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Incidence and Predictors of Any Incident Indicator of Unprotected Sex among a Cohort of HIV sero-discordant Couples in Lusaka, Zambia (1994 – 2012)

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Master of Public Health
Global Epidemiology

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2007

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An abstract of
A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University

In partial fulfillment of the requirements for the degree of
Master of Public Health
In Global Epidemiology

2015

Abstract

Incidence and Predictors of Any Incident Indicator of Unprotected Sex among a Cohort of HIV sero-discordant Couples in Lusaka, Zambia (1994-2012).

By Udodirim N. Onwubiko

Background: Under-reporting of sexual behavior in HIV prevention efforts compromise the accuracy of predicting sero-discordant couples likely to transmit infections to negative partners. Here we examine the reliability of self-reports of unsafe sexual behaviors using 4 biological measures of unprotected sex and the association between time to unprotected sex and predictors collected at enrollment and during follow up.

Methods: HIV discordant heterosexual couples were followed at a CVCT center from 1994 to 2012. Baseline and time varying factors were measured. The outcome was a time-varying composite measure of any: self-reported unprotected sex, sperm present on a wet prep, incident pregnancy, and incident HIV/STI. A repeated outcomes survival analysis with time-varying covariates explored factors associated with unprotected sex.

Results: Among 3,049 couples followed for an average of two years, any indication of unprotected sex occurred during 62% of study intervals. In the unadjusted analysis, younger age of the male partner ((M-F+: HR 0.995 p=0.002, M+F-: HR 0.99 p <0.0001), female partner (M-F+: HR 0.995 p=0.011, M+F-: HR 0.992 p =0.003), fewer number of years co-habiting (M-F+: HR 0.992 p=0.008, M+F-: HR 0.991 p =0.003), age difference (M-F+: HR 0.995 p=0.04, M+F-: HR 0.991 p=0.02), male partner use of alcohol (M+F-: HR 1.114 p=0.01), female partners number of lifetime sex partners (M-F+: HR 1.092 p=0.002), circumcision of the male partner (M+F-: HR 0.886 p=0.032), self-reported condom use by female partner (M-F+: HR 0.812 p<0.0001, M+F-: HR 0.756 p <0.0001), being pregnant at interval visits (M-F+: HR 1.304 p<0.0001, M+F-: HR 1.341 p <0.0001) were significant predictors of unprotected sex. Multivariate analyses showed that a higher number of lifetime sex partners for the female partner (aHR: 1.003, 95% CI: 1.002, 1.004), a positive history of STI in the past year for the male partner (aHR: 1.149, 95% CI: 1.062, 1.243) and being pregnant at any interval visit (aHR: 1.191, 95% CI: 1.08, 1.314) were significant predictors for the M-F+ couples while consistent protected sex with the study partner (aHR: 0.795, 95% CI: 0.658, 0.961), being pregnant at any interval visit (aHR: 1.253, 95% CI: 1.113, 1.41) and use of either oral (OCP) or injectable (INJ) contraceptives at interval visits were important predictors of unprotected sex for the M+F- couples.

Conclusions: Our study provides further evidence that gross under-reporting of unsafe sexual practices is very likely by couples in discordant relationships and caution must be exercised when using self-reports to determine a discordant couple's risk of HIV transmission to the negative partner.

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Acknowledgements

This thesis would not have been possible without the support of my husband, Mr. Emmanuel Chukwu, who extended himself in many ways to take care of the family allowing me to devote time to both my studies and this thesis. Also many thanks to my parents, Mr. and Mrs. J.I. Onwubiko who helped out with child care needs during this time in spite of their own tight schedules. Thanks to my sisters, Chinwe Udeze RN, Judith Onwubiko PharmD, Chima Onwubiko PharmD and Uchechi Onwubiko for their support. Many thanks too to my thesis advisor, Dr. Kristin Wall for her guidance and ever available support in making this possible. Finally thanks to Jesus Christ for His grace and wisdom during the course of my study.

Udodirim Onwubiko, MBBS
MPH 2015

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INTRODUCTION

Human Immunodeficiency Virus (HIV) infection is the leading cause of death in sub-Saharan Africa (SSA) and has remained so since the beginning of the twenty first century(1). Despite significant advances in methods of infection detection and strong efforts by the international community (especially by the United Nations joint programme on Acquired Immunodeficiency Syndrome (UNAIDS)) in scaling up of HIV treatment services (anti-retroviral therapy (ART)) mortality due to HIV in this region of the world has remained high. Approaches aimed at preventing infection have therefore become the major tool in mitigating the impact of the epidemic in this sub-region. Of the multiple established routes of infection known to science, the most important and common route among persons of productive age (>15 years old) globally is unprotected sexual contact between HIV sero-discordant partners (one partner HIV sero-positive and the other HIV sero-negative).

Numerous epidemiological studies have reported on the risk of HIV transmission in stable sero-discordant relationships. In all relevant literature reviewed, occurrence of unprotected sex was measured using only self-reported answers in surveys/questionnaires (2-7). Some studies have gone further to examine the reliability of the self-reported sexual behavior by test retest interview method or separate couple interviewing. However, only few studies have examined the validity of self-reported occurrence of unprotected sex in stable sero-discordant relationships by evaluating the incidence of biological indicators of unprotected sex. The use of a combination of these indicators in or study to measure the actual incidence of unprotected sex in this population may provide a more accurate estimate of the risk of

transmission of HIV among them and thereby help in identifying couples in Couples Voluntary Counseling and Testing (CVCT) programs at greater risk of HIV transmission/acquisition for targeted intervention.

BACKGROUND AND LITERATURE REVIEW

Sub-Saharan Africa is a region of the world well known for its constant struggle against high mortality rates amidst limited resources. A review of the World Health Organization's (WHO) reports on leading mortality causes in the region show that HIV infection is currently the leading cause of death in sub-Saharan Africa (SSA) and has remained so since the beginning of the twenty first century(1).

