



Published in final edited form as:

Sex Transm Dis. 2011 May ; 38(5): 429–433. doi:10.1097/OLQ.0b013e31820369f6.

Association between Participant Self-Report and Biological Outcomes Used to Measure Sexual Risk Behavior in HIV-1-Seropositive Female Sex Workers in Mombasa, Kenya

R. Scott McClelland, MD, MPH^{1,2,3,5}, Barbra A. Richardson, PhD⁴, George H. Wanje, BSc⁵, Susan M. Graham, MD, MPH^{1,7,8}, Esther Mutunga⁵, Norbert Peshu, MBChB, MPH, DTCH⁸, James N. Kiarie, MBChB, MPH⁶, Ann E. Kurth, PhD, CNM⁹, and Walter Jaoko, MBChB, PhD⁷

¹Department of Medicine, University of Washington, Seattle, USA

²Department of Epidemiology, University of Washington, Seattle, USA

³Department of Global Health, University of Washington, Seattle, USA

⁴Department of Biostatistics, University of Washington, Seattle, USA

⁵Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, Kenya

⁶Department of Obstetrics and Gynaecology, University of Nairobi, Nairobi, Kenya

⁷Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya

⁸Kenya Medical Research Institute, Kilifi, Kenya

⁹College of Nursing, New York University, New York, USA

Abstract

Background—Few studies have examined the association between self-reported sexual risk behaviors and biological outcomes in HIV-1-seropositive African adults.

Methods—We conducted a prospective cohort study in 898 HIV-1-seropositive women who reported engaging in transactional sex in Mombasa, Kenya. Primary outcome measures included detection of sperm in genital secretions, pregnancy, and sexually transmitted infections (STIs). Because three outcomes were evaluated, data are presented with odds ratios [OR] and 96.7% confidence intervals [CI] to reflect that we would reject a null hypothesis if a p-value were ≤ 0.033 (Simes' methodology).

Results—During 2,404 person-years of follow-up, self-reported unprotected intercourse was associated with significantly higher likelihood of detecting sperm in genital secretions (OR 2.32, 96.7% CI 1.93, 2.81), and pregnancy (OR 2.78, 96.7% CI 1.57, 4.92), but not with detection of STIs (OR 1.20, 96.7% CI 0.98, 1.48). At visits where women reported being sexually active, having >1 sex partner in the past week was associated with lower likelihood of detecting sperm in genital secretions (OR 0.74, 96.7% CI 0.56, 0.98). This association became non-significant after adjustment for reported condom use (adjusted OR 0.81, 96.7% CI 0.60, 1.08).

Conclusions—Combining behavioral and biological outcomes, which provide complementary information, is advantageous for understanding sexual risk behavior in populations at risk for transmitting HIV-1. The paradoxical relationship between higher numbers of sex partners and less

frequent identification of sperm in genital secretions highlights the potential importance of context-specific behavior, such as condom use dependent on partner type, when evaluating sexual risk behavior.

Keywords

HIV-1; sexually transmitted disease; women; Africa; sexual risk behavior

Introduction

Many people continue to be sexually active after receiving a diagnosis of HIV-1 infection.¹ Sexual risk behavior may change over the course of disease progression,^{1, 2} and a growing body of evidence demonstrates that the effect of antiretroviral therapy (ART) on risk behavior varies in different settings.^{3–10} Accurate measurement of sexual risk behavior in HIV-1-seropositive individuals is essential to understanding how the disease spreads in populations and for predicting and measuring the effect of interventions intended to reduce the risk of secondary transmission. However, recent biomarker validation studies have raised concern about the accuracy of self-reported behavioral data.^{11, 12}

Of necessity, data in studies of sexual risk almost invariably include participants' self-reported behavior.¹³ Biological outcomes have been proposed as a surrogate marker for high-risk sexual behavior,¹⁴ but are also subject to bias and measurement error.^{15, 16} Combining behavioral self-report with biological outcomes can be a useful strategy for understanding sexual risk behavior.¹⁷ This approach has been employed in a variety of settings,^{10, 18, 19} but there is a paucity of data evaluating the relationship between self-reported sexual risk and biological outcomes in individuals with HIV-1, particularly in high-prevalence areas such as sub-Saharan Africa.^{20, 21} Our objective was to examine the relationship between self-reported risk behavior and biological outcomes including sperm in female genital secretions, pregnancy, and sexually transmitted infections (STIs) in a cohort of HIV-1-seropositive female sex workers in Mombasa, Kenya.

