Epidemiological Linkages between Sexually Transmitted Infections

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Abstract

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INTRODUCTION: Interactions between STIs can be complex and difficult to disentangle. These conditions share a common group of sexual risk factors, which may lead to the identification of non-causal associations. Studies that aim to determine the temporal sequence of STIs require prospective data collection with frequent STI assessment, as well as careful control for potential confounding factors. We conducted three studies to further characterize the complex inter-relationships between several STIs including HIV, utilizing data from a cohort of female sex workers (FSWs) in Mombasa, Kenya. The specific aims of these studies were: 1) To explore the contribution (population attributable risk percent) of STIs to HIV acquisition among Kenyan FSWs, and how this has changed over time since the cohort was established 2) To assesses the incidence and correlates of *Chlamydia trachomatis* infection among HIV-seropositive and seronegative women enrolled in the cohort and 3) To determine the effect of incident HSV-2 on subsequent BV episodes among HIV-seronegative women.

METHODS: We conducted longitudinal follow-up and prospective cohort analyses to complete the three specific aims. The study was conducted within the Mombasa cohort, an established, open cohort study of high-risk women. Follow-up began in February 1993, with over 3500 women enrolled to date. The eligibility criteria to join the cohort are: age 16 – 50 years, residing

in the Mombasa area, self-identifying as exchanging sex for payment in cash or in kind, and able to provide informed consent. Participants were seen monthly at the research clinic, where a standardized interview addressing past medical and sexual history is administered. They also receive a physical and pelvic examination, with collection of genital samples to test for STIs.

RESULTS:

Aim 1: Between 1993 and 2012, 1,964 women contributed 6,135 person-years of follow-up. The overall PAR% for each infection was; prevalent HSV-2 (48.3%), incident HSV-2 (4.5%), BV (15.1%), intermediate microbiota (7.5%), vaginal yeast (6.4%), *T. vaginalis* (1.1%), *N. gonorrhoeae* (0.9%), non-specific cervicitis (0.7%), GUD (0.8%), genital warts (-0.2%). The PAR% for prevalent HSV-2 (40.4%, 61.8%, 58.4%, 48.3%) and BV (17.1%, 19.5%, 14.7%, 17.1%), were high but did not change significantly over time. The PAR% for trichomoniasis, gonorrhea, GUD and genital warts all remained <3% across the four study periods.

Aim 2: Between August 2006 and December 2010, 865 women contributed 2011 person-years of observation. Sixty-four women experienced 101 episodes of C. trachomatis infection (incidence rate of 5.0/100 person-years). In multivariate analyses, younger age (<25 years and 25-34 years versus ≥35 years; hazard ratio [HR] 8.49 95% CI 4.1-17.7 and HR 2.9 95% CI 1.7-5.0 respectively), depot medroxyprogesterone acetate use (HR 1.8 95% CI 1.1-3.0) and recent Neisseria gonorrhoeae infection (HR 3.3 95% CI 1.5-7.4) were significantly associated with increased risk of acquiring C. trachomatis infection.

Aim 3: Between 1993 and December 2010, one hundred and sixty four of 406 HSV-2/HIV-1-seronegative Kenyan women acquired HSV-2, incidence rate 21/100 person-years. Incident HSV-2 was associated with increased likelihood of BV (adjusted OR 1.28; 95% CI 1.05-1.56; p=0.01).

CONCLUSION: At the population level, abnormal vaginal microbiota and HSV-2 have consistently been the largest contributors to these high-risk women's risk for HIV acquisition over the past 20 years. Interventions that prevent these conditions would benefit women's health generally, and also hold potential for reducing HIV risk in women. Secondly, we found a high incidence of *C. trachomatis* among younger high-risk women suggesting the need for screening as an important public health intervention for this population. Finally, our findings strengthen the evidence for a causal link between genital HSV-2 infection and disruption of the vaginal microbiota.

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DEDICATION

For Ngari and Marigu Wamai. May you be inspired to live extraordinary lives that will make a difference in this world.

And as always, for Ngondi Wamai.

CHAPTER 1: Introduction

Despite sustained prevention efforts, HIV continues to spread globally. In 2012, 25 million adults were living with HIV-1 in sub-Saharan Africa [1]. In the same year, approximately 1.6 million became newly infected, with the highest incidence occurring among sexually active adults. Throughout the region, women account for 60% of all HIV infections. According to the Kenya AIDS Indicator Survey 2007 (KAIS), 14% of all new HIV infections in this country occurred among FSWs and their clients [2]. Female sex workers, therefore, continue to be an important group in HIV transmission dynamics in Kenya.

Sexually transmitted infections (STIs) increase susceptibility of HIV-seronegative individuals to HIV acquisition. They may also increase infectivity of HIV-seropositive individuals. HIV infection, in turn, increases susceptibility to some STIs [3-6]. Curable STIs might be particularly important in the early stages of an HIV epidemic, when people with high-risk sexual behaviors are more likely to be infected [7]. As the HIV epidemic matures, the proportion of new HIV infections attributable to curable STIs may decline. Thus, in the later stages of an HIV epidemic, incurable STIs such as herpes simplex virus type 2 (HSV-2) may take a proportionally greater role as drivers of the epidemic. Monitoring and understanding the trends in the proportion of HIV infections attributable to different STIs at different stages of an HIV epidemic may be helpful for informing the development of interventions to control the spread of HIV.

Less attention has been directed to understanding the potentially important interactions between STIs other than HIV. For example, important interactions between bacterial vaginosis (BV) and other STIs such as *Chlamydia trachomatis* and HSV-2 have been observed [8-19]. Sexually transmitted infections are associated with significant adverse reproductive health outcomes. *Chlamydia* causes tubal infertility and increases the risk for potentially life-threatening ectopic pregnancy [20]. Bacterial vaginosis is among the most frequent causes of vaginal complaints among women, causing malodorous vaginal discharge [21]. HSV-2 can cause recurrent painful herpetic ulcers. These distressing symptoms frequently prompt women to seek care, which can

be time-consuming and costly. In addition, conditions such as BV and HSV-2 are difficult to treat or incurable with frequent recurrences. Thus, understanding how these STIs influence each other will guide strategies for controlling these infections and their adverse outcomes.

Interactions between STIs can be complex and difficult to disentangle. These conditions share a common group of sexual risk factors, which may lead to the identification of non-causal associations. Studies that aim to determine the temporal sequence of STIs require prospective data collection with frequent STI assessment, as well as careful control for potential confounding factors. Taken together these factors underlie this dissertation, the aim of which was to characterize the complex inter-relationships between several STIs including HIV. The analyses presented in the subsequent chapters utilize data from a cohort of female sex workers (FSWs) in Mombasa, Kenya.

The Mombasa Cohort was established in 1993 to identify the risk factors for HIV acquisition among FSWs, utilizing funding from the US National Institutes of Health (NIH), Preparation for AIDS Vaccine Evaluation program. The cohort has been maintained for >20 years with overlapping funding from multiple NIH grants as well as funding from other resources including grants from Family Health International and the Puget Sound Partners for Global Health. Over 3000 women have been enrolled in this cohort since its initiation. Participants have regular clinic visits with frequent screening for STIs and regular collection of data on sexual risk behaviors. Access to data from this sizeable population and the collaborative infrastructure and relationships in Kenya and Seattle provided a unique opportunity to conduct the studies detailed in this dissertation. The specific aims of these studies were:

1) To explore the contribution (population attributable risk percent [PAR%]) of STIs to HIV acquisition among Kenyan FSWs, and how this has changed over a 20-year period spanning 1993 through 2012 (chapter two)

2) To assess the incidence and correlates of *Chlamydia trachomatis* infection among HIV-seropositive and seronegative women in the cohort (chapter three)
3) To determine the effect of incident HSV-2 on subsequent BV episodes among HIV-seronegative women (chapter four).

The following paragraphs provide a short description of each chapter.

Chapter 2: Changes in the contribution (population attributable risk %) of genital tract infections to HIV acquisition among Kenyan female sex workers from 1993 to 2012

Sexually transmitted infections are significant co-factors in the global spread of HIV [3, 5], and a considerable source of morbidity, especially among women of reproductive age [22, 23]. There is evidence that the contribution of different STIs to HIV acquisition is dependent upon the stage of the HIV epidemic [24]. We had access to a large dataset spanning 20 years of data collection (1993-2012) and 325 HIV seroconversion events. Access to these prospective data facilitated our exploration of how the relative contribution of different STIs, measured as their PAR%, has changed over time among HIV-seronegative women enrolled in the Mombasa Cohort. Our principal goal was to estimate the PAR% of various genital tract conditions (*Neisseria gonorrhoeae*, non-specific cervicitis, *Trichomonas vaginalis*, BV, vaginal candidiasis, HSV-2, genital ulcer disease and genital warts) to HIV acquisition in this population of high-risk Kenyan women. Findings from this study are important in at least two major respects. First, these data are helpful in interpreting the findings from numerous large trials that have evaluated STI prevention interventions as a strategy for reducing HIV incidence. Second, the findings have the potential to contribute to future HIV prevention research by focusing attention on those genital tract conditions that may contribute the most to HIV transmission dynamics at the population level.

Chapter 3: Incidence and correlates of *Chlamydia trachomatis* in a high-risk cohort of Kenyan women

Chlamydia trachomatis is caused by an obligate intracellular gram negative organism that is difficult to culture, and until recently, reliable diagnosis of this pathogen has been elusive. This has resulted in lack of testing, and therefore a lack of data, particularly in resource-limited settings such as sub-Saharan Africa. Less than 10% of those infected with *Chlamydia* develop acute signs and symptoms of infection, resulting in a substantial number of untreated cases [25, 26]. In addition, *Chlamydia* infection is associated with serious reproductive health problems such as pelvic inflammatory disease, ectopic pregnancy, tubal infertility and chronic pelvic pain [27].

In this chapter, we discuss findings from an analysis of the incidence and correlates of *C. trachomatis* infection among HIV-seropositive and seronegative women enrolled in the Mombasa Cohort. We utilized the Gen-Probe Aptima GC/CT Detection System, a nucleic acid amplification test which consists of amplifying the *C. trachomatis* ribosomal RNA sequences using transcription-mediated amplification (TMA). This test has excellent sensitivity (94.2%) and specificity (97.6%) for detection of *Chlamydia* on endocervical swabs [28]. This study adds substantial new information to a sparse body of literature on the incidence and risk factors for *Chlamydia* in African women. Identifying correlates of infection among women in sub-Saharan Africa is useful in formulating interventions to reduce the burden of this infection. Targeted screening and development of screening algorithms will continue to be useful in light of the relatively high cost and technical difficulty of performing the currently available tests. Developing inexpensive point-of-care tests that can be used in resource-limited settings will enhance the diagnosis and early management of this silent epidemic. This research has been published in the Sexually Transmitted Diseases journal [29].

Chapter 4: Incident herpes simplex virus type 2 infection increases the risk of subsequent bacterial vaginosis episodes

Evidence is mounting to support the interaction between HSV-2 and BV. Herpes simplex virus type 2 (HSV-2) infected women have a higher prevalence of bacterial vaginosis (BV) compared to HSV-2-seronegative women [9, 14, 15]. To explore the temporal relationship between HSV-2 acquisition and subsequent risk of BV, we leveraged the large amount of prospectively collected data from the Mombasa Cohort to test the hypothesis that incident HSV-2 would be associated with subsequent increases in the frequency of BV. Findings from this study have advanced our understanding of the effect of incident HSV-2 on BV acquisition. These results will be useful for designing and assessing future interventions aimed at reducing the prevalence of BV. In addition, understanding the bi-directional interactions between BV and HSV-2 could have important HIV-1 prevention implications, because both HSV-2 [30-35] and BV [36, 37] have been associated with a greater risk of acquiring and transmitting HIV-1 [18, 38, 39].This research has been published in the Journal of Infectious Diseases [40].

In summary, findings from these three prospective studies advance our knowledge of the risk factors for HIV and STI acquisition among FSWs. These data are helpful for understanding the history of genital infections' contribution to HIV acquisition and to future interventions to reduce HIV and STI incidence.

CHAPTER 2: Changes in the Contribution of Genital Tract Infections to HIV acquisition among Kenyan Female Sex Workers from 1993 to 2012

ABSTRACT

BACKGROUND: Understanding temporal trends in the contribution of different genital infections to HIV incidence over the past two decades will be important for optimizing future HIV and sexually transmitted infection (STI) prevention efforts.

METHODS: In a long-term open cohort study of HIV-seronegative high-risk women, we performed monthly evaluations for HIV, vaginal yeast, bacterial vaginosis (BV), *Trichomonas vaginalis, Neisseria gonorrhoeae*, non-specific cervicitis, herpes simplex virus type 2 (HSV-2), genital ulcer disease (GUD) and genital warts. We used Cox regression to evaluate the association between STIs and HIV acquisition over 4 time periods (1993-1997, 1998-2002, 2003-2007 and 2008-2012). Models were adjusted for age, hormonal contraceptive use, workplace, sexual risk behavior, and other STIs. The resulting hazard ratios were used to calculate population attributable risk percent (PAR%).