Of the 35 million people estimated by the United Nations joint programme on AIDS (UNAIDS) to be living with HIV globally as at 2013, more than 70% live in SSA and a slightly higher proportion (74%) of all AIDS-related deaths recorded globally in the same year occurred in this region as well (8). The scourge posed by this infection in this region is therefore undeniable and far reaching. Some countries in the region have been recorded as being more severely affected and ten of them together account for a staggering 81% of infections occurring in SSA (8).

Zambia, a highly urbanized country located in southern parts of Africa having an estimated population of 15.2 million inhabitants and a roughly equal distribution of males and females, is one of these ten countries (8-10). Per 2013 UNAID statistics, the prevalence of HIV among Zambian adults aged 15 – 49 years is 12.5% (11.9 – 13.3%) (9), a significant leap when compared to the global HIV prevalence in persons of this age group (0.8%) (10-12). Zambian HIV prevalence translates to about 1 in every 7 persons aged 15 – 49 years in the country living with HIV infection(13). The national prevalence varies by sex and location. Females have a higher prevalence (16.1%) than the males (12.3%) and prevalence of infection is appreciably worse in the urban areas (23%) compared to the rural areas

(10%)(14). With a little over 40% of the Zambian population living in the urban areas of the country's capital, Lusaka, and the industrial towns of the Copperbelt, these cities have been shown by studies to have the highest prevalence of HIV in the country(15).

Contrary to the pattern observed in more developed countries where most new HIV transmissions occur within same sex (homosexual) relationships, most incident infections in SSA occur within heterosexual relationships(8, 16). The result of this is that more women in SSA get infected with HIV than men and they get infected about 5 – 7 years, on the average, earlier than men(8). This finding is more pronounced in eastern and southern SSA countries where the prevalence of HIV among adolescent females (15 – 19 years old) is more than double the prevalence of HIV among their male counterparts and also doubles by the time they are young women (20 - 24 years)(8). Possible explanations proposed and examined in the past include the wide acceptance of intergenerational sexual relationships in these regions, early sexual debut among females with comparatively limited education about sexual health, and traditional norms and beliefs about the sexual power dynamics between men and women(17),(18).

Zambia's HIV transmission pattern is quite similar to this pattern observed in other African countries. Epidemiological studies show that unprotected heterosexual contact is the predominant route of infection in adults in Zambia (19, 20) and a significant proportion occur between partners in stable relationships like cohabiting/married couples. With about 60 - 95% of incident heterosexually transmitted cases occurring in sero-discordant (one

partner HIV sero-positive and the other negative) cohabiting/married couples (20, 21), this group are the country's largest risk group for incident HIV infections (22).

Several studies have documented the high prevalence of HIV sero-discordance among heterosexual couples in SSA (23, 24). Czaicki *et al* reported a 12% prevalence of sero-discordance (49% Male positive, Female negative [M+F-], and 53% Male negative, Female positive [M-F+]) among cohabiting couples in Ndola, one of the cities in the Copperbelt regions in Zambia (22). In a prospective cohort study done by Chomba *et al* in Lusaka, Zambia (n = 8,500 cohabiting heterosexual couples), 49% of the couples who sought HIV testing services at a Voluntary Counseling and Testing (VCT) center were found to have at least one positive partner and 23% (approximately 1 in 4 couples) of the entire cohort was sero-discordant for HIV (11% M+F-, 12% M-F+)(23). This closely matched the findings in a similar cross-sectional study done in Lusaka, Zambia (n = 3,500 cohabiting heterosexual couples) by McKenna *et al* which reported a 1 in 5 prevalence of sero-discordance among the couples (25). Outside of Zambia, similar statistics have also been reported by other studies in other countries in SSA. In an analysis of the first Demographic and Health Surveys to include results of HIV infection at the level of cohabiting couples, Damien de Walque compared the rates of sero-discordance in five African countries (Burkina Faso, Cameroon, Ghana, Kenya and Tanzania). He asserted that at least two-thirds of HIV infected couples in these countries were sero-discordant(26) showing that the high sero-discordance rate among heterosexual couples is widespread across the SSA region.

While the proportion of sero-discordant couples that got infected prior to the beginning of the current stable relationship versus the proportion that got infected while in the current relationship (i.e. via extra-relationship affairs) is largely unknown, the risk of transmission of infection from the positive partner to the negative partner has been the subject of many studies. Hugonnet *et al* estimated a 10/100 person-years (py) rate of sero-conversion for men and a 5/100 py rate for women in sero-discordant relationships(27). Compared to the estimated rates among concordant negative couples (0.45/100 py for men and 0.17/100 py for women)(27), this reflects a marked increase in risk of transmission among the sero-discordant couples ((RR_{Women} = 57.9, CI: 12.0 - 244; RR_{Men} = 11.0, CI: 1.2 - 47.5 for men)(27). A prospective cohort study of 415 sero-discordant couples in Rakai, Uganda also showed similar transmission rate estimates (12/100 py vs. 11.6/100 py for males and females respectively) (28).

The risk of transmission differs by the awareness of the couples of their sero-status. Dunkle *et al* estimated that about 20 – 25% of negative partners in a sero-discordant relationship who are unaware of their sero-status get sero-converted per year irrespective of the gender of the sero-positive partner (20). This rate pertains to a coital frequency of 2 – 3 sexual encounters per week and a risk of transmission of 1 in 500 per contact. Couple's awareness of their sero-status greatly reduced the rate of transmission to about 3 – 7% per year (20). Gray *et al* in the retrospective study among a cohort of monogamous heterosexual sero-discordant couples (n= 174 couples) in Rakai, Uganda estimated that the overall adjusted probability of HIV-1 transmission per coital act is 0.0011 (95% CI 0.0008, 0.015) in the absence of genital ulceration (and 0.0041 in the presence with genital ulceration) with the

probability increasing from 0.0001/coital act at viral loads less than 1,700 copies/ml to 0.0023/coital act at higher viral loads (38,500 copies/ml)(29).

In recent times, much emphasis has been placed on increasing couples' awareness of their sero-status through the use of CVCT services. However, it is not clear how reliable self-reports of sexual behavior, the primary medium used to assess risk per couple, collected at these clinic visits are at accurately assessing the risk of HIV transmission to the sero-discordant couple being counseled.

