys. The majorities of participants were 20~49 years old, single, Han ethnicity, Jiangsu residents and highly educated. Over 90% of participants were either homosexual or bisexual. Significantly more MSM were single and highly educated.

Trends in HIV prevalence, risk behaviors and HIV testing

The trends of HIV prevalence, sexual behaviors, STD diagnosis and HIV testing rates among MSM are presented in Table 2. The HIV prevalence among MSM range from 9.9% to 12.5%, the trend was not significant. There was a decreasing trend of current syphilis prevalence with the range from 10.6% to 5.6%. During the five years' of the study, some risk behaviors like UAI, vaginal sex and UVI in the past 6 month showed decreasing trends. On the other hand, the rate of multiple sexual partners and ever used rush popper indicated significantly increasing trends. The behaviors including anal sex, commercial anal sex, ever used drugs, diagnosed with STDs and tested for HIV showed no significant change.

Factors associated with HIV infection

A total of 3031 participants were included. Table 3 presents the results of unvariable logistic analyses of factors in related to HIV infection among participating MSM. Factors with *p*-values less than 0.10 in univariable analysis were included in the multivariable model. Because the factors of "anal sex" and "UAI" in the past 6 months were correlated with each other (r=0.400, p=0.000), "anal sex" was not included in multivariate logistic analysis. "Ever used rush popper" was also excluded from multivariate logistic analysis for missing data in 2013. Table 4 shows the results of multivariate logistic regression analysis. Participants were more likely to be infected with HIV if they had UAI in the past 6 months, sex role as receptive or dual,

diagnosed with STDs in the previous year and currently were syphilis infected. Participants tested for HIV in the previous year were less likely to be HIV infected compared with those not tested.

Discussion

Over the five annual cross sectional surveys, we found that HIV prevalence among MSM in Nanjing remained steady at a high level from 2013 to 2017. This trend is different from the period of 2008 to 2012 when a rapid increase from 6.6% (RDS sampling, 430 sample size) [10] to 13.7% (snow ball sampling, 670 sample size) [11] were reported. These three surveys were conducted by the same work team and recruited from the same venues. We also observed a 10% decline in UAI among MSM. These outcomes should be cautiously attributed to the comprehensive prevention program implementation, especially the supply of risk-reduction counseling after each test. Compared to other cities, HIV prevalence among MSM in Nanjing was much lower than in the southwestern cities of Chongqing (21.2% in 2014) [12] and Kunming (17.0% in 2014) [13]. It was higher than in Beijing[14] (6.1% in 2011) or other countries (4.4% in India [15] in 2010), 2.5% in the Republic of Cyprus[16] in 2012). However, all of these comparisons should be considered cautiously as there were differences in methodologies used to estimate prevalence. Because HIV continues to spread in Nanjing, interventions should be continued and strengthened.

We found that the HIV testing rate in the previous year remained steady at around 60%, higher than that reported in Chongqing (43.5% in 2014) [12], Beijing (42.6% in 2016) [17], and Guangzhou (53.4% in 2013) [18]. It was also higher than the national annual HIV testing rate (50.4% in 2011) among MSM [19]. HIV testing rate among MSM in Nanjing increased from only one in five in 2008 to nearly half in 2012 [20], and then to almost two thirds by 2017. The disclosure of HIV positive status can have individual health benefits and potentially reduce community spread of HIV through early diagnosis and early initiation of ART [21]. Our multivariate analysis confirmed that being tested for HIV in the previous year was a protective factor. However, there is still a gap in HIV testing rate among MSM between Nanjing and other areas, like Australia (80.5%) [22] and USA (67%) [23]. We need to continue the expanding test program further.

It is alarming that rush popper use among MSM in Nanjing rose dramatically from

12.4% to 21.7%, it just a little bit lower than that reported by Beijing (26.8%) [24] or Shenyang (26.3%) [25]. Rush popper used to be a prescription drug prescribed to relieve angina. Now MSM use it to facilitate sexual intercourse due to its mechanism of relaxing the anal sphincter and dilating capillaries [26]. Univariate analysis indicated that rush popper use was associated with a higher rate of HIV infection, which was consistent with other reports [24, 25, 27]. Two factors may explain this result. First, rush popper has the effects on sexual behaviors such as promoting sexual desire, reducing sexual inhibition and decreasing physical experiences of pain, which may further prevent users from using condoms. Second, prior research had documented that rush popper use may increase HIV transmission through their engagement in group sex and multiple sex partners [28]. Our findings underscore the need for specific intervention programs to reduce rush popper use.

