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Biomarker validation of self-reported sex among middle-aged female sex workers in China

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Introduction

Self-reported questionnaires are a central component of public health data collection. In the field of sexually transmitted infections (STIs) including HIV/AIDS, self-reporting sexual behavior is commonly practiced. However, the responses generated from questionnaire data may not always be accurate (1). Such inaccuracies may lead to misclassification of the outcome and/or exposure status of subjects (information bias) (2). Misreporting may arise from inaccurate recall, misinterpretation or incomprehension of survey items, or in response to ‘sensitive’ questions. Participants may perceive a question to be ‘sensitive’ if they feel it is inappropriate, stigmatizing, threatening, invasive, socially undesirable or inconsistent with the researcher’s expectations (3). Therefore, validation of self-reported sexual behavior is paramount.

Self-reported sexual activity can be validated by biomarkers, such as prostate specific antigen (PSA). PSA is transmitted through ejaculation and can be subsequently detected in vaginal fluid for up to 48 hours after unprotected sex (4). The PSA test is 100% sensitive and 95–96% specific to detect 1.0 ng PSA/mL of vaginal swab ejaculate immediately after semen exposure (5–7), and declines thereafter (4,7).

Earlier studies revealed that self-reported condom use was associated with lower STI rates (8). However, with the advent of biological measures, such as the PSA test, several studies in different populations of sex workers have revealed that condom use may be inaccurately reported (9–12) and that bias exists regardless of the interview mode (e.g., face-to-face interviews, audio computer assisted self-interviewing, and computer assisted self-interviewing) (13). Additionally, our previous study demonstrated that a substantial proportion of FSWs over-reported their condom use and that this over-reporting was associated with syphilitic infection (14).

High-risk individuals may be more inclined to misreport socially undesirable behaviors. However, previous studies linking misreported sexual activity to STIs are mixed (9,11,14).

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These inconsistencies may stem from different definitions of discordance, including using self-reported condom use instead of sexual activity. It is possible that less bias may be associated with reporting sexual activity in general, rather than specific sex practices (e.g., condom use) because FSWs are selected into studies based on occupation (e.g., engaging in sexual activity with clients). However, it remains unknown if misreporting sexual activity occurs, what the magnitude and direction of the bias is, and what factors contribute to misreporting.

The objective of this study was twofold: 1) to validate self-reported data with a biomarker (PSA) to determine if middle-aged FSWs accurately reported their sexual activity in the past 48 hours, and 2) to determine if discordance between self-reported sexual intercourse and biomarker data was associated with active and prevalent syphilitic infection.

Materials and Methods

Study population and location

Detailed information regarding sampling has been previously reported elsewhere (14). Briefly, a multi-site cross-sectional study was carried out in 2014 in Hefei, Nanning, and Qingdao, China. The three different cities were chosen based on their heterogeneity in prevalence estimates of HIV/STIs. From 2010–2013, Nanning had the highest HIV/STI prevalence of notifiable STIs (e.g., notifiable STIs include HIV/AIDS, syphilis, and gonorrhea (15); followed by Hefei, and Qingdao (14).

Women who were middle-aged FSWs and resided in one of the three cities for at least three months were included in the study. Middle-age was defined as 35 years and older and was based on the findings from a qualitative study (16). Women were considered FSWs if they had commercial sex at least once a week in the 30 days prior to the interview.

Recruitment of study participants

FSWs were recruited using Respondent Driven Sampling (RDS), a peer recruitment strategy used to target hidden populations (17,18). RDS methods for the sample have been previously described (14). Briefly, seeds with different durations of sex work and client solicitation location (e.g. street-based, massage parlors and karaoke bars) were purposefully selected to recruit a diverse sample of middle-aged FSWs at each site (~400 participants at each site). Applications of RDS suggest that after 5–6 recruitment waves, the RDS sample provides a broad cross-section of the hidden population (17). We successfully completed more than six waves of recruitment at each site (e.g., 8 waves of recruitment in Qingdao, 9 in Hefei, and 11 in Nanning). Convergence plots and bottleneck plots were used to determine whether the final RDS estimates were biased by the convenience sample of seeds (19) based on five variables: age, education, marital status, migration status, and client solicitation site. After 100–300 participants were recruited at each study location the five variables reached equilibrium, indicating that the RDS sample included a broad cross-section of the middle-aged FSW population. Our final sample size was 418 in Nanning, 407 in Hefei, and 420 in Qingdao.