Several factors come into play when relying on self-reported measures of sexual behavior alone especially in the African setting. A few of these include the problem of cultural and personal inhibitions in discussing sexual issues with non-intimate persons, memory recall by the subjects being interviewed (especially regarding behaviors spanning over a long period of time) and the usual human predisposition to portray oneself in a better light. Since most clinicians and HIV prevention researchers rely heavily on these self-reports in decision making, the reliability and validity of these reports is of crucial importance.

A review of literature indicate that there have been attempts in the past by epidemiological researchers to assess reliability of sexual behavior self-reports in various relationship settings and cultures by comparing separate sexual behavior recalls by the partners(30-33), short term and long term recalls(31, 33-35), test and retest results, memory recalls and biological samples assessing for presence/absence of sexually transmitted diseases (STDs)(36) with mixed results. Some have found good reliability between partners (especially regarding recent sexual activity) and others found poor reliability.

Most literature reviewed (30-36) used the inter-partner interview agreement or correlation of self-reports with STD diagnosis for assessment of the validity of self-reported sexual behavior. Very few studies have looked at the correlation between self-reports and biological markers other than sexually transmitted disease (STD) diagnosis in the HIV negative partner.

One of the few studies found, Allen *et al* (2003), found under-reporting of sexual behavior common when self-reports were measured against 3 biological markers (sperm in vaginal smears, incident pregnancy and STDs including HIV, gonorrhea, syphilis and Trichomonas vaginalis)(24). In the questionnaires collected at every 3-month visit during the follow-up period, only 10% of the cohort reported having unprotected sexual contact with their partners. However, evaluation of the biological markers revealed up to 50% of these sero-discordant couples had sperm detected in vaginal smears and 32% of pregnancies/HIV transmissions were detected when couples had reported 100% protected sexual intercourse (24).

Interestingly, they also found that the biomarker frequently used by most studies to assess reliability of self-reported sexual behavior i.e. positive laboratory tests for STDs were relatively infrequent in this study. Among the partners that sero-converted (n = 107) during the course of the study, DNA sequencing confirmed that 87% of them acquired the infection from their HIV positive spouse (24) indicating that most sero-converters got the infection from their positive partners.

Another study among women aged 18 - 24 years in Kampala, Uganda by Kelly *et al* assessed validity by comparing the self-reported sexual behavior against 2 biomarkers; Herpes Simplex Virus Type 2 (HSV-2) infection and the presence of semen in self-

collected vaginal swabs. Among women who did not report having unprotected sex in the interviews, 20% - 44.4% tested positive for HSV-2 and about half (49.4% - 67.1%) had semen detected in their vaginal smear samples(37).

Minnis *et al* demonstrated a similar trend in the observed discrepancy between self-reporting of sexual activity and biological markers in their randomized controlled study in Zimbabwe investigating the accuracy of self-reported sexual behavior in HIV prevention collected using 2 methods (face to face interviews and audio computer-assisted self-interviewing (ACASI)) by comparison with 1 biomarker (evidence of recent semen exposure, Prostate-specific antigen (PSA) in vaginal smears)(38). Among women testing positive to PSA, a combined total of 48% had reported having no unprotected sex in the past 48 hours (11.7% reported not having sex at all while 36.2% reported having only condom-protected sex). Only 52% of the women of the PSA-positive women reported having unprotected sex during that time (38). Similar trends were reported in other studies using PSA detection as a biomarker for unprotected sex in other countries in Africa that were reviewed (39-41).

A review of the studies cited above shows that majority of the studies examining the validity of self-reported sexual behavior in HIV prevention research have focused on using a few biomarkers: semen exposure (either by assessing for sperm in the vaginal samples or PSA detection) or STI/STD detection. This gap in literature is the focus of this study. To the best of our knowledge, we found only one study (Allen *et al* (2003) (24) assessed validity using other biomarkers aside these two. However, the study which at the time of publication represented the largest single-site study of heterosexual sero-discordant

couples, compared to our study was limited by the number of couples and the duration of the follow up period (n = 963 sero-discordant couples, Mean follow-up - 14 months). Given that sexual matters are frequently shrouded in secrecy, we expect that sexual behaviors between sero-discordant couples designated as being dangerous to their health will be even more concealed at visits leading to poor assessment of the actual risk for the couple in HIV transmission. Therefore, reliable and valid means of assessing HIV risk behaviors is important to identify couples in sero-discordant relationships in need of intervention and prevent transmission.

METHODS

The original cohort study was approved by the Institutional Review Board (IRB) of Emory University and the appropriate review board in Zambia. Couples signed joint written informed consent at the time of HIV counseling and testing and again at the time of enrollment into the prospective studies. This research analysis was done using de-identified data from the original prospective cohort study.

Study Population

The data being used for this study was collected from discordant couples enrolled in couples' voluntary counseling and testing (CVCT) services in Lusaka, (2010 census - 1, 269, 848)(42) Zambia between 1994 and 2012 using established Rwanda Zambia HIV Research Group (RZHRG) study sites. All couples presenting for testing at any of the RZHRG Couples Voluntary Confidential Counseling and Testing center were engaged by the study staff and invited to participate in the study. After HIV testing, consenting couples were screened by the staff based on their HIV sero-status and sero-concordant couples were eliminated from the study. A total of 3, 050 couples met the baseline criteria (sero-discordance) for inclusion in the study and written consent was obtained from both partners prior to final enrollment in the study.

Data Collection

The predictors of interest in this study were couple demographics (age, education, length of relationship, number of prior pregnancies, fertility intentions, family income, stage of HIV of the positive partner, log viral load of the positive partner) as well as time-varying covariates such as contraception methods being used by the couple, use of anti-retroviral

therapy, circumcision status of the male in the relationship, non-incident pregnancy, alcohol consumption, sex with outside partners, genital ulceration or inflammation.