RESULTS: Between 1993 and 2012, 1,964 women contributed 6,135 person-years of follow-up. In adjusted analyses, HSV-2 status (prevalent HSV-2 and incident HSV-2 versus HSV-2 seronegative; hazard ratio (HR) 2.50 95% confidence interval (CI) 1.51-4.13, and HR 2.95 95% CI 1.63-5.34 respectively), abnormal vaginal microbiota (intermediate vaginal microbiota and BV versus normal microbiota; HR 1.54 95% CI 1.13-2.09 and HR 1.86 95% CI 1.40-2.47 respectively), vulvovaginal candidiasis (HR 2.09 95% CI 1.62-2.70), gonorrhea (HR 2.05 95% CI 1.38-3.04) and GUD (HR 2.23 95% CI 1.14-4.35) were significantly associated with increased risk of acquiring HIV. The overall PAR% for each infection was; prevalent HSV-2 (48.3%), incident HSV-2 (4.5%), intermediate microbiota (7.5%), BV (15.1%), vaginal yeast (6.4%), *T. vaginalis* (1.1%), *N. gonorrhoeae* (0.9%), non-specific cervicitis (0.7%), GUD (0.8%), genital warts (-0.2%). Over the course of the four time periods studied, the PAR% for prevalent HSV-2 (40.4%, 61.8%, 58.4%, 48.3%) and BV (17.1%, 19.5%, 14.7%, 17.1%), remained relatively high and did not show a significant trend for change over time. The PAR% for trichomoniasis,

gonorrhea, GUD and genital warts all remained <3% across the four study periods. Only the PAR% of HIV attributable to vulvovaginal candidiasis changed significantly over time, beginning at 7.3% and decreasing steadily across the four time periods (5.0%, 4.1%, -0.1%, test for trend p=0.04).

CONCLUSIONS: At the population level, abnormal vaginal microbiota and HSV-2 have consistently been the largest contributors to these high-risk women's risk for HIV acquisition over the past 20 years. Interventions that prevent these conditions would benefit women's health generally, and also hold potential for reducing HIV risk in women.

INTRODUCTION

Preventing the spread of HIV continues to be a challenge worldwide, particularly in sub-Saharan Africa [41]. In this region, a substantial geographic overlap between areas with high STI and HIV prevalence has been recognized since early in the HIV epidemic. Recognition of this geographic overlap led to the hypothesis that STIs could influence HIV transmission [42]. Although evidence from observational studies has supported this hypothesis [18, 31, 34, 36-38, 43-49], treatment of STIs to reduce HIV incidence has not proven to be effective in the majority of clinical trials. Data from nine randomized trials conducted to date have been disappointing (Table 2.1) [50], with only one showing efficacy of STI treatment as a HIV prevention tool. This has raised critical questions about whether STI treatment for HIV control is a feasible prevention tool [51].

In an effort to understand the discrepancy between findings from observational studies supporting the role of STIs in HIV acquisition and negative findings from clinical trials, several theories have been advanced [7, 52-55]. Variations in the interventions, differences in STI prevalence, and other important factors such as lack of power due to declining HIV incidence and poor participant retention rates may have contributed to the striking differences in the findings from the trials [56]. The most widely-accepted and plausible factor, however, is the one proposed by Hitchcock and Fransen, suggesting that the contribution of STIs to new HIV infections may change over time as an HIV epidemic matures [55]. In their commentary, they note that the seemingly contradictory results from the first two studies (Mwanza and Rakai) were consistent with a widely accepted model of the forces necessary to establish an HIV epidemic. This mathematical tool was developed by May and Anderson to predict the spread of infectious diseases (equation 1) [57].

$$R_0 = \beta D c \qquad (1)$$

Where R_0 is the basic reproductive rate (the average number

of secondary infections generated by one primary case), **β** is the probability/efficiency of transmission, **D** is the duration of infectiousness and **c** is the mean rate of sexual partner change. If R_0>1, the infection will spread. If R_0<1, infection will be controlled. Efficiency of transmission is determined by infectiousness of the HIV infected individual and susceptibility of those at risk. STIs may increase the efficiency of HIV transmission by increasing infectiousness of HIV infected individuals and enhancing susceptibility of individuals to infection.

In this model, STIs have their greatest impact on HIV transmission during the early stages of an epidemic, when HIV spreads primarily among high-risk groups. Individuals with multiple partners are more likely to have an STI, which increases the efficiency of HIV transmission and the likelihood that the virus will be acquired during a few sexual encounters with an HIV-positive partner. In a more mature epidemic, STIs may be less important in driving transmission at the population level. This is because HIV is spreading throughout the general population, among people who have fewer sex partners but a higher number of sexual encounters per partner. After repeated exposure, transmission may eventually occur [58]. Thus, in an early epidemic, the proportion of new HIV infections attributable to STIs such as *Chlamydia*, gonorrhea, and syphilis is much higher than at later stages [59, 60]. As the epidemic matures, there is a shift towards a greater proportion of HIV transmission attributable to incurable infections such HSV-2 [61]. This theory is supported by simulation studies suggesting that role of STIs in HIV transmission may be influenced by the stage of the epidemic. Freeman et al demonstrated that time since the introduction of HIV in a community determines the contribution of STIs to HIV acquisition [24]. In this study, the authors demonstrate a fall in population attributable risk percent (PAR%) for curable STIs with a simultaneous increase in the importance of HSV-2 as the HIV epidemic matures. However, not all studies have observed this kind of transition. A recent meta-analysis assessing the trends in HIV risk concluded that risk factors for HIV

acquisition and transmission remained relatively unchanged, early versus later in the epidemic [62].

Due to the conflicting findings in the literature, we sought to determine how the relative contribution of different STIs, measured as their PAR%, has changed over time among HIV-seronegative women enrolled in a longitudinal open cohort of female sex workers in Mombasa, Kenya. Our principle goal was to estimate the PAR% of various genital tract conditions (*Neisseria gonorrhoeae*, non-specific cervicitis, *Trichomonas vaginalis*, BV, vaginal candidiasis, and HSV-2, genital ulcer disease (GUD) and genital warts) to HIV acquisition in this population of high-risk Kenyan women.

MATERIALS AND METHODS

We conducted longitudinal follow-up of women participating in an open cohort study of high-risk women in Mombasa, Kenya between February 1993 and December 2012. The Mombasa cohort was established in 1993 to identify risk factors for HIV acquisition and has subsequently conducted observational and interventional studies related to HIV and STI prevention. The eligibility criteria to join the cohort were: age 18–50 years, residing in the Mombasa area, self-identifying as exchanging sex for payment in cash or in kind, and able to provide informed consent. This study was approved by the ethics review boards of Kenyatta National Hospital and the University of Washington. All participants provided written informed consent.

Clinic Procedures

At enrollment and monthly follow-up visits, a study nurse conducted a standardized interview detailing demographic data, medical, gynecological, and sexual history. Thereafter, a study physician performed a physical examination including a pelvic speculum examination. During the pelvic examination, swabs of cervical and vaginal secretions were collected for STI testing. Swabs were also collected from the base of genital ulcerations if these were observed. Blood is

also collected for serological testing for HIV and HSV-2. Women were asked to return for their results a week later.

In the course of this study, three clinical trials were conducted in the Mombasa Cohort. The first was a phase III randomized, double-blind, placebo-controlled trial to assess the effect of intravaginal nonoxynal-9 gel use on HIV and STI acquisition, and was conducted between July 1996 and February 1998 [63]. The second was a phase II randomized, double-blind, placebo-controlled trial to test the efficacy of monthly oral periodic presumptive treatment (PPT) with 2 g of metronidazole plus 150 mg of fluconazole to reduce the incidence of BV, vaginal candidiasis, and *T. vaginalis* infection and to promote vaginal colonization with *Lactobacillus* species among women at risk for HIV-1 acquisition [64]. This trial was conducted between May 2003 and November 2005. The third was another phase II randomized, double-blind, placebo-controlled clinical trial to test the efficacy of monthly topical PPT with co-formulated vaginal suppositories containing 750g of metronidazole plus 200 mg of miconazole to reduce the incidence of BV and vaginal candidiasis, conducted between 2011 and 2013 (data analyses ongoing). For the first and second trials, eligible participants were followed as part of the Mombasa Cohort. Participants for the third trial were recruited from within the cohort as well as from the general population. For women recruited from the Mombasa Cohort, we were able to abstract data from the monthly trial visits to capture many data elements for routine Mombasa Cohort participation. These data were entered into the Mombasa Cohort dataset, but include a small proportion of systematically missing Mombasa Cohort variables due to differing study procedures. To assess the effect of trial participation on our overall results, we performed sensitivity analyses in which we excluded data from all three clinical trials from our dataset.

Treatment of Infections

All the women enrolled in the Mombasa Cohort received free outpatient medical services that included treatment of STIs according to WHO [65] and Kenyan national guidelines.

Asymptomatic cases of BV and vaginal yeast were not treated. Syndromic management was offered during examination visits if indicated. All genital specimens were collected before any medication was dispensed. At the results visit, additional treatment was provided if indicated by the laboratory test results.

Laboratory Procedures

HIV-1 serostatus was determined by ELISA (Detect HIV1/2, BioChem Immunosystems, Montreal, Canada or PT-HIV 1,2-96, Pishtaz Teb Diagnostics, Tehran, Iran). Positive tests were confirmed using a second ELISA (Recombigen, Cambridge Biotech, Worcester, MA, USA [February 1993-August 2004] or Bio-Rad HIV-1/HIV-2, Bio-Rad Laboratories, Hercules, CA, USA [August 2004-May 2006] or Vironostika HIV-1 Uniform II AG/AB, bioMerieux, Marcy l'Etoile, France [May 2006-December 2012). Vaginal Gram's stained slides were evaluated for BV using Nugent's criteria [66]. Bacterial vaginosis was defined as a score of 7-10 and intermediate microbiota as a core of 4-6. Saline wet mounts were examined at 40X power for the presence of motile trichomonads, clue cells, and yeast. Potassium hydroxide wet mounts were examined for the presence of yeast. Culture for *N. gonorrhoeae* was performed on modified Thayer Martin media. Cervical Gram's stained slides were examined microscopically for the number of polymorphonuclear lymphocytes, with a count of ≥30 being considered as non-specific cervicitis. Starting August 2006, endocervical samples were tested for the presence of *N. gonorrhoeae* and *Chlamydia trachomatis* by transcription mediated amplification using the Gen-Probe Aptima GC/CT Detection System (Gen-Probe, San Diego). Because the chlamydia data do not span the entire period of cohort participation, they were not used for this study. Serological testing for HSV-2 was performed using a type-specific HSV-2 gG based ELISA (HerpeSelect 2, Focus Diagnostics, Cypress, California, USA).

Data Analyses

This study was conducted between February 1993 and December 2012. This 20 year period was categorized into four five-year time periods: 1993–1997, 1998-2002, 2003–2007, 2008-2012. Only women who were HIV seronegative at baseline (enrolment) were included in the analyses. The exposures were each of several genital tract infections/conditions (vulvovaginal candidiasis, BV, *T. vaginalis*, *N. gonorrhoeae*, non-specific cervicitis, HSV-2, genital ulcers and genital warts). The outcome was time to HIV acquisition. We determined the incidence rates of each of the genital tract conditions for the overall study period and for each time period, allowing repeat infections for all conditions except HIV and HSV-2.

Person-time was calculated as time from enrollment until time of first positive HIV test, last clinic attendance date (for women lost to follow-up), or end of study period (for women remaining HIV-uninfected). We assumed an effect window of 60 days to capture the influence of STIs on HIV seroconversion risk [47]. That is, we assumed the effect of STI on HIV susceptibility would persist for 15 days after the infection was detected at a clinic visit. We also estimated that HIV seroconversion would be detected 45 days after HIV acquisition, assuming that infection would occur at the midpoint between visits, with visits occurring every 30 days. Thus, the total window of effect for any STI to influence HIV susceptibility was estimated to be 60 days (15+45).

To assess trends of HIV acquisition in the cohort, we used Cox regression models to determine the hazard ratios (HR) and 95% confidence intervals (CIs) for the effect of STIs on time to HIV seroconversion during the entire 20 year period and for each of the four time periods. First we determined the incidence and correlates of HIV acquisition for the entire 20 year time period. For each STI, we performed a linear test of trend to assess the change in incidence across the different time periods We conducted univariate analyses to determine whether individual risk factors were associated with HIV infection. Variables that were significantly associated with HIV in the univariate analyses were then included in the multivariate model. For each time period,

the final Cox model included the confounding factors from the multivariate model for the entire 20-year period. We decided *a priori* to include all genital tract conditions in the final multivariate model.

PAR% was calculated from the adjusted HR obtained from the final Cox models. The formula for PAR% = pc (HR-1/HR) X 100%, where pc is the proportion of cases (at the visit level) that were exposed during the study. For each STI, we performed a linear test of trend to assess the change in PAR% across the different time periods. We also prepared a graphical presentation of the change in PAR% across the five time periods. Analyses were performed using IBM SPSS 19.0 (IBM, Kirkland, WA, USA) and STATA 12 (StataCorp, College Station, TX, USA).

RESULTS

Between 1993 and 2012, we enrolled 2301 HIV seronegative women, of whom 1,964 returned for at least one follow-up visit in the cohort and were included in this analysis. Table 2.2 shows the baseline characteristics of these participants. Six hundred and ninety nine (36%) of the women were less than 25 years of age. The majority (1310, 66%) reported that they worked at a bar or restaurant. Alcohol use was common (1512, 77%). Except for BV and prevalent HSV-2 infection, which were identified in 642 (33%) and 1442 (73%) respectively, diagnosis of STIs and other genital tract conditions at baseline was rare.

The enrolled women contributed 6,135 person-years of follow-up. The median duration of follow-up was 1,136 days (interquartile range [IQR] 315-2,878). Three hundred and twenty five women acquired HIV, resulting in an overall incidence rate of 5.3 per 100 person-years (Table 2.3). The numbers of seroconverters in 1993-1997, 1998-2002, 2003-2007 and 2008-2012 were 150, 98, 56, and 21 respectively. The HIV incidence declined over the course of the study from 11.8 per 100 person-years in the first time period to 1.3 per 100 person-years in the last time period. This pattern of declining incidence across the four time periods was similar for *T.*

vaginalis and non-specific cervicitis. Incidence rates of the other genital conditions demonstrated more variability over time.