We analyzed the factors associated with HIV infection using the pooled five surveys data. Consistent with other reports [29, 30], our data confirmed that UAI is an important risk factor for HIV infection. Although there was a declining trend of UAI among MSM, slightly more than 40% of participants reported using condoms only intermittently or never. It is necessary to improve awareness of the risk of HIV infection among MSM and the need for consistent condom use during sexual activity. In the present study, anal sex role was found to be independent risk factor for HIV infection men who engaged exclusively or partially in receptive anal sex carry a higher risk of HIV infection, possibly because rectal mucosa is easily damaged during receptive anal sex, thus increasing the likelihood of HIV virus passing into their blood [31]. Therefore, both the “receptive” and “dual” role MSM should probably be considered a priority target for condom promotion.

We observed a declining trend in current syphilis prevalence among MSM during these recent five years. Compared with previous report [11], current syphilis prevalence was lower than that in 2011 (9.1%) and 2012 (11.5%). This phenomenon is consistent with the report that syphilis infection has declined in China [31]. The implementation of the syphilis prevention and control plan by the China’s Ministry of Health may have

contributed to this decline [32]. It is well known that STDs can increase the risk of HIV infection [33]. The positive association observed between syphilis and HIV, can be explained by similar risk behaviors [34]. Thus anyone presenting with syphilis should be tested for HIV and vice versa [35]. Meanwhile, screening and standardizing STDs treatment need to be continued to push this trend further.

Several limitations of our study should be noted. First, our participants were recruited from MSM venues, some MSM social platforms and a government internet site. Thus they may not be representative of MSM who do not go to these venues or visit the website. "Hidden" MSM may carry higher risk behaviors. Second, it is possible that face to face investigation may have underestimated the levels of risk behaviors due to the issue of social desirability. The study was however, anonymous; fake names and study serial number were used to match the records. Third, our cross sectional studies are inherently observational and descriptive; thus, we cannot infer causality.

Overall, our five consecutive surveys were carefully designed, implemented, and quality controlled. Under the implementation of comprehensive interventions, we observed stable HIV prevalence, steady HIV testing rate, decreasing UAI and syphilis prevalence. However, an increasing use of rush popper was observed. In response to the high HIV burden among MSM in Nanjing, HIV prevention and intervention messages must be increased about the urgent need for consistent condom use, targeting especially those MSM who engage in any receptive anal intercourse (although condom use is also important for inserters), HIV testing expanded, rush popper use reduced, STD screening increased and more widespread use of standardized treatment implemented.

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Contributors

SS Wu, YY Xu, L Liu, X Li were contributed to data collection; WJ Xu contributed to laboratory testing, F Xu contributed to the study design and quality control, ZP Zhu responsible for data analysis and manuscript writing, HJ Yan and R Detels were contributed to manuscript revision. All authors read and approved the final manuscript.

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The founders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts

The authors declare that they have no competing interests.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Ethics approval

This study was approved by the Ethics Committee of the National Center for AIDS and STD Control, China CDC. Written informed consent was obtained from each participant prior to the survey.

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Table 1. Demographic characteristics among MSM in Nanjing, 2013 to 2017

Variables	2013	2014	2015	2016	2017	*Liner by liner* χ^2	*P*
Total sample	649	669	577	633	503	-	-
Age group						0.061	0.805
<20	6.0(39)	4.3(29)	5.4(31)	2.0(18)	3.8(20)		
20～49	86.0(558)	84.9(568)	89.1(514)	91.0(557)	89.1(467)		
⩾50	8.0(52)	10.8(72)	5.5(32)	6.0(37)	7.1(37)		
Marital status						30.186	<0.001
single, divorced or widowed	73.3(476)	74.6(499)	81.3(469)	81.5(516)	84.5(425)		
married or live together	26.7(173)	25.4(170)	18.7(108)	18.5(117)	15.5(78)		
Han ethnic	97.4(632)	98.7(660)	97.9(565)	98.6(624)	97.0(488)	0.074	0.785
Residency (hukou) in Jiangsu	68.4(444)	71.4(478)	66.7(385)	69.0(437)	67.6(340)	0.437	0.509
Education						24.910	<0.001
junior high school and lower	18.0(117)	13.2(88)	11.4(66)	11.8(75)	9.5(48)		
senior high school or skill school	24.3(158)	22.1(148)	20.8(120)	18.5(117)	21.9(110)		
college and higher	57.6(374)	64.7(433)	67.8(391)	69.7(441)	68.6(345)		
Sex Orientation						1.303	0.2524
homosexual/ bisexual	94.1(611)	93.6(626)	92.7(535)	94.8(600)	95.4(480)		
heterosexual/ not sure	5.9(38)	6.4(43)	7.3(42)	5.2(33)	4.6(23)		

Table 2. Trends in HIV prevalence, risk behaviors and HIV testing among MSM in Nanjing,02013 to 2017