Couple demographics were collected at baseline using a detailed questionnaire administered separately to men and women by same-sex interviewers who maintained a neutral attitude to reduce underreporting. The time varying covariates were collected at 3-monthly intervals during follow-up period using similar data collection method as at baseline. Vaginal swabs from the female partner (self-collected) for sperm detection and *Trichomonas vaginalis* diagnosis (by microscopy) were submitted by the women while on the premises. Blood (whole blood) was drawn from both partners for other STI diagnosis (done on the premises except for gonorrhea cultures), HIV test and pregnancy tests (female samples only). Free STI treatment was offered to both partners when tests were positive and counseling provided.

Laboratory methods

Samples from the HIV negative partners were tested with rapid test and 2 ELISA tests. When sero-conversion is detected, HIV sequencing is performed to confirm epidemiological linkage. The Rapid Plasma Reagin (RPR) test was used for syphilis screening and positive test results confirmed by the *Treponema Pallidum* Hemagglutination (TPHA) test. For gonorrhea diagnosis, vaginal sample microscopy (Gram stain) and culture was used.

Statistical Analysis

All questionnaire data were verified at entry. Data from laboratory tests were merged with the questionnaire data to create the full dataset. Statistical analysis was done using Statistical Analytical Software (SAS) version 9.4 (SAS Institute, Inc., Cary N.C USA).

For the purpose of analysis, the cohort was grouped into two based on the HIV status of the partners at enrollment - male positive couples (M+F-) and female positive couples (M-F+).

The distributions of various characteristics of the individuals and couples in the cohort were described and tested using chi square test of proportions for the categorical variables and student's t-test/ANOVA for the continuous variables. Couple demographics were also described (counts and % for categorical variables, means and SDs for continuous variables) by whether or not the couple ever engaged in unprotected sex. Differences between couples engaging and not engaging in unprotected sex was evaluated via chi-square (or Fishers Exact) or t-tests, as appropriate.

Unprotected sex was defined as testing positive for or reporting any of the following five indicators: incident pregnancy in a female in a HIV discordant relationship during the interval of study, incident sero-conversion of the HIV negative partner during the interval of study, self-reported unprotected sex by either partner during the interval of study, sperm present on a wet prep during the interval of study, and self-reported or diagnosed sexually transmitted infection (syphilis, gonorrhea, chlamydia) during the interval of study. A composite measure of unprotected sex was defined for each three month interval of observation (a time-varying and repeated outcome of interest). The index created for

unprotected sex was validated by performing a cross tabulated frequency procedure in SAS.

The incidence of unprotected sex was calculated for the final composite indicator variable and for each component of the composite as a rate (number of unprotected sex events / person-time) and 95% confidence interval. Bivariate associations between baseline and time-varying covariates of interest and the outcome of interest were calculated via Anderson-Gill models or a similar model that accommodates time-varying, repeated outcome measures. Multivariate models were then built with non-collinear variables using a backward selection method. A cutoff level of $p \leq 0.05$ (two-sided) was used for assessing statistical significance.

RESULTS

A total of 3,050 HIV discordant couples were enrolled in this study and followed between 1994 and 2012. The entire cohort contributed a total of 28,352 three-monthly visits (range of visits per couple, 1 – 83; average number of visits per couple - 9 visits). More than half (54%) of these couples had positive female partners at enrollment (M-F+).

Description of selected demographic and risk factor profiles

A summary of the characteristics of the couples by couple sero-status at enrollment (M_F+ vs. M+F- couples) are shown in Table 1. The average age of men in the cohort was 35 years while the female partners were about 29 years old on the average. The average age difference between the partners was 7 years. On average the partners had lived together for 7 years and about 32% had lived together for more than 8 years. An appreciably greater proportion of men (44%) could read Nyanja (the widely spoken language in Lusaka) compared to the women (24%) and there was no significant difference in the distribution of these characteristics between the two groups of sero-discordant couples.

The average household monthly income was 274, 654 Kwachas (equivalent to $80 USD). Households with positive male partners (M+F- couples) earned slightly more than the households with negative male partners (84 USD/month vs. 76 USD/month).

Alcohol use among the women was relatively uncommon (1 in 5 women) compared to the men (about 4 in 5 men). Similarly, the male partners had a significantly higher average number of lifetime sexual partners compared to the women and more than 85% of the men were not circumcised as at enrollment or during duration of study.

At the time of enrollment, more than 90% of couples had had a prior pregnancy and the average number of pregnancies per couple was 4 (SD 2.4). The average number of living

children was 2 and 488 women (16%) of the cohort were pregnant at enrollment. Regarding future fertility intentions, more than half (56%) of the HIV negative men were certain they still wanted more children while less than half (45%) of the HIV positive men indicated that they would want to have more children. Among the women, the reverse was observed. A smaller proportion (39%) of the HIV negative women wanted more kids compared to their sero-positive counterparts (48%). Majority of the positive partners (greater than two-thirds) in the cohort were in the early stages of HIV (HIV clinical stage I or II).

During the follow-up period, the women indicated complete adherence to the use of condoms for all sexual contacts with their partners at 85% of all clinic visits. Majority of the women depended on non-hormonal (and non-IUD) methods for contraception and reported use of hormonal contraceptives at less than 35% of all follow-up visits.

Incidence of Unprotected Sex by Composite measure and the component indicators

A summary of the incidence of unprotected sex estimated using the composite variable and the component indicators is presented in Table 2. Based on the composite variable created from the 5 indicators of interest to this study (self-reported occurrence of unprotected sex, sperm in vaginal smear, incident pregnancy, clinically diagnosed STI in either partner and sero-conversion of the HIV negative partner during duration of study), unprotected sex was found to have occurred in 62% of the visit intervals for the entire cohort and there was no appreciable difference in incidence of unprotected sex between the two groups of discordant couples ($p=0.021$).

When considered individually, all 5 indicators provided varying results but significantly lower incidence of unprotected sex.

Using self-reported measure as the sole indicator per interval visit, the incidence of unprotected sex was only about 33% (36% for M-F+ couples and 30% for M+F- couple, p<0.0001).Laboratory diagnosed STI in males was positive in 4% of intervals (p=0.0003) and a similar incidence was observed for STI among the female partners (p<0.0001). Sperm was present in only 7% of vaginal smears (p=0.06) while only 3% of intervals had an incident pregnancy detected (p=0.06). There were 478 sero-conversions in the entire cohort and this represented about 15.7% of unprotected sex incidence during the follow-up period (p=0.24).