Materials and Methods

Population and Procedures

An open cohort study of female sex workers at risk for HIV-1 was initiated in February 1993.²² Detailed procedures for enrollment and follow-up HIV-1-seropositive women in the cohort have been published.¹⁰ Briefly, women who acquired HIV-1 during follow-up in the high-risk HIV-1-seronegative cohort were identified beginning in 1993. In addition, women who were HIV-1-seropositive at initial screening were offered enrollment beginning in 2001. Participants were invited to return for monthly follow-up visits including a standardized interview, physical examination, pelvic speculum examination, and collection of genital samples for diagnosis of STIs. Questionnaires were obtained by trained interviewers who stressed the confidentiality of the research data and the importance of responding truthfully to improve our understanding of HIV-1 risk in this population. In order to focus on episodic memory (i.e. recall and tally of specific episodes),¹⁶ our questions were based on a one-week recall interval. For example, women were asked, "*In the last working week, how many times did you have intercourse?*" Interviewers then asked, "*In the last working week, how many times did you have intercourse with a condom?*" Blood was collected every 3 months for CD4 lymphocyte subset analysis beginning in 1998. Urine was collected for rapid pregnancy testing if women missed a menstrual period. Antiretroviral therapy was provided to eligible participants according to the Kenyan National Guidelines beginning in 2004.

Individualized risk reduction education and free condoms were provided at each visit. Women were instructed to use condoms consistently and correctly for every episode of intercourse. Ethical review committees at the Kenya Medical Research Institute, Kenyatta National Hospital, and University of Washington approved the study. All participants provided informed consent.

Serology and Microbiology

Women were screened for HIV-1 using an ELISA (Detect HIV [BioChem ImmunoSystems]), and positive tests were confirmed using a second ELISA (Recombigen [Cambridge Biotech] or Vironostika [bioMérieux]).²³ Vaginal secretions were examined using a saline wet mount to identify sperm and motile *Trichomonas vaginalis* parasites. Cervical secretions were also examined microscopically for the presence of sperm, and were cultured for *Neisseria gonorrhoeae* on modified Thayer-Martin media. Urine β -human chorionic gonadotropin (hCG) testing was performed using a rapid assay (Plasmatec hCG [Plasmatec Laboratory Products]). Quantitation of CD4 lymphocytes was performed using a manual system (Cytosphere [Coulter]) from 1998 until 2004, and with an automated method (FACSCount [Becton Dickinson]) thereafter.

Data Analysis

We defined five self-reported sexual risk behaviors as exposures. Women were categorized as having *unprotected intercourse* if they reported one or more sex acts and had less than 100% condom use. They were considered to be *abstinent* if they reported no sex acts in the past week. Those reporting sexual activity were considered to have *100% condom use* if the number of sex acts with a condom was equal to the total number of sex acts. The *number of sex partners* and the *number of sex acts* were also determined for those who were not abstinent. The distribution of these data was skewed, with many visits having few recent sex partners or sex acts. As a result, these data were modeled as bivariate exposures, dichotomizing at their medians. We defined three biological outcomes including presence of STIs (*N. gonorrhoeae* or *T. vaginalis*), presence of sperm in genital secretions by microscopy, and pregnancy detected by urine β -hCG testing. For women who became pregnant, data were censored at the first visit at which pregnancy was detected to avoid counting the same pregnancy multiple times.