Variables	2013	2014	2015	2016	2017	*Liner by liner* χ^2	*P* value
	%(n)	%(n)	%(n)	%(n)	%(n)		
HIV infection	9.9(64)	12.3(82)	12.5(72)	9.8(62)	10.1(51)	0.181	0.670
Current syphilis infection	6.2(40)	10.6(71)	5.9(34)	4.7(30)	5.6(28)	5.043	0.025
Had anal sex, last 6 months	86.1(559)	81.0(542)	76.4(441)	83.6(529)	80.7(406)	2.958	0.085
Had UAI, last 6 months	50.7(329)	42.5(284)	43.0(248)	41.2(261)	41.7(210)	9.433	0.002
Multiple sex partners, last 6 months	55.6(361)	49.6(332)	46.3(267)	57.3(363)	62.0(312)	19.194	＜0.001
Had commercial anal sex, last 6 months	2.8(18)	3.6(24)	3.1(18)	5.6(17)	4.4(18)	3.397	0.065
Had vaginal sex, last 6 months	22.2(144)	22.4(150)	19.2(111)	19.9(126)	16.3(82)	7.034	0.008
Had UVI, last 6 months	14.0(91)	15.1(101)	11.6(67)	13.7(87)	9.1(46)	6.791	0.009
Ever used rush popper (*)	-	12.9(86)	20.8(120)	23.1(146)	21.7(109)	17.879	＜0.001
Ever used drugs	2.0(13)	0.9(6)	1.7(10)	0.9(6)	1.2(6)	1.166	0.280
Diagnosed with STD, last 12 months	6.9(45)	6.6(44)	4.7(27)	8.4(53)	7.0(35)	0.333	0.564
Tested for HIV, last 12 months	61.2(397)	64.1(429)	57.0(329)	63.2(400)	63.4(319)	0.228	0.633

*: The data of “ever used rush poppers” haven’t been collected in 2013.

Table 3. Univariate analysis of factors associated with HIV infection among MSM in Nanjing

Factors	HIV infection % (n)	OR (95% CI)	*P* value
Age group			
<20	6.6(9)	1.000	
20～49	11.1(297)	1.785(0.898-3.546)	0.098
≧50	10.9(25)	1.734(0.785-3.834)	0.174
Residency (hukou)			
Jiangsu	11.1(231)	1.000	
Others	10.6(100)	0.947(0.739-1.214)	0.668
Education level			
junior middle school and lower	15.0(59)	1.000	
senior middle school/skill school	12.7(83)	0.827(0.577-1.185)	0.300
college and higher	9.5(189)	0.598(0.436-0.819)	0.001
Sex role			
insertive	7.6(80)	1.000	
receptive	13.6(99)	1.925(1.410-2.628)	0.001
dual	9.5(189)	1.695(1.276-2.252)	0.001
Anal sex, last 6 months			
no	9.0(50)	1.000	
yes	11.3(281)	1.290(0.940-1.769)	0.114
Multiple sex partners, last 6 months			
no	10.9(158)	1.000	
yes	10.9(173)	0.996(0.792-1.251)	0.971
Had UAI, last 6 months			
no	7.9(135)	1.000	
yes	14.7(196)	1.999(1.585-2.521)	0.001
Commercial sex, last 6 months			
no	10.9(319)	1.000	
yes	12.6(12)	1.186(0.640-2.197)	0.587
Had vaginal sex, last 6 months			
no	11.2(272)	1.000	
yes	9.6(59)	0.840(0.625-1.130)	0.250
Had UVI, last 6 months			
no	11.0(272)	1.000	
yes	9.6(59)	0.937(0.663-1.304)	0.712

Table 3 Continued

Factors	HIV infection % (n)	OR (95% CI)	*P* value
Ever used rush popper			
no	10.0(192)	1.000	
yes	16.3(75)	1.750(1.311-2.336)	0.001
Ever used drugs			
no	10.7(320)	1.000	
yes	26.8(11)	3059(1.518-6.164)	0.002
Diagnosed with STD, last 12 months			
no	10.5(296)	1.000	
yes	17.2(35)	1.771(1.207-2.598)	0.003
Current syphilis infection			
no	10.1(285)	1.000	
yes	22.7(46)	2.614(1.841-3.712)	0.001
Tested for HIV, last 12 months			
no	12.9(149)	1.000	
yes	9.7(182)	0.728(0.578-0.916)	0.007

Table 4. Multivarible analysis of factors associated with HIV infection among MSM in Nanjing

Factors	OR(95% CI)	*P* value
Education level		
junior middle school and lower	1.000	
senior middle school/skill school	0.883(0.610-1.278)	0.509
college and higher	0.700(0.503-1.035)	0.501
Sex role		
insertive	1.000	
receptive	1.936(1.409-2.660)	0.001
dual	1.684(1.261-2.249)	0.001
Had UAI, last 6 months		
no	1.000	
yes	2.046(1.558-2.687)	0.001
Ever used drugs		
no	1.000	
yes	1.874(0.997-4.120)	0.053
Diagnosed with STD, last 12 months		
no	1.000	
yes	1.610(1.077-2.407)	0.020
Current syphilis infection		
no	1.000	
yes	2.219(1.531-3.217)	0.001
Tested for HIV, last 12 months		
no	1.000	
yes	0.631(0.437-0.912)	0.014

Footnote: the variables that included in multivariable analysis were below: education level, sex role, had UAI, ever used drugs, diagnosed with STD in last 12 months, current syphilis infection and test for HIV in last 12 month.

Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey The STROBE-Vet statement checklist

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Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey

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Title and Abstract Page

Trends in HIV prevalence and risk behaviors among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey

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Abstract:

Objective: To examine the trends of HIV prevalence, risk behaviors and HIV testing among MSM in Nanjing.

Design: Five consecutive cross sectional surveys.

Setting: Nanjing, China

Primary and secondary outcome measures: HIV and syphilis prevalence, HIV testing rate, and factors associated with HIV infection; demographic characteristics and behaviors

Results: 649, 669, 577, 633, 503 MSM were recruited from 2013 to 2017. HIV prevalence was 9.9%, 12.3%, 12.5%, 9.8% and 10.1%, respectively. Syphilis prevalence decreased with a range from 10.6% to 5.6%. risk behaviors like unprotected anal intercourse (UAI) and unprotected virginal sex (UVI) in the past 6 months decreased, but multiple sex partners and ever used rush popper rose significantly. MSM tested for HIV in the previous remained stable from 57.0% to 64.1%, t (P=0.633). Multivariate analysis showed that tested for HIV in the past year was protective against HIV infection. MSM who had UAI in the past 6 months, sex role as receptive and dual, diagnosed with STDs in the past year and currently syphilis infected were risk factors for HIV infection.

Conclusions: We observed stable high HIV prevalence, a steady HIV testing rate, decreasing syphilis prevalence and UAI among MSM in Nanjing. However, rush popper use rose dramatically. The HIV preventive strategies for MSM including condom promotion, HIV testing expansion, and reduction of rush popper use, STDs screening and standardized treatment should be strengthened.

Strengths and limitations of this study

- The present study is designed to assess the recent HIV prevalence, risk behaviors and HIV testing trends among MSM in Nanjing.
- We analyzed and confirmed several factors associated with HIV infection using pooled five years' data.
- Rush popper use among Nanjing MSM had a rising trend.
- Snow ball sampling and internet convenience sampling may not yield a representative sample of the general MSM population; these data should be interpreted with caution.
- Face to face investigation may under evaluated risk behaviors due to the issue of social desirability.

Introduction

In recent years, a fast-spreading HIV epidemic among men who have sex with men (MSM) presented a new challenge world-wide [1, 2]. In China, MSM transmission has surpassed both injection drug use and blood donors and has become the major HIV transmission route [3, 4]. The overall HIV prevalence, from national MSM sentinel surveillance data, demonstrated a rising trend from 0.9% in 2003 to 6.3% in 2011 [5].

Nanjing is the capital city of Jiangsu Province, eastern China, with a total population of 8.6 million. Consistent with many other metropolitan cities of China, homosexual transmission has become the major route of HIV transmission in Nanjing [6]. According to the national direct network HIV/AIDS Case Reporting System, the annual proportion of newly reported HIV cases attributed to homosexual transmission rose from 44.3% in 2008 to 63.5% in 2012 [7] and to 71.3% in 2015 in Nanjing. Recent studies have highlighted a higher prevalence of club drugs use among MSM [8], and rush popper (inhalant nitrites) is the most popular. Research has documented that rush popper use may increase HIV transmission by increasing engagement in high risk sex behaviors among MSM [9].

In response to the escalating HIV epidemic among MSM, Nanjing implemented comprehensive prevention strategies over the past decade. Condom promotion and lubricant distribution were free in MSM popular venues. Almost 40 voluntary HIV testing and counseling sites were established across the city to provide friendly HIV testing service. From 2008 to 2012, under the implementing of China-Bill Melinda Gates Foundation on HIV/AIDS program and the Global Fund to Fight AIDS program in Nanjing, a growing number of MSM Community Based Organizations (CBOs) established and provided peer-led HIV rapid testing and referrals to treatment.

In 2013, the Chinese national center for AIDS/STDs control and prevention launched Expanding HIV Testing and Scale-up Antiretroviral Therapy (ART) Program in eight cities over the country, Nanjing was one of them. The program implemented various measures. On one hand, CDC led health education programs, HIV testing days,

referrals for ART and standardized STDs treatment. On the other hand, CBOs carried out condom and lubricant distribution, behavioral interventions and provision of HIV rapid testing kits. After each test, staff of CDC or CBO provided risk-reduction counseling.