In univariate analyses, all genital tract conditions except genital warts were associated with an increased likelihood of acquiring HIV (Table 2.4). In multivariate analyses, HSV-2 status (prevalent HSV-2 and incident HSV-2 versus HSV-2 seronegative; HR 2.50 95% CI 1.51-4.13, and HR 2.95 95% CI 1.63-5.34 respectively), abnormal vaginal microbiota (intermediate vaginal microbiota and BV versus normal microbiota; HR 1.54 95% CI 1.13-2.09 and HR 1.86 95% CI 1.40-2.47 respectively), vulvovaginal candidiasis (HR 2.09 95% CI 1.62-2.70), gonorrhea (HR 2.05 95% CI 1.38-3.04) and GUD (HR 2.23 95% CI 1.14-4.35) remained significantly associated with increased risk of acquiring HIV. The association between trichomoniasis and HIV acquisition was of borderline significance (HR 1.41 95% CI 0.99-2.02).

Table 2.5 illustrates changes over the four time periods in the association between genital tract conditions and HIV acquisition. Although the increased risk in HIV acquisition across the four time periods remained for most of the genital tract conditions, there was less power for these subgroup analyses, and not all associations remained statistically significant. Nonetheless, the adjusted analyses are useful for assessing trends across time and calculating PAR%.

To calculate PAR%, we first determined the visit-level prevalence of the different genital tract conditions. Herpes simplex virus type 2, BV, and intermediate microbiota had the highest prevalences for the overall study period at 80.4%, 32.6% and 21.3% respectively (Table 2.6). These three conditions remained highly prevalent across the four time periods. The overall PAR% for each infection was; prevalent HSV-2 (48.3%), incident HSV-2 (4.5%), BV (15.1%), intermediate microbiota (7.5%), vaginal yeast (6.4%), *T. vaginalis* (1.1%), *N. gonorrhoeae* (0.9%), non-specific cervicitis (0.7%), GUD (0.8%), genital warts (-0.2%) (Table 2.7). Across the four time periods, the PAR% for prevalent HSV-2 (40.4%, 61.8%, 58.4%, 48.3%) and BV

(17.1%, 19.5%, 14.7%, 17.1%), remained high, and there was no significant temporal trend for change in the contribution of these conditions to HIV acquisition. The PAR% for trichomoniasis, gonorrhea, GUD and genital warts all remained <3% across the four study periods. Only the PAR% of HIV attributable to candida changed significantly over time, beginning at 7.3% and decreasing steadily across the four time periods (test for trend p=0.04). Figure 2.1 is a graphical presentation of the PAR% results, highlighting HSV-2 and BV as the leading contributors to HIV acquisition.

This analysis excluded 437 women who did not return for follow-up visits after enrollment. Compared to the 1,964 women who continued in follow-up, the women who were lost to follow-up were slightly younger (median age 26 versus 28, p=<0.001), more educated (9% versus 6% with 8-12 years of education, p=0.004), less likely to have ever been married (44% versus 52%, p=0.002) , had slightly fewer children (median 1 versus 2, p=0.03) and were less likely to use oral contraceptive pills (7% versus 12%, p=0.013). They were also less likely to report having more than 1 alcoholic drink per week and less likely to have non-specific cervicitis or HSV-2. These two groups did not differ in terms of sexual risk behavior and prevalence of genital tract conditions except non-specific cervicitis and HSV-2 (data not shown). Apart from age and HSV-2, none of the other factors were associated with HIV acquisition in this population.

In sensitivity analyses excluding data from visits during which participants took part in three randomized controlled trials to assess the effect of PPT on the incidence of genital tract conditions, we found that the PAR% for HSV-2 was higher, while the PAR% for BV and abnormal microbiota was lower than with the full dataset but these two conditions remained the largest contributors to HIV acquisition (data not shown).

DISCUSSION

In this 20 year prospective study of African women at high risk for HIV acquisition, HSV-2 and BV consistently were the genital tract infections contributing the greatest risk to HIV acquisition. The PAR% for these two conditions remained relatively high and stable over time, around 53% for HSV-2 and 15% for BV. Curable STIs also contributed to HIV acquisition throughout the study period, but their PAR% contributions were much lower because these conditions were present at far fewer visits. The overall PAR% for curable STIs was 9% during the study period. The contribution of curable STIs ranged from 10.7% in the first time period to 1.4% in the last time period. This finding may reflect aggressive screening and treating of STIs in the cohort as well as the counselling and behavior change communication that the women receive once they have joined the cohort. This interesting finding may also reflect transmission dynamics in Kenya. Although the HIV/STI incidence was relatively high in 1993 when the cohort was established, transmission dynamics were already slowing down in the country.

The findings from this study may be helpful in considering the results of prior trials of interventions to reduce HIV incidence by targeting STIs. Many of these RCTs targeted bacterial STIs, which appear to be less important as drivers of transmission (Table 2.1). Only 1 clinical trial of STI treatment for HIV-1 prevention demonstrated a statistically significant reduction in HIV incidence [67]. It has been suggested that epidemic phase was the major reason for the discordant results between the Mwanza trial that showed a 38% reduction in HIV incidence and the Rakai trial that showed only a 3% non-significant reduction in HIV incidence [54]. It is assumed that the relative contribution of curable STIs to HIV acquisition may decline in the later stages of the epidemic. The baseline HIV prevalence in communities included in the Mwanza trial was 4% while the baseline community HIV prevalence in the Rakai communities was 16%. In a sub-analysis of the Rakai data (mature epidemic), the PAR% for curable STI or STI symptoms in HIV-1 transmission was found to be only 10% [59]. In contrast, in the Mwanza trial

(young epidemic), 43% of HIV-1 infections among men in the comparison group could be attributed to symptomatic STIs or new episodes of syphilis [60]. Another credible explanation for success in the Mwanza trial was the syndromic STI management approach which could have had a major impact on *Haemophlilus ducreyi*. This organism is the cause of chancroid, which causes painful genital ulcers and tender suppurative inguinal adenopathy that may be particularly amenable to syndromic management [68], and was highly prevalent in East Africa in the early 1990s [69]. It has also been noted that 43% of GUD cases in Rakai were due to HSV-2 compared to <10% in Mwanza town [54]. Other plausible explanations for the failure to reduce HIV incidence involve technical issues such as low incidence in some studies, greater between community variability in clusters, contamination between intervention and control communities, and high attrition rates that led to under-powered studies [56]. Two rigorously conducted trials targeted HSV-2 suppression, and these also failed to demonstrate an effect on HIV incidence [70, 71]. This effect has been attributed to the pharmacokinetics of acyclovir, which has a short half-life and poor absorption [72]. As a result, plasma levels at the standard dose of 400mg twice daily may not have been sufficient to suppress the persistent immune activation triggered by HSV-2 infection that enhances HIV acquisition. In addition, results from an in-vitro study demonstrated that CD4+ and CD8+ T cells persist at sites of HSV-2 reactivation for months after healing, even with daily antiviral therapy [73].

Several lines of evidence support the biological plausibility of a causal association between STIs and HIV acquisition, which may be mediated through genital mucosal disruption, inflammatory responses, and changes in genital tract milieu. First, interruption of the genital mucosal barrier weakens one of the body's primary defenses against HIV. During HSV-2 reactivation, macro- or micro-ulcerations cause mucosal disruption in the genital tract, which can facilitate HIV entry during exposure to HIV-infected genital fluids [74, 75]. Secondly, inflammatory responses have been cited as a factor that may increase biological susceptibility. This is thought to occur by

infection of host CD4+ lymphocytes, macrophages and dendritic cells through chemokine receptors on these and other cell types. The predominant co-receptor is C-C chemokine receptor type 5 (CCR5). Sexually transmitted infections cause local inflammation and selectively increase recruitment of CD4 and CCR5 positive cells, which may serve as targets for HIV infection in mucosal tissue [76-79]. Finally, the healthy vagina has a number of natural protective factors against HIV. At puberty, lactobacilli begin to thrive and consume glycogen, breaking it down into lactic acid which maintains a low (acidic) pH of approximately 4.0 in the vagina. This acidic condition kills microorganisms. Lactobacilli also produce hydrogen peroxide, which kills pathogens, and has been shown to interfere with HIV *in vitro* [80]. In most women, the large number of lactobacilli in the vagina is maintained. When lactobacilli are depleted, as happens in BV, the vaginal pH rises and there is overgrowth of various anaerobic bacteria. This results in weakening of the innate defenses of the vagina facilitating infection by HIV [81].

It is interesting to note that there are important interactions between HSV-2 and BV, the two genital tract conditions that account for the greatest PAR% for HIV acquisition. Women with BV are more likely to acquire HSV-2 [8, 10-13]. There is also evidence that prevalent and incident HSV-2 infection is associated with increased prevalence of BV [9, 14, 15, 40]. In general, HSV-2 and BV appear to work together, with each driving the acquisition and/or transmission of the other. Figure 2 (from van de Perre*, Lancet Infect Dis* 2008) attempts to summarize the association between BV, HSV-2 and HIV-1. While the diagram does not capture all the complex mucosal and systemic interactions between the three diseases, it does provide a useful summary. Preventing HSV-2 and BV as distinct diseases and also devising innovative ways to mitigate the interactions between these two conditions could help to reduce women's risk of acquiring HIV.

While most studies targeting genital tract infections as a strategy to reduce HIV incidence have not demonstrated efficacy, it remains plausible that effective strategies for preventing herpes

and BV could reduce HIV incidence. Possible interventions for HSV-2 control include vaccination, and pre-exposure prophylaxis for HSV-2 negative individuals, and reducing BV. Despite the disappointing results of the Herpevac trial, a herpes vaccine remains an important research priority [82]. To be most effective, the HSV-2 vaccine would preferably be a pre-pubertal vaccine administered to males and females before they become sexually active. The efficacy of prophylaxis for HSV-2 negative individuals with an HSV-2-positive partner has not been demonstrated and would be an important area to explore. In the absence of a vaccine, pre-exposure prophylaxis could be useful for reducing the risk of herpes, particularly in high risk groups such as sex workers, and possibly in young women who are at substantial risk for both herpes and HIV.

Although standard antibiotic regimens result in high cure rates for BV, recurrences are common [83]. Interventions that allow women to maintain healthy vaginal microbiota free from BV and intermediate microbiota should be explored as a means of reducing the risk of herpes and other STIs. Periodic presumptive treatment as a strategy to reduce the incidence of vaginal infections (candida, BV and trichomoniasis) and improve vaginal health has been evaluated. Data from Mombasa, Kenya has demonstrated that monthly PPT can reduce BV, promote *Lactobacillus* colonization and maintain a healthy vaginal environment [64, 84]. Other studies have also assessed the effect of PPT on vaginal microbiota with similar conclusions that PPT can reduce the frequency of vaginal infections [85, 86]. This is a fundamental step towards the development of inexpensive, female-controlled, non-coitally dependent HIV risk reduction interventions for women. It is conceivable that clinical trials to assess BV suppression for STI prevention would be worthwhile, and if successful would provide a useful tool for STI prevention.

In light of the disappointing findings from most clinical trials that have evaluated STI interventions as a tool for HIV prevention, combined with the availability of other highly potent HIV prevention tools such as treatment as prevention [87], pre-exposure prophylaxis [88, 89]

and male circumcision [90-92] , it is difficult to envision another clinical trial of an STI intervention for HIV prevention. Such a study would only be worth considering if a highly effective intervention such as a HSV-2 vaccine were available. Even then, this would require enormous infrastructural and logistical support to meet the ethical requirements given the current availability of potent HIV prevention tools. Nonetheless, effective and affordable STI services including STI counseling, promoting condom use and referral for diagnosis and treatment of STIs are essential. Notably, HIV incidence in many countries began to decline well before antiretroviral therapy became available [93], and strong STI programs in the 1990s may have influenced these trends. Besides the potential to reduce the HIV burden, these services will reduce the burden of mortality and morbidity caused by STIs.

This study had several strengths. First, we had a relatively large dataset which allowed sufficient power to assess the associations between STIs and HIV. Second, interactions between STIs including HIV are difficult to study because precise timing of the acquisition of an infection is difficult to ascertain. However, frequent sampling to detect HIV and STIs at monthly intervals and rigorous measurement of potential confounding factors such as sexual risk behavior and contraceptive use provided a good basis for studying the associations between HIV and STIs. Finally, the Mombasa Cohort was established in 1993. Although this may not correlate perfectly with the early-phase epidemic in Kenya, HIV incidence was still relatively high at that time. As a result, we were able to assess changes in the PAR% contributions of different STIs to HIV acquisition during an extended period that incorporated a wide range of HIV incidences.

Our study also had several limitations. First, STIs and HIV share a common mode of acquisition through contact with an infected partner; hence our HR and PAR% estimates could be inflated due to confounding by sexual risk behavior. We addressed potential confounding by adjusting for reported risk behaviors including frequency of unprotected sex, number of sexual partners, and number of sex episodes. Nonetheless, we recognize the potential for residual confounding.

Second, the number of seroconverters decreased over time in the cohort. The modest number of events in the last ten years limited the precision in our PAR% estimates for the last two time periods. Finally, as with all observational studies, these results cannot definitively establish the presence causal associations between STIs and HIV acquisition. Despite these limitations, these results make a unique addition to the literature on STI and HIV interactions by directly evaluating trends in the contribution of different STIs to HIV acquisition over 2 decades of observation.

SUMMARY

Herpes simplex virus type-2 infection and BV have remained the largest contributors to HIV acquisition in this cohort of female sex workers from 1993 through 2012. While RCTs for prevention and treatment of genital tract infections have mostly failed to reduce HIV incidence, these results highlight the importance of these two conditions as persistent drivers of HIV transmission. Novel ideas such as HSV-2 vaccination, HSV-2 pre-exposure prophylaxis, and interventions that allow women to maintain healthy vaginal microbiota could reduce HIV transmission in populations.