Analyses were performed using SPSS version 15.0 and Stata version 9.2. Generalized estimating equations (GEE) with a logit link and exchangeable correlation structure were used to assess the association between self-reported behaviors and biological outcomes. Our primary analyses evaluated the associations between the exposures of interest and each of the three outcomes. We used Simes' methodology to adjust our α -level to accommodate multiple testing.²⁴ Data are presented with odds ratios and their 96.7% confidence intervals to reflect that we would reject a null hypothesis if a p-value were ≤ 0.033 . As a result, p-values less than 0.07 are reported to the third decimal place to facilitate identification of statistical significance and statistical trends ($p < 0.066$).

Multivariate GEE was used to control for additional potential confounding factors, which were considered for inclusion in adjusted models based on known or possible associations with risk behavior and the outcomes of interest. The final models for all three biological outcomes adjusted for time-varying cofactors including age, contraceptive method, and use of ART. The final model for STIs also adjusted for calendar year category (1993–1996, 1997–2000, 2001–2004, and 2005–2008) in order to account for temporal trends in STI incidence. Further adjustment for baseline educational level, marital status, workplace (bar versus nightclub), alcohol use, self-reported vaginal washing, Karnofsky score, and CD4

lymphocyte count did not substantially alter the observed associations, so these variables were not retained in the final multivariate models.

Results

Of 898 women included in this analysis, 600 (67%) were HIV-1-seropositive at enrollment. The remainder contributed HIV-1-seropositive visits after HIV-1-seroconversion in the cohort. The women contributed 2,404 person-years of HIV-1-seropositive follow-up. There were 15,926 visits, with a median of 10 (interquartile range [IQR] 3-27) visits per participant. The median time between visits was 33 (IQR 29-48) days.

Baseline characteristics of the participants are shown in Table 1. The median age of the women was 31 (IQR 26-36) years, and most had completed at least some primary education. Only 73 (8%) reported no prior pregnancies, and 386 (43%) were using a modern method of contraception other than condoms alone. During the week prior to their baseline visit, 351 (39%) participants reported unprotected intercourse. Of the 771 (86%) who were sexually active during the preceding week, 420 (55%) reported 100% condom use, 250 (32%) reported multiple sex partners, and 212 (28%) reported more than two sex acts. During follow up, there were 789 episodes of sperm detected in genital secretions (incidence 32.8/100 person-years), 61 pregnancies (incidence 2.7/100 person-years), and 896 episodes of STI (gonorrhea and/or vaginal trichomoniasis; incidence 37.3/100 person-years). One hundred and twenty-nine (14%) of these women initiated ART.

Association between Self-Reported Sexual Risk Behavior and Biological Outcomes

The unadjusted associations between reported sexual risk behaviors and biological outcomes are presented in Table 2. Women were more than twice as likely to have sperm in genital secretions (odds ratio [OR] 2.32, 96.7% confidence interval [CI] 1.93, 2.81, $P < 0.001$) and to be pregnant (OR 2.78, 96.7% CI 1.57, 4.92, $p < 0.001$) at visits when they reported unprotected sex. There was also a statistical trend suggesting a higher likelihood of STIs with unprotected sex (OR 1.20, 96.7% CI 0.98, 1.48, $p = 0.056$). For visits at which women reported sexual activity during the past week, they were less likely to have an STI if they reported 100% condom use (OR 0.76, 96.7% CI 0.58, 1.00, $p = 0.034$), although this finding was not statistically significant at $\alpha = 0.033$. There was a lower likelihood of detecting sperm in genital secretions when women reported > 1 sex partner (OR 0.74, 96.7% CI 0.56, 0.98, $p = 0.024$). To explore this seemingly paradoxical relationship, this analysis was repeated with adjustment for 100% condom use (adjusted odds ratio [AOR] 0.76, 96.7% CI 0.58, 1.01, $p = 0.041$) and any condom use (AOR 0.81, 96.7% CI 0.60, 1.08, $p = 0.118$). Neither the number of sex partners nor the frequency of sex was significantly associated with pregnancy or STIs in this population.