Unadjusted bivariate association between time to unprotected sex and baseline risk factors collected at enrollment

Details of the unadjusted association between the baseline predictors and time to unprotected sex are described in Table 3. Both the age of the man and the woman in a discordant relationship, individually, had a statistically significant effect of reducing the rate of unprotected sex. A one year increase in the age of the man slightly reduced the rate of engaging in unprotected sex by 1% (M-F+: HR 0.995 p=0.002, M+F-: HR 0.99 p <0.0001). A one year increase in the age of the woman also had similar effect on the rate of unprotected sex the couple was likely to engage in ((M-F+: HR 0.995 p=0.011, M+F-: HR 0.992 p <0.003). In both discordant groups, females in the youngest age group (15 – 25 years) were 10% more likely to engage in unprotected sex (HR 1.10 95% CI 1.03, 1.17 p=0.005) compared to the women in the oldest age group (>32years). However, female partners in male positive relationships who were between the ages of 26 and 31 years were even more likely to engage in unprotected sex (HR 1.14 95% CI 1.03, 1.26 p=0.009) than

their younger female counterparts when compared to the oldest age group. Higher age disparity between the couples and longer relationship duration had protective effects on the rate of engaging in unprotected sex for both groups.

The literacy level of both partners and the family monthly income had little to no effect on the rate of unprotected sex.

Alcohol use was associated with unprotected sex among couples in M+F- couples. The use of alcohol by the man in a male positive relationship significantly increased the rate of unprotected sex by 11.4% (p=0.01). A similar association was also observed among the women in male positive relationships but the effect was not statistically significant (HR 1.089 95% CI 0.997, 1.19, P=0.057).

The number of sex partners either partner has had in the past one year had a bigger influence on the rate of unprotected sex for both discordant groups than the number of sex partners either partner has had over the life time (See Table 3). However, the number of lifetime sex partners of the woman in an M-F+ discordant group had a very significant effect on the rate of unprotected sex ($p<0.0001$). Self-reported history of STI in the past year by the male partner collected at enrollment was significantly associated with unprotected sex among the female positive discordant couples (HR 1.09 95% CI 1.03, 1.15 p=0.002).

Among the M+F- discordant group, the circumcision status of the male was a protective factor in engaging in unprotected sex (HR 0.89 95% CI 0.79, 0.99 p=0.032). No such effect was observed among the M-F+ group (HR1.04, 95% CI 0.97, 1.12, P= 0.253).

The HIV positive partner's clinical stage of disease of the log viral load had no significant effect on the risk of unprotected sex for either discordant group.

Unadjusted bivariate association between time to unprotected sex and time varying risk factors collected during follow-up period.

Table 4 contains the detailed description of the unadjusted association between the time varying predictors and time to unprotected sex. There was a 20% decrease in likelihood of engaging in unprotected sex when women reported using condoms during intercourse with their partners ($p<0.0001$) during follow-up. This effect was slightly stronger among the male positive couples than among the female positive couples.

There was no detectable association detected between the methods of contraceptives being used by female positive couples on the couple's risk of engaging in unprotected sex during follow-up. However, use of either Injectable contraceptives or Oral contraceptives by male positive couples was associated with an increased hazard rate of at least 11% when compared to those who were on non-hormonal contraceptives ($p=0.001$ and $p=0.008$ respectively). The pregnancy status of each couple during follow-up was an important risk factor too. In both discordant groups, couples who were pregnant at any time during follow-up were at least 30% more likely to engage in unprotected sex ($p<0.0001$) in subsequent visit intervals.

Predictors of Unprotected sex (Fully adjusted multivariate model)

Table 5 presents the details of the fully adjusted predictive model for the outcome by the discordant group. For the female positive couples (M-F+), the statistically significant risk factors that predict the likelihood of the couple to engage in unprotected sex as defined by the 5 indicators include – the couple's number of years cohabiting, woman's number of

lifetime sex partners, man's age and history of STI collected at enrollment, self-reported use of condoms during intercourse and the pregnancy status of the female partner during the follow-up period. Of these six predictors, the strongest factor was the woman's pregnancy status collected at each interval visit. M-F+ couples with pregnant females were 19% more likely to engage in unprotected sex than when the female partner was not pregnant (p=0.0005). Being at least 6 months post-partum during visit reduced the likelihood by at least 14%. Male partners who reported having an STI in the past year during enrollment had a 15% increased likelihood of engaging in unprotected sex (p=0.0006) over time. The woman's lifetime number of sex partners had a very small but highly significant effect on the likelihood of engaging in unprotected sex over time (HR 1.003 95% CI 1.002, 1.004 P<0.0001).

Among the male positive discordant couples (M+F-), the important predictors were – the number of years of couple's cohabitation and male partner's age at enrollment, self-reported use of condoms at interval visits, pregnancy status of the female partner during follow-up and the method of contraception being used by the couple.

As observed among the female positive couples, the strongest predictive factor was the pregnancy status of the female partner in the relationship. M+F- couples with pregnant female partners were 25% more likely to engage in unprotected sex than when the female was not pregnant (p=0.0002). Being at least 6 months post-partum had a smaller protective effect among these couples than among the M-F+ couples (HR 0.95 95% CI .74, 1.22 p=0.68).

Self-reports of using condoms during sex by the female partners in M+F- relationships at interval visits was associated with a 20% decreased likelihood of unprotected sex (p=0.018).

Method of contraception used by these couples played a significant role in predicting the risk of engaging in unprotected sex by these couples. Couples reporting use of either oral contraceptives (COC) or Injectable contraceptives during interval visits were at least 15% more likely to have unprotected sex compared to those who were on non-hormonal contraceptives (HR 1.15, P=0.041 and HR 1.16 p=0.43 respectively). Couples reporting use of implants had a slightly reduced likelihood of unprotected sex but this was not significant (p=0.428).