However, it is unknown whether these strategies reduced the HIV prevalence among MSM, and to what extent their risk behaviors were changed. Therefore, we designed and conducted five consecutive cross-sectional surveys from 2013 to 2017, aimed to identify the trends of HIV and syphilis prevalence, risk behaviors, and HIV testing among MSM. We also analyzed the factors correlated with HIV infection using the pooled five years' survey data.

Methods

Participants

Participants met the following criteria: 16 years of age or older, male, had oral or anal sex with a male in the previous one year, could provide written informed consent and were willing to complete the study .

Sampling method and participant recruitment

We employed two methods to recruit participants: snow ball sampling and internet convenience sampling. For snow ball sampling, the participants were recruited from MSM venues such as bars, clubs, saunas, and public restrooms. Initial "seed" participants were recommended by volunteers of CBOs or staffs of MSM bars. Each initial seed was invited to participate in the study and then asked to invite other person with the same inclusion criteria. We also posted study advertisements on some MSM social platforms and the Nanjing CDC official website, inviting MSM to participate in our study. These recruit processes continued from April to July in each year.

Study procedures

We designated two survey sites in Nanjing. They were VCT clinics in the Nanjing municipal CDC and Qinhuai district CDC. All interviewers were CDC staff members who were well trained. We screened for duplicate reported telephone numbers and excluded those repeated participants during a same survey year. The survey sites, main interviewers and recruit methods were consistent during the five years surveys. After the qualification screening, every participant signed a consent form before the formal investigation. Face to face interviews were used to collect information including demographic characteristics, sexual behaviors in the past 6 months (with males and females), drugs use, STD diagnoses, and HIV testing history. No name or identifying information was collected. Five ml of whole blood was collected for HIV and syphilis test. After testing, we offered HIV risk-reduction counseling for each participant. Confirmed HIV cases were subsequently referred to the designated free ART clinic for treatment, and current syphilis cases were referred to standardized

STDs clinics for Treatment.

Laboratory testing

Blood samples were screened for HIV-1 antibody with a rapid test (Determine HIV1/2, Alere Medical Co., Ltd. Chiba Prefecture, Japan). Positive samples were retested by an enzyme-linked immunoassay (HIV Ag/Ab ELISA KIT 96T, Zhuhai Livzon Diagnostics Ins, China), ELISA positive cases were confirmed with a Western blot assay (HIV BLOT 2.2, MP, Singapore 627885). Syphilis screening was performed by treponema pallidum particle assay (TPPA) (Alere Medical Co, Ltd, Japan), and confirmed by the rapid plasma regain test (RPR) (Diagnosis; Shanghai, Kehua, China). TPPA and RPR positive participants were determined to be currently infected.

Variable definitions

"UAI (unprotected anal sex)" was defined as inconsistent use of condoms during anal sex with male partners in the past six months; "UVI ((unprotected vaginal sex)" was defined as inconsistent use of condoms during vaginal sex with female partners in the past six months. We defined "multiple sexual partners" as having had two or more sexual partners in the past 6 months. "Ever used drugs" as ever used heroin or opium.

Statistical analysis

Data were double entered and checked for accuracy using Epi Data software (Version 3.0: Epi Data Association, Odense, Denmark). Descriptive statistics were used to describe participant's demographic characteristics and prevalence rate. Trend tests were performed using χ^2 tests with linear-by-linear association. Univariate and multivariate logistic regression were conducted to adjust ORs for potential confounding. Only variables that were significant in univariate analyses at $P<0.1$ were included in the multivariate logistic regression models. A P value <0.05 (two tailed) was considered to be statistically significant. All statistical analyses were conducted using SPSS software (version 20; IBM, Armonk, NY, USA).

Patient involvement

Patients were not involved in setting the research question, the outcome measures, the design, or the implementation of the study. No patients were asked to advise on interpretation or writing up of results. No patients were advised on dissemination of the present study and its main results.

Results

Demographic and social characteristics

Demographic characteristics of the participants in the five-year surveys are presented in Table 1. The number of self-reported MSM was 649, 669, 577, 633 and 503 respectively. The composition of the samples and their corresponding characteristics were comparable in the three surveys. The majorities of participants were 20~49 years old, single, Han ethnicity, Jiangsu residents and highly educated. Over 90% of participants were either homosexual or bisexual. Significantly more MSM were single and highly educated.

Trends in HIV prevalence, risk behaviors and HIV testing

The trends of HIV prevalence, sexual behaviors, STD diagnosis and HIV testing rates among MSM are presented in Table 2. The HIV prevalence among MSM range from 9.9% to 12.5%, the trend was not significant. There was a decreasing trend of current syphilis prevalence with the range from 10.6% to 5.6%. During the five years' of the study, some risk behaviors like UAI, vaginal sex and UVI in the past 6 month showed decreasing trends. On the other hand, the rate of multiple sexual partners and ever used rush popper indicated significantly increasing trends. The behaviors including anal sex, commercial anal sex, ever used drugs, diagnosed with STDs and tested for HIV showed no significant change.