Interviews

All eligible subjects participated in a face-to-face anonymous interview in a private room conducted by trained interviewers. Interviewers were either local public health staff who provided free STI counseling and testing or were well-trained graduate students. All interviewers completed training on interviewing techniques, establishing and maintaining rapport with participants, and ensuring participant confidentiality. To aid subject's comprehension interviewers used computer-assisted personal interviewing (CAPI).

Ethical Considerations

Since sex work in China is illegal and stigmatized, we used three approaches to ensure participant confidentiality. First, interviewers received training on confidentiality. Second, interview sites were chosen for their capability to maintain confidentiality and included clinics, hotels, and the local Centers for Disease Control and Prevention. Third, FSWs were recruited using RDS, a peer referral method that serves to build rapport between researchers and potential participants and address fears related to study participation. Trained research team members explained the study protocol and procedures and answered questions prior to obtaining informed consent. This study was approved by the Institutional Review Boards of the University of Maryland, Shandong University School of Public Health, and the Centers for Disease Control and Prevention in Hefei and Guangxi.

Measurement

Prostate Specific Antigen Test (PSA)—PSA is detectable in vaginal discharge for up to 48 hours after acts of unprotected vaginal intercourse and therefore, can be used as a biological indicator of unprotected sex (5,20,21). Before interviewing consenting participants, trained lab technicians collected vaginal secretions and administered rapid PSA tests at the interview sites. Presence of PSA was tested using the rapid ABACard p30 test (Abacus Diagnostics, West Hill, CA), which has been shown to be reliable, sensitive, specific (20,21) and comparable to the quantitative PSA assay(5). Extraction of specimens from fresh vaginal swabs was performed in the extraction buffer provided with the testing kit and swabs were soaked for at least 5 minutes. ABACard p30 was tested using 200 µL of swab extraction and was loaded directly into the well of the immunochromatographic test strip according to the manufacturer's instructions.

Discordance—FSWs were asked to report their sexual activity in the past 48 hours. The CAPI questionnaire automatically generated the 48-hour window based on the starting time of the interview. Those who indicated no sexual intercourse in the past 48 hours on the questionnaire, but tested positive for the PSA test, were considered discordant. Those who indicated no sexual intercourse in the past 48 hours and tested negative on the PSA test were considered concordant (Figure 1). FSWs who reported having intercourse in the past 48 hours were not included in the study because negative PSA results may be due to reasons other than misreporting. For example, a well-controlled clinical trial demonstrated that almost all women (98%) tested positive immediately after being inseminated with their partner's semen, but that the proportion testing PSA positive dropped to 29% by 24 hours and declined to only 3% 48 hours after insemination (4). Women self-reporting semen

exposure within 48 hours may test negative due to PSA clearance and therefore, would not be considered a true misreport.