Table 2.1: Randomized controlled trials to assess effectiveness of STI treatment for HIV prevention

Author, year	*Population/Setting*	*Intervention*	*STI targeted*	*Effect on STI incidence/prevalence*	*Effect on HIV Incidence (95% CI)*
Grosskurth et al [a], 1992-1994 [67]	Men and women, Mwanza, Tanzania	Syndromic STI management	NG, CT, syphilis,	↔ NG, CT, GUD	aRR 0.58 (0.42–0.79)
Wawer et al [a], 1994 – 1998 [94]	Men and women, Rakai, Uganda	Mass treatment: azithromycin, ciprofloxacin, metronidazole	NG, CT, HD, TV, syphilis, BV GUD, genital discharge, dysuria	↓ syphilis, TV, ↔ BV, NG, CT ↔ GUD, genital discharge, dysuria	aIRR 0.97 (0.81-1.16)
Ghys et al [b], 1994 – 1998 [95]	FSW, Cote d'Ivoire	Intensive STI management	NG, TV, CT, GUD	↓ NG, TV ↔CT, GUD	IRR 0.70 (0.25-1.90)
Kamali et al [a], 1994 – 2000 [96]	Men, Women, Youth, Masaka, Uganda	Syndromic STI management	HSV-2, syphilis, NG, CT, GUD, genital discharge	↓ HSV-2, NG, syphilis ↔CT, GUD and genital discharge	aIRR 1.0 (0.63-1.58)
Kaul et al [b], 1998 – 2002 [97]	FSW, Nairobi, Kenya	Azithromycin	NG, CT, HD, TV, syphilis, BV	↓ CT, NG and TV ↔BV, syphilis	IRR 1.2 (0.6-2.5)
Gregson et al [a], 1998 – 2003 [98]	Men and women, Zimbabwe	Syndromic STI management	GUD, genital discharge	↔ GUD, ↔ genital discharge	aIRR 1.27 (0.92-1.75)
Watson-Jones et al [b], 2004 – 2007 [71]	HSV-2 positive women, Tanzania	Acyclovir	HSV-2	↔ GUD	IRR 1.01 (0.61-1.66)
Celum et al [b], 2003 – 2007 [70]	HSV-2 positive women, MSM, Peru, Southern Africa, USA	Acyclovir	HSV-2	↓ GUD	HR 1.16 (0.83-1.62)
Celum et al[c], 2004 – 2008 [99]	HIV-1 discordant couples, Eastern & Southern Africa	Acyclovir	HSV-2	↓ GUD	HR 0.92 (0.60 – 1.41)

STI - Sexually Transmitted Infection; NG – *Neisseria gonorrhoeae*; CT - *Chlamydia trachomatis*; TV – *Trichomonas vaginalis*; HD – *Hemophilus ducreyi*, HSV-2 Herpes Simplex Virus type 2; GUD – Genital Ulcer Disease; - aRR – Adjusted Relative Risk; aIRR – adjusted Incident Rate Ratio; IRR – Incident Rate Ratio; HR – Hazard Ratio

↓ - significant reduction

↔ - non-significant change

[a] Community Randomized Control Trial (RCT); [b] Individual Randomized Control Trial;

[c] Tested effect of STI treatment on HIV-1 transmission rather than acquisition

Table 2.2: Baseline characteristics of 1964 participants

Characteristic	Median (IQR) or Number (percent)
Demographics	
Age	
<25 years	**699 (35.6)**
25 – <35 years	**959 (48.8)**
≥35 years	**306 (15.6)**
Education	
≤8 years	**1192 (60.7)**
>8 – ≤12 years	**658 (33.5)**
≥12 years	**114 (5.8)**
Ever married[a]	**1029 (52.4)**
Work place	
Bar/Restaurant	**1310 (66.3)**
Night club	**507 (25.8)**
Home based/other	**147 (7.5)**
Gynecological	
Parity	**2 (1-3)**
Hormonal contraceptive use	
OCP	**229 (11.7)**
DMPA/Norplant	**467 (23.9)**
Sexual risk behavior reported in an average week	
Unprotected intercourse	**178 (9.1)**
>1 sex partner[b]	**919 (47.4)**
>1 sex encounters[b]	**1307 (67.4)**
Drug use reported at enrollment in the cohort	
Alcohol (≥1 drink per week)	**1512 (77.0)**
Tobacco (≥1 cigarette per day)	**338 (17.2)**
Laboratory diagnosis of genital tract conditions	
Vulvovaginal candidiasis	**280 (14.3)**
Bacterial vaginosis	**642 (32.7)**
Trichomonas vaginalis	**95 (4.8)**
Neisseria gonorrhoeae	**78 (4.0)**
Non-specific cervicitis	**220 (11.2)**
Herpes simplex virus type 2	**1442 (73.4)**
Genital ulcer disease	**36 (1.8)**
Genital warts	**26 (1.3)**
Reported vaginal washing	**1841 (93.7)**
Water only	**491 (26.7)**
Soap/Other	**1350 (73.3)**

[a] Included 30 currently married, and 999 widowed or divorced women
[b] Analyzed only among 1938 women reporting any sexual activity in the past week

Table 2.3: Incidence per 100 person-years of HIV and other genital tract conditions by time period

Genital tract condition	*Overall incidence*	*1993-1997*	*1998-2002*	*2003-2007*	*2008-2012*	*P value for test of trend*
HIV	5.30	11.83	6.19	3.28	1.33	0.03
Candidiasis	62.56	72.11	48.88	71.73	59.93	0.82
Intermediate microbiota	109.10	200.13	94.59	98.49	70.17	0.34
Bacterial vaginosis	168.97	243.28	147.36	186.07	119.21	0.20
Trichomonas vaginalis	18.63	36.22	21.02	15.76	6.58	0.02
Neisseria gonorrhoeae	8.74	21.75	8.64	3.31	5.39	0.15
Non-specific cervicitis	26.88	105.24	10.58	10.14	5.64	0.20
HSV-2	19.58	31.40	23.33	11.87	13.44	0.08
Genital ulcer disease	7.49	9.19	6.74	6.20	8.46	0.75
Genital warts	11.19	27.37	3.70	4.57	14.55	0.56

Table 2.4: Univariate and multivariate analyses of the correlates of HIV infection

Characteristic	*HIV infections/ Person-years*	*Incidence / 100 person-years*	*Univariate Analysis*		Multivariate Analysis	
			HR (95% CI)	*p value*	HR (95% CI)	p value
Age (years)						
<25	73/814	8.96	1.76 (1.23-2.53)	0.006	2.01 (1.36-2.97)	<0.001
25 to 34	175/2797	6.25	1.49 (1.12-1.98)	0.002	1.35 (1.00-1.83)	0.05
≥ 35	77/2522	3.05	1.00		1.00	
Education						
≤ 8 years	209/3933	5.31	1.00			
>8 – ≤12 years	104/1890	5.50	1.00 (0.79-1.26)	0.98		
≥ 12 years	12/312	3.84	0.70 (0.40-1.26)	0.24		
Ever married	173/3417	5.06	0.92 (0.74-1.15)	0.46		
Work place						
Bar/Restaurant	273/4226	6.46	1.00		1.00	
Night club	39/1638	2.38	0.37 (0.27-0.52)	<0.001	0.41 (0.28-0.61)	<0.001
Home based/Other	13/270	4.81	0.63 (0.35-1.10)	0.11	1.49 (0.82-2.70)	0.19
Hormonal contraceptive method						
None	182/4354	4.18	1.00		1.00	
OCP	37/462	8.01	1.59 (1.14-2.21)	0.006	1.27 (0.88-1.81)	0.20
DMPA/Norplant	105/1238	8.48	1.93 (1.52-2.46)	<0.001	1.85 (1.43-2.39)	<0.001
Sexual risk behavior						
Unprotected intercourse in last week	86/1548	5.56	1.01 (0.79-1.29)	0.93		
>1 sex partner/week	37/1040	3.56	0.56 (0.40-0.78)	0.001	0.81 (0.56-1.18)	0.27
>1 sex encounter/week	121/2272	5.33	0.86 (0.69-1.09)	0.21		
Presence of genital tract						

conditions						
Candidiasis	89/957	9.30	1.90 (1.49-2.42)	<0.001	2.09 (1.62-2.70)	<0.001
Abnormal vaginal microbiota						
Normal microbiota	87/2641	3.29	1.00		1.00	
Intermediate vaginal microbiota	95/1361	6.98	1.94 (1.46-2.58)	<0.001	1.54 (1.13-2.09)	0.01
Bacterial vaginosis	141/2040	6.91	1.95 (1.50-2.54)	<0.001	1.86 (1.40-2.47)	<0.001
Trichomonas vaginalis	39/345	11.29	2.08 (1.50-2.89)	<0.001	1.41 (0.99-2.02)	0.06
Neisseria gonorrhoeae	31/174	17.82	3.00 (2.08-4.32)	<0.001	2.05 (1.38-3.04)	<0.001
Non-specific cervicitis	54/446	12.10	1.99 (1.48-2.67)	<0.001	1.14 (0.81-1.60)	0.45
HSV-2						
Prevalent HSV-2	265/4817	5.50	2.51 (1.58-3.96)	<0.001	2.50 (1.51-4.13)	<0.001
Incident HSV-2	34/575	5.91	3.02 (1.72-5.29)	<0.001	2.95 (1.63-5.34)	<0.001
Genital ulcer disease	10/69	14.53	2.62 (1.43-4.82)	0.002	2.23 (1.14-4.35)	0.02
Genital warts	7/75	9.33	1.26 (0.60-2.62)	0.50	0.92 (0.41-2.10)	0.85
Cervical ectopy	60/680	8.81	1.54 (1.16-2.03)	0.003	1.10 (0.81-1.48)	0.54
Alcohol use	259/4911	5.27	0.99 (0.77-1.30)	1.00		
Tobacco use	38/1252	3.04	0.55 (0.39-0.76)	0.001	0.98 (0.94-1.02)	0.29
Calendar year						
1993-1997	150/1267	11.83	6.39 (3.97-10.26)	<0.001	5.17 (3.03-8.83)	<0.001
1998-2002	98/1583	6.19	3.87 (2.40-6.25)	<0.001	3.01 (1.79-5.05)	<0.001
2003-2007	56/1707	3.28	2.19 (1.31-3.64)	0.003	1.84 (1.09-3.11)	0.02
2008-2012	21/1577	1.33	1.00		1.00	

Table 2.5: Association between different genital tract conditions and HIV acquisition*

Genital tract condition	1993-1997	1998-2002	2003-2007	2008-2012
Candidiasis	3.04 (2.12-4.36) p = <0.001	1.65 (0.92-2.95) p = 0.09	1.47 (0.69-3.016) p = 0.32	0.99 (0.21-4.64) p = 0.99
Intermediate microbiota	1.76 (1.10-2.82) p = 0.02	1.08 (0.53-2.21) p = 0.83	1.30 (0.50-3.36) p = 0.60	2.35 (0.53-10.35) p = 0.26
Bacterial vaginosis	1.90 (1.19-3.02) p = 0.01	2.07 (1.13-3.79) p = 0.02	1.81 (0.90-3.63) p = 0.10	3.32 (0.98-11.17) p = 0.05
Trichomonas vaginalis	1.18 (0.70-1.97) p = 0.53	2.10 (1.07-4.10) p = 0.03	2.22 (0.66-7.51) p = 0.20	2.22 (0.25 -19.88) p = 0.48
Neisseria gonorrhoeae	1.50 (0.84-2.65) p = 0.17	1.91 (0.80-4.54) p = 0.14	7.39 (1.68-32.49) p = 0.01	3.60 (0.41-31.24) p = 0.25
Non-specific cervicitis	1.09 (0.74-1.61) p = 0.68	1.64 (0.70-3.83) p = 0.25	-**	-**
Prevalent HSV-2	1.98 (0.98-4.00) p = 0.06	4.74 (1.11-20.19) p = 0.04	3.70 (0.86-15.93) p = 0.08	2.46 (0.30-19.95) p = 0.40
HSV-2	3.26 (1.35-7.85) p = 0.01	6.69 (1.46-30.60) p = 0.01	3.42 (0.61-19.24) p = 0.16	1.13 (0.07-19.15) p = 0.93
Genital ulcer disease	2.13 (0.67-6.75) p = 0.20	1.80 (0.53-6.18) p = 0.35	-**	6.09 (0.71-51.98) p = 0.10
Genital warts	0.83 (0.33-2.05) 0.68	-**	3.54 (0.46-27.08) 0.22	-**

*All analyses are adjusted for age, place of work, hormonal contraceptive use, number of sexual partners, tobacco use and all sexually transmitted infections/conditions listed in the table.