Factors associated with HIV infection

A total of 3031 participants were included. Table 3 presents the results of unvariable logistic analyses of factors in related to HIV infection among participating MSM. Factors with *p*-values less than 0.10 in univariable analysis were included in the multivariable model. Because the factors of "anal sex" and "UAI" in the past 6 months were correlated with each other (r=0.400, p=0.000), "anal sex" was not included in multivariate logistic analysis. "Ever used rush popper" was also excluded from multivariate logistic analysis for missing data in 2013. Table 4 shows the results of multivariate logistic regression analysis. Participants were more likely to be infected with HIV if they had UAI in the past 6 months, sex role as receptive or dual,

diagnosed with STDs in the previous year and currently were syphilis infected. Participants tested for HIV in the previous year were less likely to be HIV infected compared with those not tested.

Discussion

Over the five annual cross sectional surveys, we found that HIV prevalence among MSM in Nanjing remained steady at a high level from 2013 to 2017. This trend is different from the period of 2008 to 2012 when a rapid increase from 6.6% (RDS sampling, 430 sample size) [10] to 13.7% (snow ball sampling, 670 sample size) [11] were reported. These three surveys were conducted by the same work team and recruited from the same venues. We also observed a 10% decline in UAI among MSM. These outcomes should be cautiously attributed to the comprehensive prevention program implementation, especially the supply of risk-reduction counseling after each test. Compared to other cities, HIV prevalence among MSM in Nanjing was much lower than in the southwestern cities of Chongqing (21.2% in 2014) [12] and Kunming (17.0% in 2014) [13]. It was higher than in Beijing[14] (6.1% in 2011) or other countries (4.4% in India [15] in 2010), 2.5% in the Republic of Cyprus[16] in 2012). However, all of these comparisons should be considered cautiously as there were differences in methodologies used to estimate prevalence. Because HIV continues to spread in Nanjing, interventions should be continued and strengthened.

We found that the HIV testing rate in the previous year remained steady at around 60%, higher than that reported in Chongqing (43.5% in 2014) [12], Beijing (42.6% in 2016) [17], and Guangzhou (53.4% in 2013) [18]. It was also higher than the national annual HIV testing rate (50.4% in 2011) among MSM [19]. HIV testing rate among MSM in Nanjing increased from only one in five in 2008 to nearly half in 2012 [20], and then to almost two thirds by 2017. The disclosure of HIV positive status can have individual health benefits and potentially reduce community spread of HIV through early diagnosis and early initiation of ART [21]. Our multivariate analysis confirmed that being tested for HIV in the previous year was a protective factor. However, there is still a gap in HIV testing rate among MSM between Nanjing and other areas, like Australia (80.5%) [22] and USA (67%) [23]. We need to continue the expanding test program further.

It is alarming that rush popper use among MSM in Nanjing rose dramatically from

12.4% to 21.7%, it just a little bit lower than that reported by Beijing (26.8%) [24] or Shenyang (26.3%) [25]. Rush popper used to be a prescription drug prescribed to relieve angina. Now MSM use it to facilitate sexual intercourse due to its mechanism of relaxing the anal sphincter and dilating capillaries [26]. Univariate analysis indicated that rush popper use was associated with a higher rate of HIV infection, which was consistent with other reports [24, 25, 27]. Two factors may explain this result. First, rush popper has the effects on sexual behaviors such as promoting sexual desire, reducing sexual inhibition and decreasing physical experiences of pain, which may further prevent users from using condoms. Second, prior research had documented that rush popper use may increase HIV transmission through their engagement in group sex and multiple sex partners [28]. Our findings underscore the need for specific intervention programs to reduce rush popper use.

We analyzed the factors associated with HIV infection using the pooled five surveys data. Consistent with other reports [29, 30], our data confirmed that UAI is an important risk factor for HIV infection. Although there was a declining trend of UAI among MSM, slightly more than 40% of participants reported using condoms only intermittently or never. It is necessary to improve awareness of the risk of HIV infection among MSM and the need for consistent condom use during sexual activity. In the present study, anal sex role was found to be independent risk factor for HIV infection men who engaged exclusively or partially in receptive anal sex carry a higher risk of HIV infection, possibly because rectal mucosa is easily damaged during receptive anal sex, thus increasing the likelihood of HIV virus passing into their blood [31]. Therefore, both the “receptive” and “dual” role MSM should probably be considered a priority target for condom promotion.