Table 3 presents the associations between reported risk behaviors and biological outcomes with adjustment for potential confounding factors. The results were similar to the unadjusted analyses, although the statistical trend suggesting an association between unprotected sex and STIs was eliminated (AOR 1.13, 96.7% CI 0.91, 1.40, $p = 0.217$).

Discussion

Data from this prospective cohort study of HIV-1-seropositive women in Kenya highlight the complementary information gained by combining behavioral self-report and biological outcomes in studies of sexual risk behavior. In this population, visits at which women reported recent unprotected intercourse, which incorporates information on both the frequency of sexual activity and the level of condom use, there was a > 2 -fold higher likelihood of concurrent detection of sperm in genital secretions. This highly significant

association suggests that as a population, the women were responding accurately and with satisfactory recall of their recent behavior. Stated another way, the biological measurement provides an internal validation of the self-reported behavior.

There was a robust association between unprotected sex and increased likelihood of pregnancy at the same visit. This might reflect the fact that these women were trying to become pregnant. Alternatively, if women knew that they were pregnant, they might have chosen not to use condoms. We do not have data regarding women's knowledge of their pregnancy status prior to the test in our clinic, so it is difficult to distinguish between these possibilities.

The association between unprotected sex and a higher risk of STIs was modest, and did not achieve statistical significance. On the other hand, among the subgroup of visits where women were sexually active, 100% condom use was associated with a >20% lower likelihood of STIs. Recognizing the complex relationship between risk behavior and STIs and the importance of context-specific behavior is crucial when interpreting these associations.^{13, 15-18, 25-27} For example, women may be more likely to use a condom with partners they believe may have an STI. The results of a recent qualitative behavioral study in our cohort highlight this point.²⁸ Many women reported making decisions about condom use based on the perceived risk of sex partners. This finding parallels reports from a variety of populations globally, including populations of non-sex workers.²⁹⁻³¹

At visits when women were sexually active, having a higher number of partners in the past week was associated with approximately 30% lower likelihood of detecting sperm in genital secretions. This association was attenuated, and no longer statistically significant, after adjustment for condom use, further emphasizing the importance of context-specific behavior. In semi-structured interviews, women from our cohort reported that condom use is easier to negotiate with casual or short-term sex partners.²⁸

It is important to consider the effect of measurement error in evaluating the relationships between self-reported behaviors and biological outcomes. Women were asked about risk behavior in the last week to optimize recall.^{32, 33} Nonetheless, some recall bias is possible. Perhaps more importantly, none of the biological outcomes is a perfect measure of unprotected intercourse. Microscopic identification of sperm in genital secretions has been used as a marker for unprotected sex, but is not highly sensitive and is present for a variable duration after intercourse.^{34, 35} Alternatives including detection of prostate specific antigen (PSA) have been used for identification of recent unprotected sex (past 2 days), whereas detection of Y chromosome DNA has been used as a marker for unprotected sex during the past several weeks.³⁵⁻³⁷ Each of these methods has limitations, and the search for better biomarkers for semen exposure continues. Similarly, pregnancy and STIs do not occur with every episode of unprotected sex, and could have occurred more than a week before the risk behavior assessment (i.e. outside of the measured interval). Measurement error in both self-reported behaviors and biological outcomes would be expected to attenuate the observed relationships between them. Stated another way, the design of this study tends to underestimate the true associations between reported risk behaviors and biological outcomes.

These data are unique, as we have been unable to identify prior studies comparing behavioral and biological outcomes among HIV-1-seropositive individuals in Africa. The large number of participants, prospective design, long-term follow-up, and careful measurement and control for potential confounding factors are important strengths of the study. The α -level used to define statistical significance was adjusted to avoid increasing the likelihood of type-1 statistical error with multiple comparisons.