**There were no cases therefore it was not possible to calculate an incidence estimate

Table 2.6: Prevalence genital tract conditions by time period (proportion of visits exposed)

Genital tract condition	*Overall prevalence*	*1993-1997*	*1998-2002*	*2003-2007*	*2008-2012*
Candidiasis	12.19	10.91	12.73	12.73	12.42
Intermediate microbiota	21.25	29.52	24.54	17.44	14.55
Bacterial vaginosis	32.66	36.07	37.78	32.82	24.53
Trichomonas vaginalis	3.66	5.44	5.38	2.81	1.40
Neisseria gonorrhoeae	1.79	3.51	2.23	0.61	1.11
Non-specific cervicitis	5.47	16.09	2.70	1.82	1.19
Prevalent HSV-2	80.42	81.70	78.29	80.00	81.37
Incident HSV-2	6.81	3.50	7.95	8.67	7.03
Genital ulcer disease	1.49	1.48	1.74	1.09	1.82
Genital warts	2.15	3.93	0.94	0.81	3.00

Table 2.7: Contribution (PAR%) of various genital tract conditions to HIV acquisition by time period

Genital tract condition	*Overall Contribution*	*1993-1997*	*1998-2002*	*2003-2007*	*2008-2012*	*P value for test of trend*
Candidiasis	6.36	7.32	5.01	4.07	-0.13	0.035
Intermediate microbiota	7.45	12.75	1.82	4.02	8.36	0.65
Bacterial vaginosis	15.10	17.09	19.53	14.69	17.14	0.68
Trichomonas vaginalis	1.06	0.83	2.82	1.54	0.77	0.87
Neisseria gonorrhoeae	0.92	1.17	1.06	0.53	0.80	-
Non-specific cervicitis	0.67	1.33	1.05	-*	-*	-
HSV-2	48.25	40.44	61.77	58.38	48.29	0.74
Incident HSV-2	4.50	2.43	6.76	6.13	0.81	0.82
Genital ulcer disease	0.82	0.79	0.77	-*	1.52	0.21
Genital warts	-0.19	-0.80	-*	0.58	-*	-

*There were no cases therefore it was not possible to calculate PAR%

**There were few cases therefore it was not possible to calculate the test of trend

Figure 2.1: Contribution (PAR%) of various genital tract conditions to HIV acquisition by time period

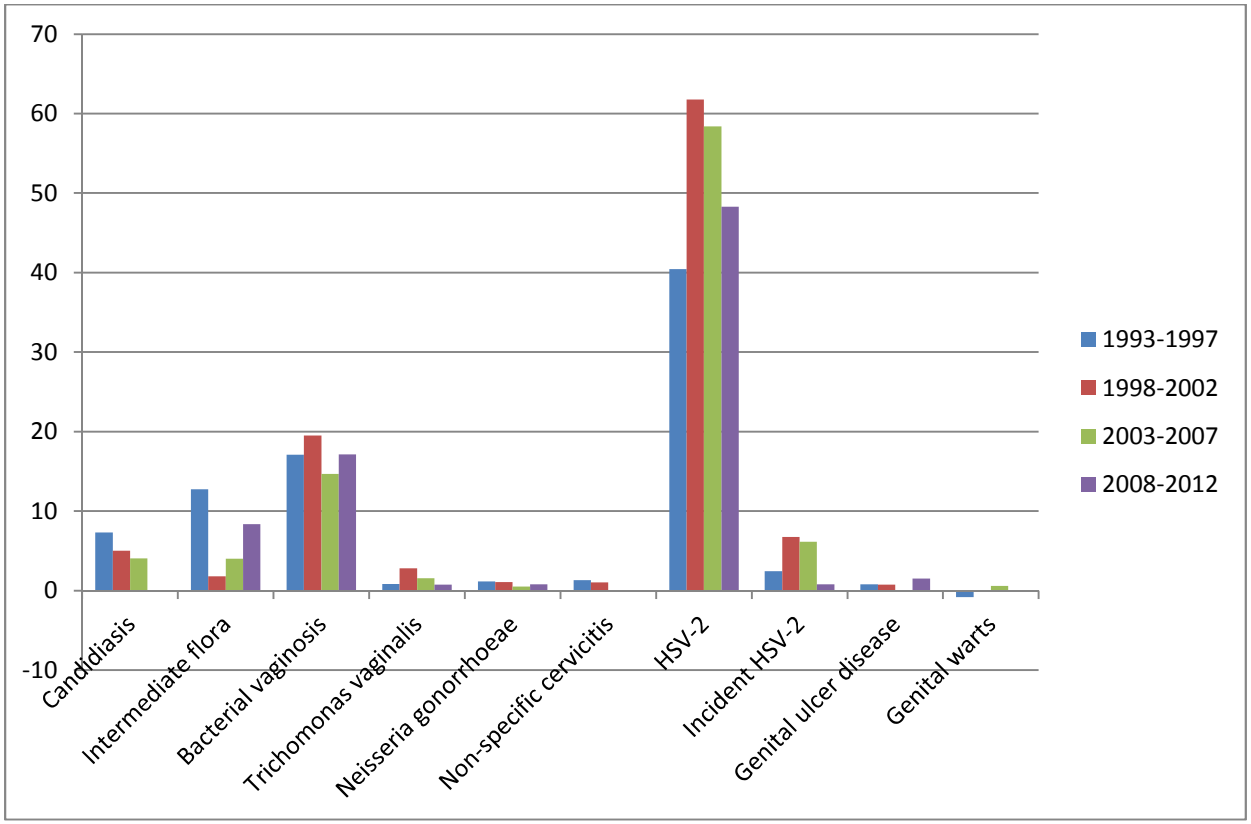
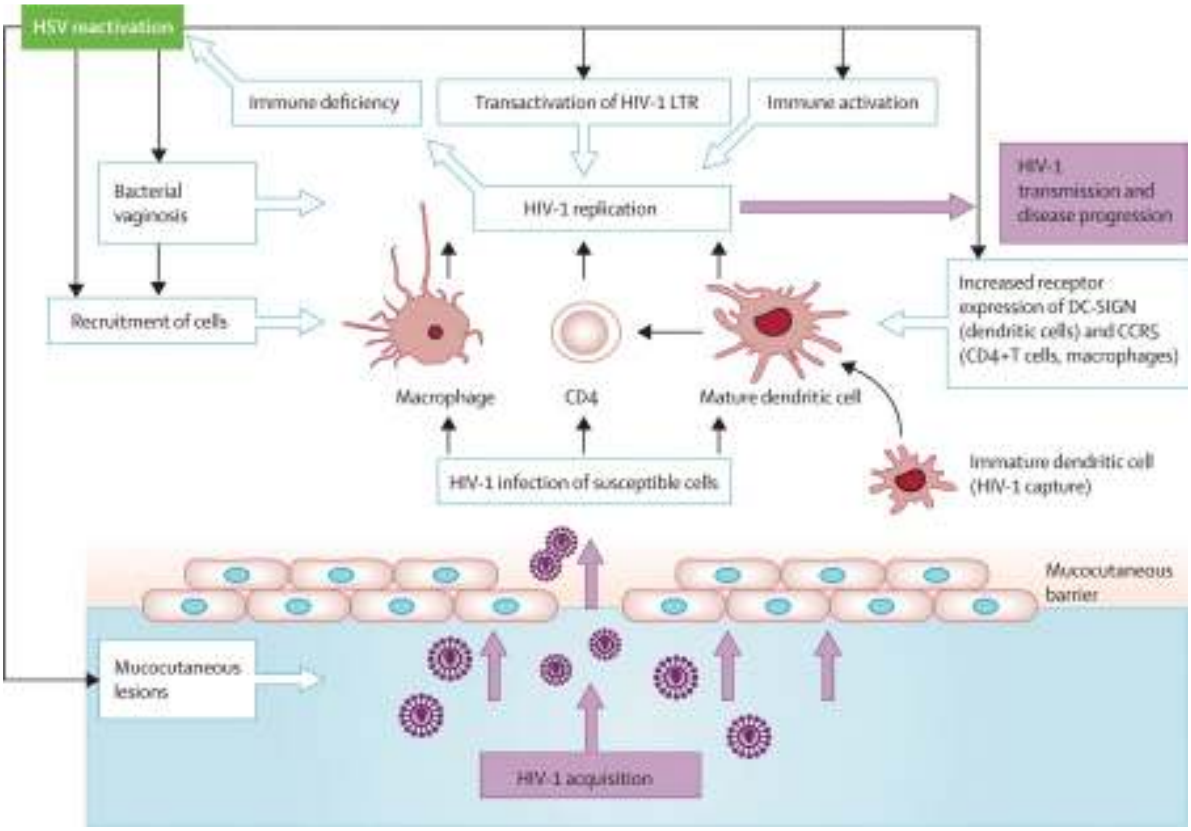


Figure 2.2: Mucosal interactions between BV, HSV-2 and HIV-1



Source: Van de Perre et al. Lancet Infect Dis 2008;8: 490-97 [100]

CHAPTER 3: Incidence and Correlates of *Chlamydia trachomatis* Infection in a High Risk Cohort of Kenyan Women

ABSTRACT

BACKGROUND: In Africa, data on *Chlamydia trachomatis* infection are scarce because reliable diagnosis is costly and not widely available. Our objective was to evaluate the incidence and correlates of *C. trachomatis* infection among high-risk Kenyan women.

METHODS: We conducted prospective cohort analyses using data from a cohort of women who reported transactional sex. *C. trachomatis* testing was performed using the Gen-Probe Aptima GC/CT Detection System. We used Andersen-Gill proportional hazards modeling to evaluate correlates of *C. trachomatis*.

RESULTS: Between August 2006 and December 2010, 865 women contributed 2011 person-years of observation. Sixty-four women experienced 101 episodes of *C. trachomatis* infection (incidence rate of 5.0/100 person-years). There was a large difference in incidence by age group: those below 25 years had an incidence of 27.6 per 100 person-years (95% CI 16.3 – 46.5), those 25 to 34 years old had an incidence of 8.4 per 100 person-years (95% CI 6.4 – 11.0), and those 35 years old and above had an incidence of 2.6 per 100 person-years (95% CI 1.8 – 3.6). In multivariate analyses, younger age (<25 years and 25-34 years versus ≥35 years; hazard ratio [HR] 8.49 95% CI 4.1-17.7 and HR 2.9 95% CI 1.7-5.0 respectively), depot medroxyprogesterone acetate use (HR 1.8 95% CI 1.1-3.0) and recent *Neisseria gonorrhoeae* infection (HR 3.3 95% CI 1.5-7.4) were significantly associated with increased risk of acquiring *C. trachomatis* infection.

CONCLUSIONS: The high incidence of *C. trachomatis* among younger high-risk women suggests the need for screening as an important public health intervention for this population.

INTRODUCTION

In 1999, the World Health Organization (WHO) estimated the global incidence of *Chlamydia trachomatis* infection to be 92 million cases worldwide, with 16 million (17%) occurring in Africa [101]. However, estimates from resource-constrained settings including most of Africa are imprecise due to lack of surveillance, limited laboratory infrastructure and diagnostic capacity, and the widespread use of syndromic management. The few community-based studies that have measured *C. trachomatis* in sub-Saharan Africa suggest a low prevalence in the general population (1.6-3.2%) [102, 103]. A cross-sectional study among female adolescents in Uganda estimated *C. trachomatis* prevalence at 4.5% [104]. Prevalence is much higher among high-risk groups when compared with community data. For example, prevalence estimates among female sex workers range from 9% in Nairobi, Kenya [97] to 28.5% in Dakar, Senegal.[105]

Incidence studies are particularly valuable in understanding the risk of a disease. Studies of the incidence of *C. trachomatis* infection in Africa are few [48, 97, 106]. Among women attending family planning clinics in Zimbabwe and Uganda, the incidences of *C. trachomatis* were 3.6 and 4.1 per 100 person-years respectively [48]. In the past decade, two studies of female sex workers in Kenya have reported incidence rates of 6.5 and 9.0 per 100 person-years respectively [97, 106].

Less than 10% of women infected with *C. trachomatis* develop acute signs and symptoms of infection [25]. The absence of symptoms in the majority of those infected results in a substantial number of cases that are unrecognized, untreated, and persist as a reservoir for ongoing transmission. In addition, immunity to *C. trachomatis* infection is partial and short-lived [107], so reinfection is common. *Chlamydia trachomatis* is associated with a number of serious reproductive health problems such as pelvic inflammatory disease (PID), tubal infertility, ectopic pregnancy, and chronic pelvic pain [27]. In addition, *C. trachomatis* infection has been associated with increased risk of HIV-1 acquisition [46, 48]. These data highlight the importance

of screening and treatment for *C. trachomatis* in order to prevent the spread of this infection and related complications.

To add to the limited data characterizing the epidemiology of *C. trachomatis* in sub-Saharan Africa, we sought to determine the incidence and correlates of *C. trachomatis* infection among HIV-1-seropositive and seronegative women enrolled in a prospective cohort study of women at high risk for sexually transmitted infections (STIs).

MATERIALS AND METHODS

We conducted longitudinal follow-up in an open cohort of female sex workers in Mombasa, Kenya. The eligibility criteria to join the cohort are: age 18–50 years, residing in the Mombasa area, self-identifying as exchanging sex for payment in cash or in kind, and able to provide informed consent. The present analysis utilized data collected between August 2006 and December 2010. This study was approved by the ethical review committees at Kenyatta National Hospital and the University of Washington. All participants provided written informed consent.

Clinic Procedures

At enrollment and monthly follow-up visits, a study nurse conducted a standardized interview covering demographic data, medical, gynecological, and sexual history. A study physician performed a physical examination including a pelvic speculum examination. Swabs of cervical and vaginal secretions were collected for laboratory diagnosis of STIs. After April 2008, the visit schedule for HIV-1 seropositive women who were not on antiretroviral therapy changed from monthly to every three months, consistent with local clinical standards for this group.

Between August 2006 and April 2008, *C. trachomatis* testing was performed monthly. After April 2008, testing was performed every three months. All participants received free outpatient

medical services including treatment of STIs. Diagnosed STIs were treated according to WHO [65] and Kenyan national guidelines. Women received doxycycline 100mg twice daily for seven days if they were diagnosed with *C. trachomatis* infection. Syndromic management was offered during examination visits if indicated. When participants returned to receive their tests results, additional treatment was provided if infections were diagnosed by laboratory testing that had not been treated syndromically at the prior visit.

Laboratory Procedures

Endocervical samples were tested for the presence of *Neisseria gonorrhoeae* and *C. trachomatis* by transcription mediated amplification (TMA) using the Gen-Probe Aptima GC/CT Detection System (Gen-Probe, San Diego, California, USA). Culture for *N. gonorrhoeae* was performed on modified Thayer-Martin media. Cervical Gram's stained slides were examined microscopically for Gram-negative intracellular diplococci consistent with a diagnosis of *N. gonorrhoeae* infection. Vaginal Gram stained slides were evaluated using Nugent's criteria, with bacterial vaginosis (BV) being defined as a score of 7-10 [66]. Nugent scoring was performed by laboratory technicians with over 10 years' experience with this technique. Internal quality assurance was performed weekly and external quality assurance was performed semiannually. Saline and potassium hydroxide wet mounts were examined at 40X power for the presence of motile trichomonads, clue cells, *Lactobacillus* morphotypes, and yeast. HIV-1 serostatus was determined by ELISA (Detect HIV1/2, BioChem Immunosystems, Montreal, Canada or PT-HIV 1,2-96, Pishtaz Teb Diagnostics, Tehran, Iran). Positive tests were confirmed using a second ELISA (Recombigen, Cambridge Biotech, Worcester, MA, USA or Vironostika HIV-1 Uniform II AG/AB, bioMerieux, Marcy l'Etoile, France). For the confirmatory tests, the cut-off values used were as suggested by the manufacturers. In addition to the manufacturers' recommendations, we took into consideration a grey zone. This grey zone range was determined by identifying

10% of readings above and 10% of readings below the cut-off. We performed repeat testing for any results that fell within this range.