Prevalent and Active Syphilitic Infection—Trained laboratory technicians collected venipuncture blood specimens to test for syphilis. Syphilitic infection was first assessed with a qualitative immunoassay to detect antibodies to *Treponema pallidum* (Alere Determine™ TP test; Alere Medical Co., Ltd, Chiba Prefecture, Japan) and was confirmed by a *Treponema pallidum* particle agglutination test (TPPA); Fujirebio Inc., Tokyo, Japan or ABON Biopharm Co., Ltd., Hangzhou, China). Confirmed TPPA positive samples were tested for *non-Treponemal antilipoidal* antibodies, using Tolidine Red Unheated Serum Test (TRUST; Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China). TPPA has good sensitivity (88% in the primary stage, 100% in the secondary stage, and 100% in the latent stage) and is comparable with other early treponemal tests (22). Positivity to TPPA is indicative of either a previous syphilitic infection (e.g., cured syphilis) and/or active syphilitic infection because antibodies to *Treponema pallidum* are lifelong. To distinguish between truly active syphilitic infections and past or prevalent cases, a third test (TRUST) was used. TRUST is a nontreponemal test with good sensitivity (85% in the primary stage, 100% in the secondary stage, and 100% in the latent stage) and is comparable to other nontreponemal tests (22). FSWs who sero-tested positive for TPPA were classified as prevalent cases of syphilis (e.g., both previous and active syphilis). FSWs that tested positive for TPPA and TRUST tests were considered active/recent cases of syphilitic infection.

Measurement scales—Scales were constructed to measure HIV knowledge, sex work-related stigma, and attitudes towards condom use based on our previous studies (23,24). All scales asked in the questionnaire were drafted in Chinese and then translated into English by research team members fluent in both languages. The scale items and psychometric properties are described in Appendix A. All scales were summed with higher scores indicating higher HIV knowledge, perceived stigma, and less favorable attitudes toward condom use.

FSWs were also asked to report the length of time (in months) they worked in the commercial sex industry and whether they had any previous STIs (yes/no).

Statistical Analysis

Covariates were selected based on the literature and our previous studies with FSW populations (10,12,14). Means and proportions were calculated to describe the distributions of continuous and categorical variables (Table 1). To assess the relationship between syphilitic infection and reporting discordance, analyses with one predictor and multivariable logistic regression were conducted to estimate crude and adjusted odds ratios and their 95% confidence intervals (95% CI) (Table 2). Socio-demographic variables were adjusted in the multivariable analysis. Study locations were evaluated as effect modifiers using interaction terms to determine if the association varied by study site. All interaction terms were not significant and were removed from the models. The study sites were included as covariates to adjust for potential cluster effects(25). SAS version 9.3 (SAS Institute, Cary, NC) was used to conduct all analyses.

Results

Among the 1,245 middle-aged FSWs, 320 self-reported no sexual intercourse in the past 48 hours. Among those who indicated not having sex in the past 48 hours on the questionnaire, 25.3% (81/320) had a positive PSA test (e.g., discordant) and 74.7% (239/320) had a negative PSA test (e.g., concordant). 22.4% of discordant FSWs (15/67) and 6.0% (13/216) of concordant individuals tested positive for active syphilis, while 35.8% (29/81) of discordant FSWs and 15.1% (36/239) of concordant FSWs tested positive for prevalent syphilis. The majority of discordant FSWs received a primary school education or less (60.5%), were rural to urban migrants (63.0%), lived in Hefei (59.3%) and were employed in the sex industry for shorter periods of time. Discordant FSWs had significantly lower HIV knowledge scores, but had similar attitudes toward condom use and perceived stigma.

In unadjusted models, discordant FSWs had 4.5 times the odds of active syphilis (95% confidence interval (CI): 2.0–10.05) and 3.2 times the odds of prevalent syphilis (95% CI: 1.78–5.60) compared to concordant FSWs. Discordance was significantly associated with divorced/widow status (odds ratio (OR): 1.7, 95% CI: 1.03–2.88), receiving a middle school education (OR: 0.5, 95% CI: 0.28–0.81), living in Nanning (OR: 0.5, 95% CI: 0.26–0.81), and having a lower HIV knowledge score (OR: 0.9, 95% CI: 0.82–0.97).

After adjustment for covariates, FSWs who had active syphilis (OR: 3.8, 95% CI: 1.52–9.30) or prevalent syphilis (OR: 2.6, 95% CI: 1.37–5.02) were more likely to be discordant compared to concordant FSWs (Table 2).