It is interesting to contrast these results with those of a recent study in HIV-1-seronegative women in Zimbabwe.¹¹ Overall, compared to PSA detection in vaginal fluid, self-report was a poor predictor of recent sexual activity and condom use. In this population of Zimbabwean women participating in a cohort study of the effects of contraceptive methods on HIV-1 acquisition, frequency of reported condom use was predictive of pregnancy, but not STIs.¹² This difference from our findings may be a result of numerous factors. The populations of women in family planning clinics in Zimbabwe versus female sex workers in Kenya are likely to have differed in terms of their reasons for condom use (for contraception versus STI prevention), familiarity and skill with use of condoms, ability to negotiate condom use, and willingness to disclose sexual risk behavior. Differences in specific interview questions could also contribute to different findings in these studies. We feel that both the Zimbabwe study and our present results highlight the value of combining behavioral and biological markers, since they provide complementary information that may lead to a more complete understanding of sexual risk behavior.

There are some limitations with this study. First, because sexual behaviors generally cannot be observed directly in research, there remains a possibility of measurement error due to recall and social desirability bias.³⁸ On the other hand, there are also limitations to using biological outcomes as markers of sexual risk behavior.^{15, 16} In this context, it is important to note that the evaluation of multiple behaviors provided an opportunity to compare and contrast the associations of specific reported risk behaviors with three distinct biological markers; presence of sperm, pregnancy, and STIs. A second important point is the fact that female sex workers represent a somewhat unique population. However, it should be noted that the majority of women in our cohort supplement income from bar work with occasional payment for sex in cash or in kind.¹⁰ With their relatively low frequency of sex and numbers of sex partners, we feel that these women are likely to be representative of a broader population of highly disadvantaged HIV-1-seropositive women in Africa. The relationship between reported risk behavior and biological outcomes is likely to vary in different social and cultural settings, highlighting the need to develop a broad database. Third, an inherent limitation in epidemiological studies of risk behavior is the fact that while quantitative data can provide valuable information about the relationships between behaviors and biological outcomes, this methodology may not be ideal for understanding the motivations underlying participants' behavior. To address this issue, we conducted a parallel qualitative study, providing valuable complementary information about women's perceptions of sexual risk behavior and HIV-1.²⁸ Further qualitative studies are needed to explore the relationship between women's fertility desires and risk behavior.

Recently, the National Institute of Mental Health Collaborative HIV/STD Prevention Trial Group considered the challenges of selecting outcome measures for behavioral intervention trials. They concluded that behavioral self-reports and biological endpoints yield different information, and both types of data should be considered for the evaluation of a wide variety of interventions.¹⁷ The data presented in this paper provide empirical evidence in support of this position, highlighting the importance of assessing complementary behavioral and biological markers to gain a greater understanding of HIV-1 transmission risk.

Acknowledgments

Sponsorship: This study was supported by National Institutes of Health (grant R01-AI58698) and by the Fogarty International Center (grant 5D43-TW000007 to S.M.G. and G.H.W.). Additional support for the Mombasa Field Site was received from the University of Washington Center for AIDS Research (CFAR) an NIH funded program (P30-AI027757) which is supported by the following NIH Institutes and Centers (NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NCCAM). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

We wish to acknowledge the study participants, who contributed their time and effort to make this study a success. We also wish to recognize the contributions made by our clinical, laboratory, and administrative staff. We thank the Mombasa Municipal Council for providing clinical space, and Coast Provincial General hospital for providing laboratory space. This manuscript was approved for publication by the Director, Kenya Medical Research Institute.

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Table 1

Baseline characteristics of HIV-1-Seropositive Women Followed in a High-Risk Cohort in Mombasa, Kenya

Variable	N = 898
Age (years)	31 (26 – 36)
Education (years)	8 (7 – 10)
Marital Status	
Married	9 (1.0)
Widowed, divorced, or separated	554 (61.7)
Never married	335 (37.3)
Prior pregnancy	825 (91.9)
Using modern contraceptive other than condoms ^a	386 (43.0)
Percent condom use in past week ^b	100 (0 – 100)
Number of sex acts in past week ^b	2 (1 – 2)
Number of sex partners in past week ^b	1 (1 – 2)

The values are median (interquartile range) or number (%).