Data Analyses

All women who had more than one visit at which *C. trachomatis* testing was performed were included in the analyses. The outcome was time to *C. trachomatis* infection. The exposures of interest were known or suspected risk factors for *C. trachomatis* infection including age, hormonal contraceptive use (oral contraceptive pills [OCP] or depot medroxyprogesterone acetate [DMPA] versus no hormonal contraception), vaginal microbiota (intermediate vaginal microbiota or bacterial vaginosis versus normal vaginal microbiota), place of work (bar versus night club or home-based/other), educational level, marital status, sexual risk behavior in the past week (unprotected intercourse, number of sex partners), vaginal washing, presence of other genital tract infections (*Trichomonas vaginalis, Candida albicans, N. gonorrhoeae*), HIV-1 serostatus, and cervical ectopy. These were assessed as predictors of infection using Andersen-Gill proportional hazards models and the Efron method for ties. Participants were included in the analyses beginning in August 2006 (when Gen-Probe *C. trachomatis* screening was initiated), or from the date of enrollment for those who enrolled after August 2006. Data were censored at a participant's last follow-up visit or at the end of the analysis period in December 2010. We first conducted univariate analyses to determine whether individual risk factors were associated with *C. trachomatis* infection. Variables that were associated with *C. trachomatis* (alpha = 0.10) in the univariate analyses were then included in the multivariate model.

As in previous analyses, we estimated that the effect of oral or injectable hormonal contraception would persist for 70 days after discontinuation of use [108]. We assumed that *C. trachomatis* was acquired at the midpoint between the pre-infection visit and the visit at which

the infection was detected. Because visits were, on average, every 30 days, we used an exposure interval of 85 (70+15) days for women who changed their method of hormonal contraception. We used a 45 day exposure interval for other STIs and abnormal vaginal microbiota or BV (assuming approximately 30 days for a persistent effect and 15 days incubation period). The lag period included the current visit. Analyses were performed using PASW 18.0 (PASW Inc., Chicago, USA) and STATA 11 (StataCorp, College Station, TX, USA).

RESULTS

Between August 2006 and December 2010, 865 women had more than one visit where *C. trachomatis* testing was performed. Baseline characteristics for these participants are presented in Table 3.1. Their median age was 35 years (interquartile range [IQR] 30-40). One hundred and eighty-one women (20.9%) were using DMPA. At baseline, the prevalence of *C. trachomatis* was low (1.9%). With the exception of HIV-1 (N=457; 52.8%), the prevalence of other STIs at baseline was also low.

Participants in this study contributed a total of 2011 person-years of follow-up. The median duration of follow-up was 3.8 years (IQR 2.7-4.1). Sixty-four women experienced 101 episodes of *C. trachomatis* infection, resulting in an incidence of 5.0 per 100 person-years. Twenty women had more than one episode of *C. trachomatis* infection (range: 2-5 episodes). There was a large difference in incidence by age group. Women below 25 years had an incidence rate of 27.6 per 100 person-years (95% CI 16.3 – 46.5), those 25 to 34 years old had an incidence rate of 8.4 per 100 person-years (95% CI 6.4 – 11.0), and women 35 years old and above had an incidence rate of 2.6 per 100 person-years (95% CI 1.8 – 3.6). While *C. trachomatis* infection was associated with a higher likelihood of reporting symptoms (lower abdominal pain and/or vaginal discharge) (OR 1.7 95% CI 1.0-3.0; p=0.05), only a minority *C. trachomatis* episodes were symptomatic (N=16, 15.8%). Of 101 episodes of *C. trachomatis*, 6 (5.9%) included co-infection with *N. gonorrhoeae*.

Several exposures including younger age, use of DMPA, more recent enrollment in the research cohort, having >1 sex partner in the last week, having >1 sexual encounter in the last week, being HIV-1-seropositive, and having recent or concurrent *N. gonorrhoeae* infection were associated with an increased likelihood of acquiring *C. trachomatis* infection in univariate analyses (Table 3.2). In multivariate analyses, younger age (<25 years and 25-34 years versus ≥35 years; hazard ratio [HR] 8.5 95% CI 4.1-17.7 and HR 2.9 95% CI 1.7-5.0 respectively), DMPA use (HR 1.8 95% CI 1.1-3.0) and recent or concurrent *N. gonorrhoeae* infection (HR 3.3 95% CI 1.5-7.4) remained significantly associated with increased risk of acquiring *C. trachomatis* infection.

Although age was associated with the number of sexual partners and hormonal contraceptive use, there were no statistically significant interactions between age and either of these additional covariates. Condom use was not different among hormonal contraceptive users compared to women not using hormonal contraception, and the interactions between condom use and hormonal contraception were also not statistically significant (data not shown). Thus, only a non-stratified model is presented.

DISCUSSION

The overall incidence of *C. trachomatis* infection among female sex workers in Mombasa was 5.0/100 person-years, which is similar to incidence estimates from studies conducted among female sex workers in Nairobi between 1998 and 2002 and in Mombasa between 1993 and 2003 [97, 106]. The incidence of *C. trachomatis* was markedly higher among younger women (18-25 years), suggesting the need for expansion of screening to address this problem among young at-risk women. Other factors associated with *C. trachomatis* infection were use of DMPA and recent or concurrent infection with *N. gonorrhoeae*.

Studies of risk factors for *C. trachomatis* have mostly been conducted in developed countries, and have identified similar risk factors including younger age, higher number of sexual partners, and use of hormonal contraception [109-113]. Recent prospective studies have also highlighted the potential importance of BV as a risk factor for infection with *C. trachomatis* [14, 17]. In contrast, we did not find an association between abnormal vaginal microbiota or BV and *C. trachomatis* infection. Further research is needed to explore the association between vaginal microbiota and incident STIs.

Susceptibility to *C. trachomatis* infection may be influenced by a number of biological factors. First, cervical ectopy occurs when the squamocolumnar junction lies outside the endocervix, resulting in exposed columnar cells. This anatomical characteristic has been associated with increased risk for numerous pathogens, including *C. trachomatis* [114]. Cervical ectopy tends to be greatest during adolescence and decreases with age [114]. This age-dependent phenomenon may help to explain the higher incidence of *C. trachomatis* infection in younger women. A second important biological factor is exposure to DMPA, which induces a systemic hypo-estrogenic state associated with decreased vaginal colonization with hydrogen peroxide producing *Lactobacillus* species [115]. This decrease in protective vaginal bacteria may, in turn, increase the risk of *C. trachomatis* infection. In our study, DMPA was associated with a nearly 2-fold increase in risk of *C. trachomatis* infection, adding to data suggesting a possible link between hormonal contraception and acquisition of STIs including HIV-1 [116]. We did not find an association between OCP use and *C. trachomatis* infection, possibly due to the relatively small number of women (N=41 [4.7%]) using OCP in this cohort. Condom use among women on hormonal contraception was similar to condom use among women not using any hormonal contraception. Moreover, we have previously noted that not every STI risk is increased in contraceptive users. A study conducted within this same cohort demonstrated that women using DMPA had a significantly decreased risk of bacterial vaginosis (hazard ratio, 0.7; 95%

confidence interval, 0.5-0.8) and trichomoniasis (hazard ratio, 0.6; 95% confidence interval, 0.4-1.0) [108]. These findings argue against condom use as the mediating factor for *C. trachomatis* infection among hormonal contraceptive users.

Women with a recent or concurrent *N. gonorrhoeae* infection were three times more likely to acquire *C. trachomatis* infection. Prior STI has previously been described as a risk factor for infection by other sexually transmitted pathogens [117]. Although there may be biological interactions, it is likely that this effect is also mediated through exposure to higher risk sexual networks, where a variety of STIs are circulating.

The strengths of our study include the large sample size and longitudinal follow-up that enabled us to assess the correlates of *C. trachomatis* infection. In addition, we used the Gen-Probe Aptima GC/CT Detection System, which has excellent sensitivity (94.2%) and specificity (97.6%) for detection of *C. trachomatis* on endocervical swabs [28]. This study also had limitations. Sexual risk behavior was self-reported, making these data subject to recall and social desirability bias. The questions on sexual risk behavior were limited to the past one week to mitigate recall bias. In addition, we have recently demonstrated that within this cohort, self-reported behaviors are associated with biological outcomes including STIs and sperm in genital secretions [118]. Nonetheless, some misreporting of sexual risk behaviors should be anticipated. Secondly, twenty women experienced more than one episode of *C. trachomatis* infection. We did not perform molecular testing to distinguish between treatment failure and re-infection. Future studies should consider molecular characterization to improve our understanding of *C. trachomatis* re-infection or persistence.

Findings from this study add to a sparse body of literature on the incidence and risk factors for *C. trachomatis* in Africa. Data from this study suggest that the risk of *C. trachomatis* among high-risk women under 25 years old could be substantial. Studies on the incidence of *C.*

trachomatis among young women in sub-Saharan Africa should be prioritized in view of potentially severe sequelae including PID, tubal infertility, and increased HIV-1 susceptibility. Tubal infertility is especially of concern in Africa, where prevalences of infertility as high as 27% have been reported, [119] and motherhood may be closely associated with a woman's status in the community [120]. Studies from West Africa have shown that *C. trachomatis* antibodies were more likely to be detected among infertile women compared with fertile women [121, 122]. Development of inexpensive point-of-care tests that can be used in resource-limited settings would enhance the diagnosis and early management of this largely silent epidemic.

CONCLUSION

We found a high incidence of *C. trachomatis* infection among high risk women less than 25 years old, suggesting the need for screening as an important public health intervention for younger high-risk women. In addition, data from general population women are urgently needed to gain a greater understanding of the extent to which the epidemic crosses over into the general population.

Table 3.1: Baseline characteristics of the 865 participants

Characteristic	Median (IQR) or Number (percent)
Demographics	
Age (years)	35 (30-40)
Education (years)	8 (7-10)
Ever married[a]	559 (64.6)
Work place	
Bar/Restaurant	626 (72.4)
Night club	123 (14.2)
Home based/other[b]	116 (13.4)
Gynecological history	
Parity	2 (1-3)
Hormonal contraceptive use	
OCP	41 (4.7)
DMPA	181 (20.9)
Sexual risk behavior reported in the past week	
Unprotected intercourse	195 (22.5)
>1 sex partner[c]	186 (31.0)
>1 sex encounter[c]	379 (63.1)
Clinical conditions	
Vaginal discharge	80 (9.3)
Abdominal pain	66 (7.6)
Vulval itch	94 (10.9)
Presence of GUD	23 (2.7)
Cervical ectopy	48 (5.5)
HIV-1 seropositive	457 (52.8)
Laboratory diagnosis of genital tract conditions	
Candidiasis	111 (12.8)
Bacterial vaginosis	313 (36.2)
Trichomonas vaginalis	36 (4.2)
Neisseria gonorrhoeae	22 (2.5)
Chlamydia trachomatis	16 (1.9)
Reported vaginal washing	834 (96.4)
Water only	359 (43.1)
Soap/Other[d]	475 (56.9)

[a] Included 15 currently married and 544 widowed or divorced women

[b] 22 (2.5%) women were home based and 94 (10.9%) reported “other” as their place of work

[c] Analyzed in the subgroup of 601 women who reported any sexual activity in the past week

[d] 451 (54.1%) women reported using soap, 18 (2.1%) reported using antiseptic, 4 (0.5%) reported using detergent, and 2 (0.2%) reported using “other” substances for vaginal washing

Table 3.2: Univariate and multivariate analyses of the correlates of *C. trachomatis* infection

Characteristic	*Chlamydia infections/ Person-years*	*Incidence / 100 person-years*	*Univariate Analysis*		Multivariate Analysis	
			HR (95% CI)	*p value*	HR (95% CI)	p value
Age (years)						
<25	14/51	27.6	10.1 (4.8-21.1)	<0.001	8.5 (4.1-17.7)	<0.001
25 to 34	53/630	8.4	3.2 (1.9-5.5)	<0.001	2.9 (1.7-5.0)	0.001
≥ 35	34/1331	2.6	1.0		1.0	
Hormonal contraceptive method						
None	67/1515	4.4	1.0		1.0	
OCP	1/78	1.3	0.3 (0.0-1.8)	0.2	0.2 (0.0-1.7)	0.2
DMPA	33/386	8.5	1.9 (1.1-3.2)	0.02	1.8 (1.1-3.0)	0.03
Abnormal vaginal microbiota						
Nugent score 0-3	47/998	4.7	1.0			
Nugent score 4-6	23/382	6.0	1.2 (0.6-2.2)	0.6		
Nugent score 7-10	31/631	4.9	1.1 (0.6-1.9)	0.8		
Education (> 8 years)	41/730	5.6	1.2 (0.7-2.0)	0.5		
Marital status						
Never married	43/694	6.2	1.0			
Ever married	58/1318	4.4	0.7 (0.4-1.2)	0.2		
Work place						
Bar/Restaurant	73/1471	5.0	1.0			
Night club	18/280	6.4	1.3 (0.7-2.5)	0.4		
Home based/Other	10/261	3.8	0.8 (0.3-2.0)	0.6		
Sexual risk behavior						
Unprotected intercourse	26/378	6.9	1.5 (0.9-2.5)	0.17		
>1 sex partner	24/297	8.1	1.7 (1.1-2.8)	0.02	1.3 (0.8-2.0)	0.4
HIV-1 positive	47/1224	3.8	0.6 (0.3-1.0)	0.04	0.7 (0.4-1.1)	0.1
Presence of genital tract conditions						
Candidiasis	19/316	6.0	1.2 (0.7-2.3)	0.5		
Trichomoniasis	4/86	4.7	0.9 (0.3-2.4)	0.8		
Neisseria gonorrhoeae	8/45	17.9	3.8 (1.7-8.4)	0.001	3.3 (1.5-7.4)	0.004
Reported vaginal washing						

None	4/126	3.2	1.0	
Water	56/1082	5.2	1.4 (0.5-4.1)	0.5
Soap/Other	41/802	5.1	1.4 (0.5-4.1)	0.6
Cervical ectopy	5/182	2.7	0.6 (0.2-1.6)	0.3

CHAPTER 4: Incident Herpes Simplex Virus Type 2 Infection Increases the Risk of Subsequent Episodes of Bacterial Vaginosis

ABSTRACT

Herpes simplex virus type 2 (HSV-2) infected women have a higher prevalence of bacterial vaginosis (BV) compared to HSV-2-seronegative women. To explore the temporal association between these conditions, we evaluated the frequency of BV episodes before and after HSV-2 acquisition in a prospective study of 406 HSV-2/HIV-1-seronegative Kenyan women, of whom 164 acquired HSV-2. Incident HSV-2 was associated with increased likelihood of BV (adjusted OR 1.28; 95% CI 1.05-1.56; p=0.01). Our findings strengthen the evidence for a causal link between genital HSV-2 infection and disruption of the vaginal microbiota.