DISCUSSION

Various epidemiologic and molecular studies have shown that most new HIV infections in Sub-Saharan Africa and the African continent as a whole occur among sero-discordant cohabiting couples many of whom are often unaware of their sero-status. With the growing use of Couples Voluntary Counseling and Testing services in Africa as the main tool for HIV detection and prevention, the need for greater accuracy in determining/predicting discordant couples at most risk for unsafe sexual behaviors is paramount.

The cohort

More than half of our cohort discordant couples were M-F+ (female partner positive). A greater proportion of the discordant couples were M-F+. This observation is in agreement with previous reports that a higher proportion of females in SSA are living with HIV compared to males (8, 9, 21). Many factors could explain this including the significant age difference between couples on the average (bringing up the issue of power dynamics in the relationship) and the significantly higher number of lifetime sex partners for the males compared to the females, both of which were evident in this study (See Table 1).

Incidence of Unprotected Sex

In this study, the incidence of unprotected sex when measured by the combination of 5 common indicators showed that study participants engaged in unprotected sex almost two-thirds of the time, a proportion that was almost twice the incidence detected by use of the self-reports of sexual behavior alone. This finding provides a stronger statistical backing for the suspicion mentioned in previous studies that there is usually gross under-reporting in studies of sexual behavior (24).

While our composite measure suggests a gross disparity between self-reported measure of sexual behavior and actual behavior, it does not negate the place of self-reports as a potent measure of sexual behavior. Among the individual indicators (biological and self-reported) assessed in this study, self-report of unprotected sex provided the closest estimate of the actual incidence of unprotected sex in this cohort estimated by the composite variable. Thus in the absence of other indicators, it may still serve as the best measure to assess a discordant couple's risk of HIV transmission. Emphasis must however be placed on the risk of missing couples who might also be at higher risk of disease transmission due to under-reporting of risky sexual behavior.

We did not find any significant advantage in using STD diagnosis as the sole measure of unprotected sex. Unlike prior sexual behavior studies which used STD diagnosis to assess reliability of self-reported sexual behavior, a positive STD diagnosis was seen in 3% of visits by men and 4% of visits by the female partners (no significant difference between the couple groups) through the duration of the study. Same observation was noted for incident pregnancy and negative partner's sero-conversion. These indicators however contributed to detecting the overall incidence of unprotected sex in the cohort and therefore should not be disregarded as important contributors in assessing the actual risk or unprotected sex for a discordant couple.

Predictors of Unprotected Sex

Irrespective of the gender of the HIV positive partner, the significant predictors of a couple's likelihood to engage in unprotected sex in unadjusted analyses were – younger age of the male partner, fewer number of years cohabiting, self-reported condom use, and being pregnancy status at each clinic visit.

The female positive couples had the number of sex partners had by the female partner over the course of her life and the male partner's history of STI in the past year as additional predictors of interest. For the male positive couples, the method of contraception being used by the couple was an additional predictor in the couple's likelihood to practice unprotected sex.

In multivariate models, several predictors of unprotected sex, including contraception, alcohol use, pregnancy status, and male circumcision, were different from the predictors of HIV sero-conversion (which we have previously explored in this cohort, manuscript in preparation).

Among M+F- couples, hormonal method use was (namely Oral Contraceptive Pills (OCP) and Injectable contraceptives (INJ)) were predictive of unprotected sex, potentially explaining why we and others have seen marginal to significant associations between hormonal contraceptive method use and HIV sero-conversion (43-46). Re-enforced condom counseling may be needed during INJ and OCP use.

Among all couples, prevalent pregnancy was significantly associated with unprotected sex (though again, pregnancy was not associated with HIV sero-conversion). Re-enforced condom counseling may be needed during pregnancy.

STRENGTHS AND WEAKNESSES

We have previously examined attrition and loss to follow-up which may limit generalizability and found that residence far from the clinic, younger age of the couple, and women's younger age at first intercourse are predictive of loss to follow-up among M+F- couples (47). Loss to follow-up among M-F+ couples is associated with residence far from the clinic, younger age, and younger women's age at first intercourse, low income, fewer lifetime sex partners, no history of STI in women, and male partners having a recent concurrent partner (47). Information bias in self-reported exposure variables could be differential by risk profile, basing our results in an unknown direction. Differences in the type of data captured over time due to funding changes means that we do not have measures like baseline Viral loads or fertility intentions for the entire cohort – however, we do not expect those systematic differences to be differential by HIV sero-conversion outcome.

TABLES

Table 1. Demographic and selected risk factor profiles for discordant couples in Lusaka Zambia, 1994 – 2012

	All couples		M-F+ couples		M+F- couples	
Variables	**N intervals**	**%**	**N intervals**	**%**	**N intervals**	**%**
Demographic Profile						
Male Partner Age, y (mean, SD)	35	8.1	35	8.6	35	7.6
Female Partner Age, y (Mean, SD)	29	6.8	29	6.7	28	6.8
Woman age (tertiles)						
15 – 25 years	10,789	38%	5,311	38%	5,478	39%
26 – 31 years	8,754	31%	4,420	31%	4,334	31%
32 – 62 years	8,787	31%	4,406	31%	4,381	31%
Age disparity, y (Mean, SD)	7	4.8	7	5.1	7	4.5
Years cohabiting, y (Mean, SD)	7	6.2	6	5.8	8	6.5
Monthly family income, USD*(Mean, SD)	80	98.6	76	92	84	104.6
Education						
Woman reads Nyanja*						
Yes, easily	6,641	24%	3,368	24%	3,273	23%
With difficulty/not at all	21,263	76%	10,491	76%	10,771	77%
Man reads Nyanja						
Yes, easily	12,210	44%	5,797	42%	6,413	46%
With difficulty/not at all	15,666	56%	8,052	58%	7,614	54%
Social and Sexual History						
Woman alcohol use last year						
Yes	5,605	20%	3,269	24%	2,336	17%
No	22,139	80%	10,530	76%	11,609	83%
Man alcohol use last year						
Yes	19,453	70%	10,146	74%	9,307	67%
No	8,217	30%	3,603	26%	4,614	33%
Woman lifetime sex partners (Mean, SD)	3	7	4	10	3	3
Woman last year sex partners (Mean, SD)	1	0.5	1	0.4	1	0.7