^aIncluded 81 oral contraceptive pills, 258 depot medroxyprogesterone acetate, 11 Norplant, 10 intra-uterine contraceptive device, 22 bilateral tubal ligation, 2 hysterectomy, and 2 reporting use of an unidentified method.

^bAmong visits where women were sexually active (86% of sample).

Table 2Associations between Self-Reported Sexual Risk Behavior and Biological Outcomes^a

	Sperm		Pregnancy ^b		STI	
	OR (96.7% CI) (P value)	(P value)	OR (96.7% CI) (P value)	(P value)	OR (96.7% CI) (P value)	(P value)
Unprotected Sex	2.32 (1.93, 2.81)	<0.0001	2.78 (1.57, 4.92)	<0.0001	1.20 (0.98, 1.48)	(0.056)
Abstinence	0.35 (0.29, 0.43)	<0.0001	0.53 (0.31, 0.93)	(0.016)	1.03 (0.88, 1.21)	(0.7)
100% Condom Use ^c	0.65 (0.53, 0.80)	<0.0001	0.41 (0.21, 0.81)	(0.0005)	0.76 (0.58, 1.00)	(0.034)
>1 Sex Partner ^c	0.74 (0.56, 0.98)	(0.024)	1.61 (0.75, 3.45)	(0.2)	1.05 (0.80, 1.37)	(0.7)
>2 Sex Acts ^c	1.16 (0.92, 1.46)	(0.2)	1.14 (0.51, 2.52)	(0.7)	1.02 (0.80, 1.31)	(0.9)

CI, confidence interval; OR, odds ratio; STI, sexually transmitted infection.

^aN=898^bFor women who became pregnant, data were censored after the first visit at which pregnancy was detected.^cAmong visits where women were sexually active.

Table 3

Associations between Self-Reported Sexual Risk Behavior and Biological Outcomes, Adjusted for Potential Confounding Factors^a

	Sperm		Pregnancy ^c		STI	
	AOR ^b (96.7% CI) (P value)	AOR ^b (96.7% CI) (P value)	AOR ^b (96.7% CI) (P value)	AOR ^b (96.7% CI) (P value)	AOR ^b (96.7% CI) (P value)	AOR ^b (96.7% CI) (P value)
Unprotected Sex	2.31 (1.91, 2.80) (<0.001)	2.41 (1.31, 4.46) (0.002)	1.13 (0.91, 1.40) (0.2)	1.09 (0.92, 1.29) (0.3)	0.79 (0.60, 1.04) (0.07)	1.09 (0.83, 1.44) (0.5)
Abstinence	0.35 (0.28, 0.43) (<0.001)	0.66 (0.36, 1.21) (0.1)	1.51 (0.70, 3.26) (0.3)	0.96 (0.42, 2.19) (0.9)	1.01 (0.78, 1.30) (1.0)	
100% Condom Use ^e	0.65 (0.52, 0.80) (<0.001)	0.40 (0.20, 0.83) (0.007)				
>1 Sex Partner ^e	0.74 (0.56, 0.98) (0.021)					
>2 Sex Acts ^e	1.15 (0.91, 1.45) (0.2)					

AOR, Adjusted odds ratio; CI, confidence interval; STI, sexually transmitted infection.

^aN=898

^bMultivariate analyses were used to adjust for age (time varying), any contraceptive use (time varying), and use of antiretroviral therapy (time varying).

^cFor women who became pregnant, data were censored after the first visit at which pregnancy was detected

^dMultivariate analyses were used to adjust for calendar year category (1993–1996, 1997–2000, 2001–2004, and 2005–2008), age (time varying), any contraceptive use (time varying), and use of antiretroviral therapy (time varying).

^eAmong visits where women were sexually active.