INTRODUCTION

Bacterial vaginosis (BV) is a polymicrobial condition characterized by depletion of hydrogen-peroxide producing vaginal lactobacilli and overgrowth of *Gardnerella vaginalis* and other anaerobic bacteria [123]. Although BV is the most common cause of abnormal vaginal discharge, 50–75% of women with BV remain asymptomatic. Bacterial vaginosis is common worldwide among women of reproductive age. In the United States, the estimated prevalence of BV among women aged 14-49 is 29% [124]. Among African women, BV prevalence has been reported to be as high as 51% [37]. Bacterial vaginosis has been associated with increased risk of sexually transmitted infections (STIs) including human immunodeficiency virus type-1 (HIV-1), and with adverse reproductive health outcomes.

Herpes simplex virus type-2 (HSV-2) is a common STI worldwide and the leading cause of genital ulcer disease [125]. Most HSV-2 infections are asymptomatic, with over 80% of HSV-2 seropositive individuals asymptomatically shedding virus. It is estimated that 23% of women in the United States [126] and over 50% of women in sub-Saharan Africa are infected with HSV-2 [127]. In 2003, 536 million people were infected with HSV-2 globally [125], and HSV-2 incidence was 23.6 million new cases per year. HSV-2 infection is more common in women than men. The prevalence of this chronic infection increases with age. Among high-risk groups, HSV-2 incidence can be remarkably high. For example, we reported an annual HSV-2 incidence of 23% among high risk women in Mombasa, Kenya [128]. Like BV, HSV-2 has been found to be a significant risk factor for HIV-1 acquisition [48].

Several studies have observed associations between HSV-2 and BV. Women with BV are more likely to acquire other STIs including HSV-2 [10]. In addition, women with prevalent HSV-2 infection have a higher incidence of BV compared to HSV-2 uninfected women [14]. This observation could suggest that HSV-2 increases the risk of BV. Alternatively, women with more

frequent BV may simply be more likely to acquire HSV-2. To distinguish between these two possibilities, we compared women's likelihood of having BV before and after HSV-2 acquisition.

MATERIALS AND METHODS

We conducted longitudinal follow-up of women participating in an open cohort study of high-risk women in Mombasa, Kenya between February 1993 and February 2011. The eligibility criteria to join the cohort were: age 18–50 years, residing in the Mombasa area, self-identifying as exchanging sex for payment in cash or in kind, and able to provide informed consent. This study was approved by the ethics review boards of Kenyatta National Hospital and the University of Washington. All participants provided written informed consent.

Clinic Procedures

At enrollment and monthly follow-up visits, a study nurse conducted a standardized face-to-face interview covering demographic data and medical and sexual history. A study physician performed a physical examination including a pelvic speculum examination. Swabs of cervical and vaginal secretions were collected for laboratory diagnosis of STIs. Blood samples were collected for HSV-2 and HIV-1 testing. Participants were provided free outpatient medical services including treatment of STIs according to Kenyan national guidelines. If indicated, syndromic management was offered during the examination visit. Participants were asked to return for test results after 7 days. At the results visit, additional treatment was provided for infections diagnosed by laboratory testing that had not been treated with syndromic management at the prior visit.

Laboratory Procedures

Serological testing for HSV-2 was performed using a type-specific HSV-2 gG based ELISA (HerpeSelect 2, Focus Diagnostics, Cypress, California, USA) on archived samples. An index

value of less than 2.1 (the ratio of the optical density [OD] of the sample to the OD of the standard calibrator) was recorded as negative. Index values greater than or equal to 2.1 were considered to be positive. We selected this cut-off as likely providing the best balance of sensitivity and specificity, based on a prior study that found 2.1 to be the optimum assay cut-off in African populations similar to our own. The study demonstrated that this cut-off had 93.9% sensitivity and 90.5% specificity, against a gold standard HSV-2 western blot. This was in comparison to the manufacturer's cut-off of >1.1 which had a sensitivity and specificity of 98.3% and 80.3% respectively [129]. Vaginal Gram's stained slides were scored using Nugent's criteria. Scores ≥7 were classified as BV. Vaginal saline wet preparations were assessed for the presence of motile trichomonads, and yeast. Culture for *Neisseria gonorrhoeae* was performed on modified Thayer Martin media. Cervical Gram's stained slides were examined microscopically for the presence of Gram negative intracellular diplococci. Beginning in 2006, endocervical samples were tested for the presence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by transcription mediated amplification (TMA) using the Gen-Probe Aptima GC/CT Detection System (Gen-Probe, San Diego, California, USA).HIV-1 serostatus was determined by ELISA (Detect HIV1/2, BioChemImmunosystems, Montreal, Canada or PT-HIV 1,2-96, PishtazTeb Diagnostics, Tehran, Iran). Positive tests were confirmed using a second ELISA (Recombigen, Cambridge Biotech, Worcester, MA, USA or Vironostika HIV-1 Uniform II AG/AB, bioMerieux, Marcy l'Etoile, France).

Data Analyses

We included all HIV-1-seronegative women in the cohort who were initially HSV-2 seronegative. For women who acquired HIV-1 during the study, we censored visits following HIV-1 infection. The primary exposure of interest was incident HSV-2 infection. Women were considered HSV-2 uninfected prior to a positive HSV-2 test, and positive thereafter. The outcome was BV, dichotomized according to the presence or absence of BV (Nugent score 7-10 versus0-6). The

prevalence of BV was compared during HSV-2-seronegative versus HSV-2-seropositive follow-up visits. The outcome (BV) was measured at multiple time points on participants in our study thus we used generalized estimating equations (GEE) modeling to allow us to assess the association between incident HSV-2 and BV while accounting for the correlation induced by having multiple observations per individual participant. We used GEE with a logit link, exchangeable correlation structure, and robust variance estimates. Results were expressed as odds ratios (OR) with 95% confidence intervals. We considered known and suspected potential confounding factors including age, place of work (bar/restaurant versus nightclub or home-based/other), education level, marital status, sexual risk behaviors, STIs and other genital tract infections (*Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Candida albicans*) hormonal contraceptive use, vaginal washing, alcohol consumption, and tobacco use. We assessed the effect of potential confounding factors one at a time for BV. In the final adjusted model, we included variables that changed the regression coefficients for HSV-2 serostatus as a predictor of BV by 10% or more. We also conducted a sensitivity analysis restricting the data to the subset of women who acquired HSV-2.Analyses were performed using PASW 18.0 (PASW Inc., Chicago, USA) and STATA 11 (StataCorp, College Station, TX, USA).

RESULTS

Between February 1993 and February 2011, 406 women who were both HIV-1 and HSV-2 seronegative contributed 809 person-years of follow-up at 5,650 visits. The median duration of follow-up was 652 days (interquartile range [IQR] 147-1852). The median age of the participants at baseline was 24 years (IQR 22-28). Most women worked in bars (N=244, 60%), while the remainder worked in nightclubs (N=145, 36%) or at other venues (N=17, 4%). One hundred and nineteen women (29%) reported unprotected intercourse at baseline. Bacterial vaginosis was

present at baseline in 116 (29%) of participants. Ninety-two percent of the women reported that they performed vaginal washing.

There were 164 incident HSV-2 infections (incidence rate 21/100 person-years). The prevalence of BV was higher during visits after HSV-2 seroconversion compared to visits before HSV-2 seroconversion (Figure 1).After adjustment for age, incident HSV-2 infection was associated with 1.28-fold increase in the odds of BV (95% confidence interval [CI] 1.05-1.56; p=0.01)(Table 4.1).The magnitude of this association was similar in sensitivity analyses limited to the 164 women who acquired HSV-2 (adjusted odds ratio1.25, 95% CI 1.00-1.57; p=0.05).

DISCUSSION

In this cohort of HIV-1-seronegative women, we found that incident HSV-2 infection was associated with a ~30% increase in the odds of episodes of BV. These findings advance our understanding of the association between HSV-2 infection and the vaginal microbiota, highlighting the temporal relationship between incident HSV-2 infection and a subsequent increase in the frequency of BV. By characterizing the temporal relationship between HSV-2 acquisition and increased episodes of BV, this study makes a valuable contribution that extends beyond earlier prospective studies[9, 14].The magnitude of the association between HSV-2 infection and increased risk of BV that was observed in this study was relatively similar to that observed in prior studies relating prevalent HSV-2 infection to BV[9, 14].

The biological mechanisms that might be responsible for increases in BV following HSV-2 infection are not clear. One possible mechanism is that intermittent HSV-2 reactivation may lead to immune activation in the genital mucosa, altering the vaginal microbiota [100]. Another plausible biological mechanism is that *G. vaginalis* depends on having a source of iron to thrive [130]. This may be particularly important between menses, when availability of iron could be a limiting factor. More consistent availability of iron may create an environment that facilitates the

growth of *G. vaginalis*. Additional studies will be required to elucidate the biological link between HSV-2infectionand BV.

It is interesting to note that the increased likelihood of BV following HSV-2 infection could serve as a mechanism for enhancing further herpes transmission, since BV increases genital shedding of HSV-2[9, 10, 131]. In addition, both HSV-2 and BV have been associated with a greater risk of acquiring and transmitting HIV-1[48]. Thus, understanding the synergistic interactions between BV and HSV-2 could have important HIV-1 prevention implications. Immunodeficiency caused byHIV-1 infection also increases the frequency and severity of HSV-2 reactivations, which could result in increased BV episodes in HIV-1-positive women. Thus HIV-1 status is an important consideration when assessing the association between BV and HSV-2 infection.

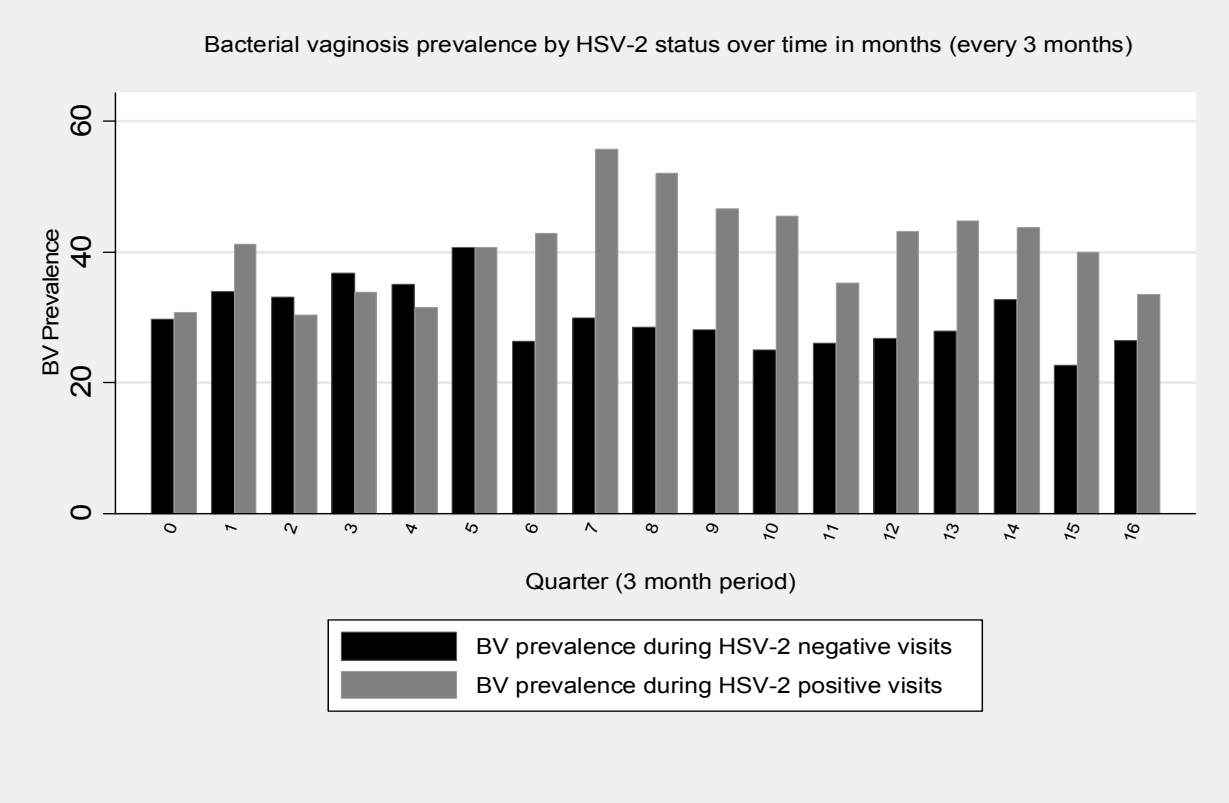
Our study had several strengths. First, these data were prospectively collected from a large population, allowing us to accrue a substantial number of incident cases of HSV-2 infection. The large sample and prolonged follow-up provided statistical power, which allowed us to establish the temporal relationship between HSV-2 infection and increased detection of BV. Second, we had a relatively homogenous population, such that women who acquired HSV-2 were similar to those who did not. Moreover, our analyses provided similar results even when we restricted only to those women who acquired HSV-2. Third, frequent cohort visits allowed us to identify the timing of HSV-2 infection with a high level of precision.