We observed a declining trend in current syphilis prevalence among MSM during these recent five years. Compared with previous report [11], current syphilis prevalence was lower than that in 2011 (9.1%) and 2012 (11.5%). This phenomenon is consistent with the report that syphilis infection has declined in China [31]. The implementation of the syphilis prevention and control plan by the China’s Ministry of Health may have

contributed to this decline [32]. It is well known that STDs that cause ulcers or inflammation greatly increase the efficiency of HIV transmission, by increasing both the infectiousness and the susceptibility to HIV infection [33]. The positive association observed between syphilis and HIV, can be explained by similar risk behaviors [34]. Thus anyone presenting with syphilis should be tested for HIV and vice versa [35]. Meanwhile, screening and standardizing STDs treatment need to be continued to push this trend further.

Several limitations of our study should be noted. First, our participants were recruited from MSM venues, some MSM social platforms and a government internet site. Thus they may not be representative of MSM who do not go to these venues or visit the website. "Hidden" MSM may carry higher risk behaviors. Second, it is possible that face to face investigation may have underestimated the levels of risk behaviors due to the issue of social desirability. The study was however, anonymous; fake names and study serial number were used to match the records. Third, our cross sectional studies are inherently observational and descriptive; thus, we cannot infer causality.

Overall, our five consecutive surveys were carefully designed, implemented, and quality controlled. Under the implementation of comprehensive interventions, we observed stable HIV prevalence, steady HIV testing rate, decreasing UAI and syphilis prevalence. However, an increasing use of rush popper was observed. In response to the high HIV burden among MSM in Nanjing, HIV prevention and intervention messages must be increased about the urgent need for consistent condom use, targeting especially those MSM who engage in any receptive anal intercourse (although condom use is also important for inserters), HIV testing expanded, rush popper use reduced, STD screening increased and more widespread use of standardized treatment implemented.

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Contributors

SS Wu, YY Xu, L Liu, X Li were contributed to data collection; WJ Xu contributed to laboratory testing, F Xu contributed to the study design and quality control, ZP Zhu responsible for data analysis and manuscript writing, HJ Yan and R Detels were contributed to manuscript revision. All authors read and approved the final manuscript.

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The founders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts

The authors declare that they have no competing interests.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Ethics approval

This study was approved by the Ethics Committee of the National Center for AIDS and STD Control, China CDC. Written informed consent was obtained from each participant prior to the survey.

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Table 1. Demographic characteristics among MSM in Nanjing, 2013 to 2017

Variables	2013	2014	2015	2016	2017	*Liner by liner* χ^2	*P*
Total sample	649	669	577	633	503	-	-
Age group						0.061	0.805
<20	6.0(39)	4.3(29)	5.4(31)	2.0(18)	3.8(20)		
20～49	86.0(558)	84.9(568)	89.1(514)	91.0(557)	89.1(467)		
⩾50	8.0(52)	10.8(72)	5.5(32)	6.0(37)	7.1(37)		
Marital status						30.186	<0.001
single, divorced or widowed	73.3(476)	74.6(499)	81.3(469)	81.5(516)	84.5(425)		
married or live together	26.7(173)	25.4(170)	18.7(108)	18.5(117)	15.5(78)		
Han ethnic	97.4(632)	98.7(660)	97.9(565)	98.6(624)	97.0(488)	0.074	0.785
Residency (hukou) in Jiangsu	68.4(444)	71.4(478)	66.7(385)	69.0(437)	67.6(340)	0.437	0.509
Education						24.910	<0.001
junior high school and lower	18.0(117)	13.2(88)	11.4(66)	11.8(75)	9.5(48)		
senior high school or skill school	24.3(158)	22.1(148)	20.8(120)	18.5(117)	21.9(110)		
college and higher	57.6(374)	64.7(433)	67.8(391)	69.7(441)	68.6(345)		
Sex Orientation						1.303	0.2524
homosexual/ bisexual	94.1(611)	93.6(626)	92.7(535)	94.8(600)	95.4(480)		
heterosexual/ not sure	5.9(38)	6.4(43)	7.3(42)	5.2(33)	4.6(23)		

Table 2. Trends in HIV prevalence, risk behaviors and HIV testing among MSM in Nanjing,02013 to 2017

Variables	2013	2014	2015	2016	2017	*Liner by liner* χ^2	*P* value
	%(n)	%(n)	%(n)	%(n)	%(n)		
HIV infection	9.9(64)	12.3(82)	12.5(72)	9.8(62)	10.1(51)	0.181	0.670
Current syphilis infection	6.2(40)	10.6(71)	5.9(34)	4.7(30)	5.6(28)	5.043	0.025
Had anal sex, last 6 months	86.1(559)	81.0(542)	76.4(441)	83.6(529)	80.7(406)	2.958	0.085
Had UAI, last 6 months	50.7(329)	42.5(284)	43.0(248)	41.2(261)	41.7(210)	9.433	0.002
Multiple sex partners, last 6 months	55.6(361)	49.6(332)	46.3(267)	57.3(363)	62.0(312)	19.194	＜0.001
Had commercial anal sex, last 6 months	2.8(18)	3.6(24)	3.1(18)	5.6(17)	4.4(18)	3.397	0.065
Had vaginal sex, last 6 months	22.2(144)	22.4(150)	19.2(111)	19.9(126)	16.3(82)	7.034	0.008
Had UVI, last 6 months	14.0(91)	15.1(101)	11.6(67)	13.7(87)	9.1(46)	6.791	0.009
Ever used rush popper (*)	-	12.9(86)	20.8(120)	23.1(146)	21.7(109)	17.879	＜0.001
Ever used drugs	2.0(13)	0.9(6)	1.7(10)	0.9(6)	1.2(6)	1.166	0.280
Diagnosed with STD, last 12 months	6.9(45)	6.6(44)	4.7(27)	8.4(53)	7.0(35)	0.333	0.564
Tested for HIV, last 12 months	61.2(397)	64.1(429)	57.0(329)	63.2(400)	63.4(319)	0.228	0.633