Man lifetime sex partners (Mean, SD)	11	15	11	13	11	17
Man last year sex partners (Mean, SD)	2	2	2	2	2	2
Woman history of STI last year						
Yes	9,806	35%	6,007	42%	3,799	27%
No	18,542	65%	8,146	58%	10,396	73%
Man history of STI last year						
Yes	9,921	35%	4,101	29%	5,820	41%
No	18,426	65%	10,051	71%	8,375	59%
Family History and Fertility Intentions						
Number of prior pregnancies (Mean, SD)	4	2.4	4	2.3	4	2.5
Pregnant at baseline						
Yes	4,397	16%	2,254	16%	2,143	15%
No	23,955	84%	11,899	84%	12,056	85%
Fertility intentions of man						
Yes, next year	1,654	15%	1,045	17%	609	13%
Yes, but not next year	3,867	36%	2,350	39%	1,517	32%
Don't know/No	5,237	49%	2,587	43%	2,650	55%
Fertility intentions of woman						
Yes, next year	2,413	20%	1,466	23%	947	18%
Yes, but not next year	2,716	23%	1,609	25%	1,107	21%
Don't know/No	6,728	57%	3,412	53%	3,316	62%
Clinical Risk Factors						
HIV stage of positive partner						
Stage I	9,674	34%	5,746	41%	3,928	28%
Stage II	9,686	34%	4,469	32%	5,217	37%
Stage III	7,101	25%	3,274	23%	3,827	27%
Stage IV	1,891	7%	664	5%	1,227	9%
Log viral load of +ve partner*(Mean, SD)	4.5	0.9	4.7	0.8	4.3	0.9
Circumcised male partner						
Yes	3,766	13%	2,352	17%	1,414	10%
No	24,539	87%	11,777	83%	12,762	90%
Time varying Factors						
Method of Contraception						

Implants	1,735	6%	721	5%	1,009	7%
Injectable	3,813	14%	1,873	14%	1,940	14%
Oral Contraceptive Pills	3,890	14%	1,920	14%	1,970	14%
Non-Hormonal	18,291	66%	9,078	67%	9,213	65%
Pregnancy Status						
Not pregnant	211,885	88%	10,972	90%	10,913	87%
Pregnant	2,089	8%	924	8%	1,165	9%
Post-Partum	860	3%	346	3%	514	4%
Sex with study partner with a condom in past 3 months reported by woman						
Yes	23,389	85%	11,568	85%	11,821	86%
No	3,980	15%	2,031	15%	1,949	14%

*USD: United States Dollar *Nyanja: Commonly spoken language in Lusaka, Zambia

*Fertility Intentions collected from 2001 – 2011. *Viral load collected from 1999 (per log10 copies/ml increase)

*Implants include Intra-uterine Device (Copper IUD)

Table 2. Measures of Unprotected Sex for discordant couples in Lusaka Zambia, 1994 – 2012

	All Couples		M-F+ couples		M+F- couples		
Outcome Measure	**N intervals**	**%**	**N intervals**	**%**	**N Intervals**	**%**	**p-value**
Composite Outcome							
Any unprotected sex*							
Yes	11,025	62%	5,859	63%	5,166	61%	0.0205
No	6,753	38%	3,468	37%	3,285	39%	
Component Outcomes							
Self-Reported occurrence of unprotected sex by woman							
Yes	9,146	33%	4,960	36%	4,186	30%	<0.0001
No	18,230	67%	8,645	64%	9,585	70%	
Sperm present on wet prep							
Yes	1,756	7%	899	7%	857	6%	0.0604
No	24,288	93%	11,871	93%	12,417	94%	
Incident pregnancy							
Yes	592	3%	270	3%	322	3%	0.0613
No	19,353	97%	9,582	97%	9,771	97%	
HIV Sero-conversion							
Yes	478	2%	226	2%	252	2%	0.2446
No	27,874	98%	13,927	98%	13,947	98%	
Incident STI (men)							
Yes	432	3%	183	2%	249	3%	0.0003
No	14,905	97%	7,636	98%	7,269	97%	
Incident STI (women)							
Yes	989	4%	572	5%	417	3%	<0.0001
No	23,252	96%	10,989	95%	12,263	97%	

*Defined as having no indication of unprotected sex (having no indication of sperm on a wet prep, no sero-conversion, no incident pregnancy, no self-reported unprotected sex, and no incident STI during the study intervals)
*STIs: gonorrhea, *Trichomonas vaginalis* infection, chlamydia, herpes, syphilis

Table 3. Unadjusted association between time to unprotected sex and the risk factors collected at enrollment (fixed factors) for discordant couples in Lusaka Zambia, 1994 – 2012

	M-F+ couples				M+F- couples			
Variables	**HR**	**95%CI**		**p-value**	**HR**	**95%CI**		**p-value**
Demographics								
Man age (per year increase)	0.995	0.991	0.998	0.0019	0.99	0.985	0.995	0.0001
Woman age (per year increase)	0.995	0.991	0.999	0.0117	0.992	0.987	0.997	0.0028
Woman age (tertiles)								
15 – 25	1.095	1.028	1.167	0.0052	1.101	1.003	1.208	0.0438
26 – 31	1.047	0.975	1.124	0.2076	1.14	1.033	1.259	0.0092
32 – 62	ref				ref			
Age disparity (per year increase)	0.995	0.99	1	0.0423	0.991	0.983	0.999	0.0226
Years cohabiting (per year increase)	0.992	0.987	0.997	0.0008	0.991	0.985	0.997	0.0025
Monthly family income (per USD increase)	1	1	1	0.4521	1	1	1	0.9391
Education								
Woman reads Nyanja								
Yes, easily	ref				ref			
With difficulty/not at all	1.004	0.943	1.068	0.9093	0.998	0.913	1.091	0.968
Man reads Nyanja								
Yes, easily	ref				ref			
With difficulty/not at all	1.001	0.946	1.058	0.9836	1.028	0.956	1.104	0.4606
Social and Sexual History								
Woman alcohol use last year								
Yes	0.986	0.929	1.046	0.6429	1.089	0.997	1.19	0.0572
No	ref				ref			
Man alcohol use last year								
Yes	1.051	0.984	1.123	0.1419	1.114	1.027	1.21	0.0097
No	ref				ref			
Woman lifetime sex partners (per partner increase)	1.004	1.003	1.006	<0.0001	1.008	0.999	1.017	0.0665
Woman last year sex partners (per partner increase)	1.035	0.994	1.078	0.0975	1.027	0.973	1.084	0.3273
Man lifetime sex partners (per partner increase)	1.001	0.999	1.003	0.2422	0.999	0.997	1.002	0.6879
Man last year sex partners (per partner increase)	1.017	0.996	1.038	0.1167	1.011	0.998	1.024	0.0948
Woman history of STI last year								
Yes	1.016	0.963	1.072	0.5611	0.938	0.872	1.008	0.0824