Discussion

Results from this multicenter study suggest that a significant proportion of FSWs underreport their sexual activity within the past 48 hours and that underreporting was associated with both active and prevalent syphilis. Our study extends the current literature by demonstrating that bias associated with misreporting encompasses self-reported sexual behaviors broadly, not just within the scope of condom use, and that misreporting is particularly strong among FSWs who practice more risky behaviors. These findings have serious public health implications as a significant proportion of responses will be misclassified by respondents. Such misclassification will distort the strength and direction of the measure of association, possibly leading to inaccurate conclusions and compromising the efficacy of interventions.

One fourth of the sample misreported their sexual activity in the past 48 hours. This is consistent with other studies that show 6% to 39% of FSWs misreport their sexual activity in the past two days (9,11,26,27). Our results were higher than that of Minnis et al. (2009), who indicated that 11.9% of Zimbabwean women misreported their sexual activity. The differences in misreporting may be attributed to the populations used (e.g., FSWs versus non-FSWs) (13).

Previous literature reporting the association between discordance and STI infection is somewhat inconsistent, with the majority of studies pointing to a positive association (9,14,26,27). In our study, discordance was strongly associated with incident and prevalent

syphilis, and this association remained after adjusting for confounding variables. One study in Guinea showed that STI prevalence was not associated with condom use (9). The differing results may be due to the definition of discordance. For example, in the Guinea study “PSA not detected” was used as one of the conditions to define discordance. This classification may have attenuated the association between discordance and STI presence, since PSA starts to decline immediately after initial semen exposure (4). Another difference is related to the measure of STIs. In our study, we used biospecimens to test for syphilis, while the Guinea study used self-reported measures. Other literature supports an association between discordance and STIs. Gallo et al., (2007) showed that discordance, defined as reporting no unprotected sex within the past 48 hours and testing PSA positive, was associated with prevalent gonorrhea, chlamydia, and trichomoniasis (11). Liu et al., (2016) demonstrated that over-reported condom use and PSA positivity was linked to syphilitic infections (14). Collectively, these results generate consistent findings that self-reported measures (either sexual intercourse or condom use) have substantial information bias and cannot validly measure engagement in sexual risks for STIs (10).

Previous studies suggest that any biases arising from misreporting is non-differential, meaning the measurement error occurs at random and the degree of misreporting is similar (28). However, discordance has been previously associated with young age and several STIs (12). In our study, discordance was associated with middle school education and STIs. Therefore, misreporting is not a random process and non-differential misclassification is unlikely (29). An association between discordance and active and prevalent syphilis signifies that differential misclassification may either overestimate or underestimate the true efficacy of interventions. Since PSA begins to clear immediately after exposure (4) the number of discordant cases in our study is likely an underestimate, suggesting that a stronger positive association exists.

Given the variation in misreporting in the literature, identifying the situational factors that give rise to discrepant reporting may improve research design and implementation. Our study shows that sex worker stigma and attitudes towards condom use were not associated with discordance. FSWs are a vulnerable population who regularly face threats and incarceration (16). Misreporting likely occurs to safeguard their identity, irrespective of stigmatization or beliefs about safe sex practices. Divorced, middle-aged FSWs with low levels of education may be more likely to practice unsafe sexual behaviors because they face financial adversity and lack adequate occupational network support (23). Therefore, interventions which address low education and focus on building social cohesion and network support among the FSW community may promote trust between researchers and FSWs, which could potentially reduce fears and anxiety surrounding participation in research studies and encourage accurate reporting.

The strengths of our study include using multiple study locations in China with varying levels of STI prevalence. Additionally, we used biomeasures to investigate the validity of self-reported sexual activity with active and prevalent syphilitic infection. However, despite these strengths, our study is not without limitations. First, a temporal association cannot be established due to the cross-sectional study design. The extent of misreporting has been shown to vary over time and therefore, we were unable to account for this variation (30).

Second, knowledge of PSA testing may have influenced correct reporting of sexual activity; however, one study demonstrated no association between prior knowledge of the PSA test and reporting unprotected sex (31). Any potential influence would likely lead to a higher proportion of FSWs over-reporting intercourse within the past 48 hours.