*: The data of “ever used rush poppers” haven’t been collected in 2013.

Table 3. Univariate analysis of factors associated with HIV infection among MSM in Nanjing

Factors	HIV infection % (n)	OR (95% CI)	*P* value
Age group			
<20	6.6(9)	1.000	
20～49	11.1(297)	1.785(0.898-3.546)	0.098
≧50	10.9(25)	1.734(0.785-3.834)	0.174
Residency (hukou)			
Jiangsu	11.1(231)	1.000	
Others	10.6(100)	0.947(0.739-1.214)	0.668
Education level			
junior middle school and lower	15.0(59)	1.000	
senior middle school/skill school	12.7(83)	0.827(0.577-1.185)	0.300
college and higher	9.5(189)	0.598(0.436-0.819)	0.001
Sex role			
insertive	7.6(80)	1.000	
receptive	13.6(99)	1.925(1.410-2.628)	0.001
dual	9.5(189)	1.695(1.276-2.252)	0.001
Anal sex, last 6 months			
no	9.0(50)	1.000	
yes	11.3(281)	1.290(0.940-1.769)	0.114
Multiple sex partners, last 6 months			
no	10.9(158)	1.000	
yes	10.9(173)	0.996(0.792-1.251)	0.971
Had UAI, last 6 months			
no	7.9(135)	1.000	
yes	14.7(196)	1.999(1.585-2.521)	0.001
Commercial sex, last 6 months			
no	10.9(319)	1.000	
yes	12.6(12)	1.186(0.640-2.197)	0.587
Had vaginal sex, last 6 months			
no	11.2(272)	1.000	
yes	9.6(59)	0.840(0.625-1.130)	0.250
Had UVI, last 6 months			
no	11.0(272)	1.000	
yes	9.6(59)	0.937(0.663-1.304)	0.712

Table 3 Continued

Factors	HIV infection % (n)	OR (95% CI)	*P* value
Ever used rush popper			
no	10.0(192)	1.000	
yes	16.3(75)	1.750(1.311-2.336)	0.001
Ever used drugs			
no	10.7(320)	1.000	
yes	26.8(11)	3059(1.518-6.164)	0.002
Diagnosed with STD, last 12 months			
no	10.5(296)	1.000	
yes	17.2(35)	1.771(1.207-2.598)	0.003
Current syphilis infection			
no	10.1(285)	1.000	
yes	22.7(46)	2.614(1.841-3.712)	0.001
Tested for HIV, last 12 months			
no	12.9(149)	1.000	
yes	9.7(182)	0.728(0.578-0.916)	0.007

Table 4. Multivarible analysis of factors associated with HIV infection among MSM in Nanjing

Factors	OR(95% CI)	*P* value
Education level		
junior middle school and lower	1.000	
senior middle school/skill school	0.883(0.610-1.278)	0.509
college and higher	0.700(0.503-1.035)	0.501
Sex role		
insertive	1.000	
receptive	1.936(1.409-2.660)	0.001
dual	1.684(1.261-2.249)	0.001
Had UAI, last 6 months		
no	1.000	
yes	2.046(1.558-2.687)	0.001
Ever used drugs		
no	1.000	
yes	1.874(0.997-4.120)	0.053
Diagnosed with STD, last 12 months		
no	1.000	
yes	1.610(1.077-2.407)	0.020
Current syphilis infection		
no	1.000	
yes	2.219(1.531-3.217)	0.001
Tested for HIV, last 12 months		
no	1.000	
yes	0.631(0.437-0.912)	0.014

Footnote: the variables that included in multivariable analysis were below: education level, sex role, had UAI, ever used drugs, diagnosed with STD in last 12 months, current syphilis infection and test for HIV in last 12 month.

Trends in HIV prevalence and risk behaviours among men who have sex with men from 2013 to 2017 in Nanjing, China: A consecutive cross sectional survey

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	-

Outcome data	15*	Report numbers of outcome events or summary measures	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-9
		(*b*) Report category boundaries when continuous variables were categorized	8-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.