No	ref				ref			
Man history of STI last year								
Yes	1.092	1.034	1.154	0.0015	1.042	0.971	1.119	0.2499
No	ref				ref			
Family History and Fertility Intentions								
Number of previous pregnancies (per pregnancy increase)	0.992	0.98	1.003	0.1555	0.983	0.968	0.999	0.0385
Pregnant at baseline								
Yes	1.038	0.974	1.106	0.2511	0.941	0.86	1.03	0.182
No	ref				ref			
Fertility intentions of man								
Yes, next year	ref				ref			
Yes, but not next year	1.069	0.978	1.169	0.1408	1.133	0.966	1.329	0.1255
Don't know/No	0.96	0.87	1.059	0.4129	1.127	0.974	1.304	0.109
Fertility intentions of woman								
Yes, next year	ref				ref			
Yes, but not next year	1.089	0.985	1.205	0.0947	1.033	0.9	1.186	0.6415
Don't know/No	1.022	0.937	1.115	0.6249	1.028	0.911	1.159	0.6541
Clinical Risk Factors								
HIV stage of positive partner								
Stage I	ref				ref			
Stage II	1.005	0.942	1.072	0.8854	0.985	0.897	1.082	0.7548
Stage III	1	0.935	1.069	0.9914	0.96	0.866	1.063	0.4314
Stage IV	0.969	0.851	1.103	0.6334	0.964	0.84	1.107	0.6067
Log viral load of positive partner (per log10 copies/ml increase)	0.998	0.956	1.041	0.9101	0.949	0.9	1.002	0.0576
Circumcised male partner								
Yes	ref				ref			
No	1.043	0.971	1.12	0.2528	0.886	0.793	0.99	0.0322

HR – unadjusted Hazard Ratio

Table 4. Unadjusted association between time to unprotected sex and the risk factors collected during follow-up† (time varying factors) for discordant couples in Lusaka Zambia, 1994 – 2012

	M-F+ couples				M+F- couples			
Variables	HR	95%CI		p-value	HR	95%CI		p-value
Number of times sex with partner in project with a condom*	1.001	1	1.002	0.018	1.002	1.001	1.004	0.0009
Sex with study partner with a condom* (categorized)								
Yes	ref							
No	0.812	0.756	0.872	<0.0001	0.756	0.688	0.83	<0.0001
Method of Contraception								
Implanted Devices	0.918	0.804	1.049	0.2091	0.94	0.793	1.113	0.4714
Injectable	1.009	0.929	1.096	0.8269	1.14	1.053	1.234	0.0013
Oral Contraceptive Pills	0.998	0.935	1.066	0.954	1.113	1.028	1.204	0.008
Non-Hormonal	ref							
Pregnancy Status								
Not pregnant	ref							
Pregnant (not incident)	1.304	1.232	1.381	<0.0001	1.341	1.251	1.437	0.0015
Post-Partum (up to 6 months)	0.857	0.751	0.978	0.0219	0.8	0.696	0.981	<0.0001
Post-Partum (Up to 6 months post-delivery)								
Yes	Ref							
No	1.194	1.051	1.355	0.0064	1.299	1.131	1.492	0.0002

† - All factors are as reported by the woman at each 3 month follow-up visit.
HR – unadjusted Hazard Ratio

Table 5. Multivariate Model of Predictors of Unprotected sex for discordant couples in Lusaka Zambia, 1994 – 2012

Variables	aHR*	95%CI		p-value
M-F+ couples				
Man age (per year increase)	1.001	0.995	1.006	0.8524
Years cohabiting (per year increase)	0.999	0.99	1.008	0.8009
Woman lifetime sex partners (per partner increase)	1.003	1.002	1.004	<0.0001
Man history of STI last year				
Yes	1.149	1.062	1.243	0.0006
No	ref			
Sex with study partner with a condom in past 3 months reported by woman				
Yes	ref			
No	0.903	0.809	1.007	0.0674
Pregnancy status				
Not pregnant/ not post-partum/ incident pregnant	ref			
Pregnant (prevalent, not incident, pregnancy)	1.191	1.08	1.314	0.0005
Post-partum (up to 6 months)	0.859	0.682	1.082	0.1967
M+F- couples				
Man age (per year increase)	1	0.99	1.009	0.9218
Years cohabiting (per year increase)	1.002	0.991	1.014	0.7
Sex with study partner with a condom in past 3 months reported by woman				
Yes	ref			
No	0.795	0.658	0.961	0.0177
Pregnancy status				
Not pregnant/ not post-partum/ incident pregnant	ref			
Pregnant (prevalent, not incident, pregnancy)	1.253	1.113	1.41	0.0002
Post-partum (up to 6 months)	0.949	0.737	1.221	0.6821
Method of contraception				
IMP	0.902	0.698	1.165	0.4284
INJ	1.16	1.004	1.339	0.0433
OCP	1.15	1.006	1.316	0.0413
Non-hormonal	ref			

*aHR – Adjusted Hazard Ratio

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