Conclusions

In light of these limitations, these results have significant implications for public health research conducted in the field of HIV/STIs. Our findings suggest that high-risk FSWs tend to misreport their sexual behavior on sensitive questions. Researchers should take caution when asking respondents to self-report sexual behaviors and understand their threats to validity. The consequences of using self-reported measures may bias the intervention results and leave some high-risk individuals unidentified for referral into sexual health programs and untreated for STIs. Our recommendations to public health researchers are to use objective measures to obtain sensitive health information, when feasible and appropriate. Since PSA only detects unprotected sex, a combination of PSA in conjunction with questionnaire interviews may be necessary until better biomeasures are available to detect both protected and unprotected sex.

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Appendix A. Description of measurement scales

HIV Knowledge Scale (Cronbach's alpha: 0.68 in Nanning, 0.82 in Hefei, and 0.79 in Qingdao)

Please indicate whether you believe the following 11 questions about HIV are true or false.

- 1 Eating food cooked by an AIDS patient will give you HIV.
  - 2 Sharing utensils such as bowls, chopsticks, and cups with a HIV positive people will give you HIV.
  - 3 You can identify HIV from looking at your clients' genitals.
  - 4 If a client's penis is washed clean, then you are protected from HIV transmission, even if you do not use a condom.
  - 5 Sharing needles to inject illicit drugs with AIDS patients will give you HIV.
  - 6 A HIV positive mother can transmit the HIV virus to her embryo or newborn.
  - 7 Being exposed to droplets by AIDS patients when they cough or sneeze can give you HIV.
  - 8 Having sex with HIV infected people without a condom can give you HIV.
  - 9 Going to school with HIV infected children will give you HIV.
  - 10 If a mosquito bites an AIDS patient and then bites you, it is possible to get HIV/AIDS.
  - 11 Sleeping in the same bed with an HIV positive person will give you the HIV virus.
-

Sex worker-related stigma scale (Cronbach's alpha: 0.72 in Nanning, 0.87 in Hefei, and 0.89 in Qingdao)

Please indicate whether you strongly disagree (0), disagree (1), agree (2), strongly agree (3) with the following statements:

- 1 My husband or boyfriend would leave me if he found out that I was a sex worker.
  - 2 My family would think that I had hurt their reputation and dignity if they knew that I was a sex worker.
  - 3 My family would be ashamed of me if they knew I was a sex worker.
  - 4 My children would be ashamed of me if they knew I was a sex worker.
  - 5 If my non-sex worker friends knew that I was a sex worker, they would not be friends with me anymore.
  - 6 If my non-sex worker friends knew I was a sex worker, they would look down on me.
  - 7 If people around me (colleagues or neighbors) knew I was a sex worker, they would exclude me.
  - 8 If people around me (colleagues and neighbors) knew I was a sex worker, they would look down on my family.
-

Attitudes towards condom use scale (Cronbach's alpha: 0.64 in Nanning, 0.80 in Hefei, and 0.70 in Qingdao)

Please indicate whether you strongly disagree (0), disagree (1), agree (2), strongly agree (3) with the following statements:

- 1 It is not necessary to use condoms if you trust your partner.
  - 2 You do not need to use condoms with intimate sex partners.
  - 3 There is no need to use condoms with stable sex partners (e.g., husbands or boyfriends).
  - 4 There is no need to use condoms with regular clients.
  - 5 Requesting to use condoms will make sex partners think you don't trust them.
  - 6 It is very difficult to request to use condoms with sex partners.
  - 7 If you insist on using condoms, it is possible to lose sex partners.
  - 8 Using condoms reduces satisfaction during sex.
-

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Figure 1.
Flow chart describing discordance

Table 1

Sociodemographic variables by discordant status

	Discordant		Concordant		Binary Analysis	
	N	%	N	%	cOR ^a	95% CI ^b
Incident Syphilis						
Yes	15	22.4	13	6.0	4.5	2.02–10.05
No (ref)	52	77.6	203	94.0	–	–
Prevalent Syphilis (TPPA)						
Yes	29	35.8	36	15.1	3.2	1.78–5.60
No (ref)	52	64.2	203	84.9	–	–
Education						
Primary school or less (ref)	49	60.5	87	36.4	–	–
Middle school	26	32.1	119	49.8	0.5	0.28–0.81
High school or above	6	7.4	33	13.8	0.5	0.20–1.24
Marital Status						
Single	5	6.2	29	12.1	0.5	0.18–1.28
Married (ref)	38	46.9	129	54.0	–	–
Divorced/Widowed	38	46.9	81	33.9	1.7	1.03–2.88
Residence						
Rural	51	63.0	140	58.6	1.2	0.72–2.02
Urban (ref)	30	37.0	99	41.4	–	–
Previous STI diagnosis						
Yes	4	4.9	17	7.1	0.68	0.22–2.08
No (ref)	77	95.1	222	92.9	–	–
City						
Nanning	21	25.9	103	43.1	0.5	0.26–0.81
Hefei (ref)	48	59.3	113	47.3	–	–
Qingdao	12	14.8	23	9.6	1.6	0.77–3.45
Continuous Variables	Median	IQR	Median	IQR ^c	cOR	95% CI
Age	41.2	35.7–45.2	38.7	35.4–43.0	1.0	1.0–1.09

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	Discordant		Concordant		Binary Analysis	
	N	%	N	%	cOR ^a	95% CI ^b
HIV knowledge	8.0	4.0–10.0	9.0	7.0–10.0	0.9	0.82–0.97
Sex worker stigma	16.0	15.0–18.0	16.0	15.0–17.0	1.0	0.95–1.13
Attitudes toward condom use	12.0	11.0–13.0	11.0	9.0–13.0	1.1	0.98–1.15
# months in sex work	24.0	12.0–48.0	30.0	12.0–58.0	1.0	0.99–1.01

^aCrude Odds Ratio

^b95% Confidence Interval

^cInterquartile Range is presented as the 25th and the 75th percentiles.

Table 2

Logistic regression of discordance and incident and prevalent syphilitic infection

	Model 1^c		Model 2^d	
	aOR^a	95% CI^b	aOR	95% CI
Incident Syphilis				
Yes	3.8	1.52–9.30	–	–
No (ref)	–	–	–	–
Prevalent Syphilis (TPPA)				
Yes	–	–	2.62	1.37–5.02
No (ref)	–	–	–	–
Age	1.0	0.95–1.07	1.00	0.95–1.06
Education				
Primary school or less (ref)	–	–	–	–
Middle school	0.5	0.23–0.89	0.47	0.26–0.86
High school or above	0.5	0.15–1.45	0.48	0.17–1.33
Marital Status				
Single	0.8	0.24–2.57	0.93	0.31–2.76
Married (ref)	–	–	–	–
Divorced/Widowed	1.6	0.84–2.99	1.28	0.71–2.31
Residence				
Rural	1.1	0.57–2.04	1.21	0.68–2.18
Urban (ref)	–	–	–	–
Previous STI diagnosis				
Yes	0.3	0.06–1.74	0.44	0.12–1.62
No (ref)	–	–	–	–
# months in sex work	1.0	0.99–1.01	1.00	0.99–1.01
HIV Knowledge	1.0	0.87–1.09	0.93	0.84–1.04
Sex worker stigma	0.9	0.85–1.06	0.97	0.88–1.07
Attitudes towards condom use	1.0	0.94–1.15	1.03	0.94–1.13
City				
Nanning	0.8	0.37–1.59	0.79	0.39–1.56
Hefei (ref)	–	–	–	–
Qingdao	1.6	0.48–5.39	1.92	0.67–5.53

^a Adjusted Odds Ratio^b 95% Confidence Interval^c Model includes active syphilis as a predictor^d Model includes prevalent syphilis as a predictor

Bold indicates p<0.05