Our results should be interpreted in the context of a number of limitations. First, this was an observational study. Thus, it is not possible to definitively prove that HSV-2 infection caused an increase in BV episodes. Second, of the 406 participants in the study, 35 (8.6%) had an initial index value between 1.1 (manufacturer's recommended cut-off) and 2.1, and then progressed to an index value greater than 2.1. Unfortunately, we do not have Western blot data for these

samples. Thus, it is possible that the cut-off of 2.1 resulted in some participants with index values between 1.1 and 2.1 being falsely classified as negative. Third, we did not collect monthly specimens for HSV-2 detection. This would have served to strengthen our argument that increases in BV may result from intermittent HSV-2 reactivation. Future studies assessing the association between HSV-2 and vaginal microbiota should consider measuring HSV-2 shedding at the time of BV assessment, and more frequently if feasible. Finally, our study population was composed of high-risk women who reported exchanging sex for payment in cash or in kind. These women's sexual risk behavior is expected to be different from the general population, and this could limit the generalizability of our findings.

By demonstrating the temporal sequence of HSV-2 infection followed by an increase in the likelihood of BV, these results strengthen the evidence for a causal link between genital herpes infection and disruption of the vaginal microbiota. Additional studies are needed to improve our understanding of the biological basis of increased BV prevalence among women who become infected withHSV-2. It will also be important to determine whether prevention or suppression of HSV-2 infection is associated with less frequent episodes of BV.

Figure 4.1: Prevalence of BV by HSV-2 status over time in months*



* Time in months since enrollment into the cohort. All women were HSV-2 negative at baseline. As the number of months in follow-up increases, there is an increase in the proportion of women withHSV-2. The proportion of women in follow-up who were HSV-2 positive at 6, 12, 18, 24, 30, 36, 42, and 48 months is 19%, 23%, 23%, 29%, 33%, 38%, 47%, and 66%respectively. The prevalence of BV in HSV-2 negative and HSV-2 positive women is shown for every three months. Data are collapsed after month 48 due to sparse data after 4 years.

Table 4.1: Prevalence of BV during HSV-2 negative vs. HSV-2 positive follow-up

	HSV-2 negative follow-up visits	*HSV-2 positive follow-up visits*	*OR (95% CI)*	*P-value**	*aOR** (95% CI)*	*P-value**
	N=3769	*N=1881*				
BV prevalence, all women[a]	1173 (31.1%)	689 (36.6%)	1.19 (0.99-1.44)	0.07	1.28 (1.05-1.56)	0.01
	N=1296	*N=1881*				
BV prevalence, HSV-2 seroconverting women[b]	424 (32.7%)	689 (36.6%)	1.12 (0.91-1.36)	0.29	1.25 (1.00-1.57)	0.05

* P-values generated from models using generalized estimating equations with a logit link, exchangeable correlation structure and robust errors.

** Model adjusted for age. Additional covariates considered for the multivariate model included place of work, education level, marital status, sexual risk behaviors, sexually transmitted infections, hormonal contraceptive use, vaginal washing, alcohol consumption, and tobacco use. However, these covariates did not confound the association between incident HSV-2 infection and BV prevalence, so were not retained in the final model.

[a] Nugent score 7-10 (versus 0-6)

[b] Nugent score 7-10 (versus 0-6), limited to the 164 women who acquired HSV-2

CHAPTER 5: Conclusion

The findings presented in this dissertation highlight the importance of understanding the epidemiological relationships between STIs including HIV. In this high-risk population of Kenyan women, we described the contribution of different genital tract conditions to HIV acquisition, concluding that HSV-2 and BV were consistently the main contributors to HIV acquisition across 20 years of follow-up (chapter 2). We conducted one of the few studies of incidence and correlates of *C. trachomatis* in sub-Saharan Africa. Similar to findings from developed countries, young age was a significant risk factor for chlamydia infection. There was a strikingly high incidence of *C. trachomatis* in younger high-risk women, with those <25 years 9 times more likely to acquire chlamydia compared to women ≥35 years (chapter 3) [29]. Finally, we contributed an important new study showing an increase in BV prevalence following HSV-2 infection (chapter 4) [40]. In this final chapter of the dissertation, we discuss the implications of our findings and future directions.

Chapter 2: Changes in the contribution (population attributable risk %) of genital tract infections to HIV acquisition among Kenyan female sex workers from 1993 to 2012

Sexually transmitted infections are an enormous source of morbidity and mortality worldwide, making effective STI control an essential public health objective. In terms of potential HIV-prevention impact, findings from our study indicate that HSV-2 and BV have persisted as the main contributors to HIV acquisition in this population of high-risk Kenyan women over a 20 year period. Although STIs vary with time, location, and population, high prevalence rates of HSV-2 and BV are characteristic of many populations [37, 125]. Thus, our findings may have wide applicability. Our results highlight the continued need to identify novel ways to control HSV-2 and BV, as this would add to the available HIV prevention tools.

HSV-2 Prevention

Controlling HSV-2 could have a large impact on HIV incidence in areas of high HSV-2 prevalence [132]. The prevalence of HSV-2 in randomly selected and population-based samples of women of reproductive age in Africa has been found to be 29–71% [133]. This range of prevalence rates and the summary relative risk values calculated in a meta-analysis were used to determine that 38–60% of new HIV infections were attributable to prevalent HSV-2 infection in these women [32]. These findings are similar to what we reported in chapter 1, demonstrating that HSV-2 is a ubiquitous problem spanning both general and high-risk populations.

The disappointing results from trials of HSV-2 suppressive therapy to reduce HIV acquisition do not disprove a causal link between HSV-2 and HIV, but suggest that mechanisms of action, and the design and implementation of interventions need to be better understood. Traditional tools for HSV-2 prevention such as condoms may offer only 40-50% protection [134]. Although acyclovir reduces HSV-2 reactivations, it failed to reduce HIV acquisition at the standard dose. Innovative HSV-2 prevention interventions are urgently needed. A few of the possibilities are discussed below.

Vaccines: If successful, a prophylactic vaccine would be the ideal HSV-2 prevention tool, the aim of which would be to reduce or eliminate viral replication in the mucosa and prevent entry into nerves, eliminating disease. However, findings from vaccine trials thus far have been disappointing. The most recently completed study was the Herpevac trial [82]. This trial assessed the prophylactic efficacy and safety of gD-Alum/MPL vaccine in the prevention of genital herpes disease in young women who were HSV-1 and HSV-2-seronegative. The trial demonstrated a 20% vaccine efficacy that was not statistically significant. Fortunately, there are several promising HSV-2 vaccine candidates that are currently under investigation [135, 136]. Prior efforts to develop a HSV-2 vaccine were primarily focused on non-replicating HSV-2 vaccine candidates such as gD_2-protein subunits [137, 138], and replication-defective HSV-2 viruses [139, 140]. Other novel vaccine ideas are now being pursued and researchers are

revisiting the live attenuated herpes vaccine approach. For example, it has been shown that deletion of conserved regions of HSV-2 ICP0(-) protein results in mutant viruses that are avirulent and immunogenic, and could be considered for HSV-2 prevention [141]. However, in animal models, this approach carries the risk of infection with the challenge virus [142].

HSV-2 prophylaxis: The objective of HSV-2 prophylaxis would be to prevent herpetic disease or infection by aborting the first episode of disease. Prophylaxis could be delivered orally or as a microbicide. Owing to transmission dynamics and socio-economic factors [22], women are more vulnerable and disproportionately affected by STIs, and have limited control over safer sexual practices. Hence, female-controlled prevention methods are urgently needed, particularly for women in the developing world. Results from the CAPRISA 004 trial were encouraging. This was a randomized, placebo-controlled trial of vaginally applied 1% tenofovir gel to assess the efficacy and safety of an antiretroviral-based microbicide in reducing HIV incidence among high-risk women in South Africa [143]. The tenofovir gel reduced HIV acquisition by 39% compared to placebo. Importantly but unexpectedly, tenofovir gel also reduced HSV-2 risk in active product recipients by 51% compared to placebo. Findings from the Partners pre-exposure prophylaxis study data also show that oral tenofovir reduces HSV-2 acquisition by 33% (personal communication, manuscript in press). The follow-on consortium for tenofovir studies (FACTS) consortium is currently conducting a phase III, multi-centre, double-blind, randomized, placebo controlled trial that will assess if tenofovir gel, when used before and after sex, is safe and effective at preventing sexually transmitted HIV and HSV-2. If successful, these findings present an important opportunity that could reduce women's risk of acquiring HSV-2 [144].

BV Prevention

Bacterial vaginosis is also of importance to HIV acquisition. Among women of reproductive age at risk of acquiring HIV, the prevalence of BV ranges from about 20% to 40% [18, 36, 38, 48,

49]. This high prevalence of BV translates into a large PAR% for HIV infection, as we (chapter 1) and others have demonstrated (Table 5.1).

Periodic presumptive treatment: The periodic administration of antimicrobials to a population or core target group, not based on symptoms, signs or laboratory tests, but rather on the likelihood of the group having a genital infection, is referred to as periodic presumptive treatment (PPT). Especially among high-risk groups such as FSWs, PPT can complement other STI control measures. It can also help reduce prevalence of STIs beyond the core group, for example among clients and the general population. One of the key limitations of PPT is the possibility of development of drug resistance. Thus, careful monitoring of the changing epidemiology of STIs and the resistance patterns to drugs commonly used to treat STIs should be performed, and combination therapy is recommended to minimize development of resistance. In addition, using drugs which have a high genetic barrier to development of resistance may be helpful. Periodic presumptive treatment has been recommended by the WHO as an interim measure for STI control among high-risk populations [145].

In a prior clinical trial we demonstrated that PPT using oral 2g metronidazole plus 150mg fluconazole monthly for 12 months was successful in reducing the incidence of BV by 45%, promoting *Lactobacillus* species colonization, and maintaining a healthy vaginal environment [64, 84]. However, this protective effect was lost after PPT was discontinued [146]. We recently completed a second RCT using a co-formulated suppository containing 750mg metronidazole and 200mg miconazole inserted nightly for 5 consecutive nights every month for 12 months. Results from this trial will be released at the Infectious Diseases Society of Obstetrics and Gynecology annual meeting in August 2014.

Only 1 of 9 clinical trials of STI treatment for HIV-1 prevention demonstrated a statistically significant reduction in HIV incidence. With the availability of other potent HIV prevention tools

such as treatment as prevention [87], pre-exposure prophylaxis [88, 89] and male circumcision [90-92], another clinical trial to assess STI treatment for HIV prevention is unlikely. A possible exception would be a successful HSV-2 vaccine, though the logistics of such a trial would be complicated. One option would be to use the stepped wedge trial design that would allow the vaccine to be introduced in trial units in stages and over time. For BV, the most likely way forward would be clinical trials to assess BV treatment as a method for reducing the incidence of non-HIV STIs including HSV-2, human papillomavirus, chlamydia, gonorrhea, and *Mycoplasma genitalium*.

Chapter 3: Incidence and correlates of *Chlamydia trachomatis* in a high-risk cohort of Kenyan women

We found an incidence of 27.6 per 100 person-years of *C. trachomatis* infection among high risk women less than 25 years old, suggesting the need for screening as an important public health intervention for younger high-risk women. Since the etiologic approach to STI management is costly, targeted screening that involves counseling and testing those at greatest risk of chlamydia could be implemented. Targeted screening strategies can identify more high-risk individuals and are less costly than mass screening efforts [147]. These strategies would be particularly useful for chlamydia, given that sensitive tests for detecting chlamydia infection remain too expensive for widespread use in most lower income countries. Targeted, laboratory-based chlamydia screening can be part of a comprehensive STI control program in developing countries. This approach will continue to be useful in light of the costly tests and as development of inexpensive point of care tests continues.

In addition to screening efforts, data from general population women are urgently needed to gain a greater understanding of the extent to which the epidemic crosses over into the general population. As a follow-up to the work presented in this dissertation, we are conducting a pilot

study to assess the feasibility of screening for *C. trachomatis, N. gonorrhoeae* and *T. vaginalis* among young general population women between 15 and 24 years old in Mombasa, Kenya. This is a sequential mixed methods study employing qualitative and quantitative methods to understand the feasibility of STI screening among young general population women. The qualitative component is complete, and included in-depth interviews and focus group discussions among adolescents, young women, parents of female adolescents and institutional leaders. The quantitative component will be starting soon, and will involve screening of young women for STIs using nucleic acid amplification tests of urine samples. An initial rapid assessment of the data from the qualitative components of our research provided results to inform development of the approach to our STI screening study and the question structure. In brief, young women are willing to be screened for STIs and parents are willing to allow their daughters who are under 18 years to be screened. School settings, however, are not a preferred testing location, due to stigma. Hence, we will conduct testing at our clinic. In the course of our qualitative work, a complex question about parental notification of STI test results arose. The parents felt strongly that they would want to know their daughters' STI results. In contrast, the girls were divided, with some willing to share their results but others not. With help from the Kenyatta National Hospital Ethics and Research Committee, we determined that the best approach will be to share results with a parent only if a young woman gave verbal assent. This study is funded by a grant from the Global Center for Integrated Health of Women, Adolescents, and Children (Global WACh) at the University of Washington.

Chapter 4: Incident herpes simplex virus type 2 infection increases the risk of subsequent bacterial vaginosis episodes

In this chapter we demonstrated the temporal sequence of HSV-2 infection followed by an increase in the likelihood of BV. These findings add support to the hypothesis that there is a causal link between genital herpes infection and disruption of the vaginal microbiota. Follow-up

studies are needed to improve our understanding of the biological basis for increased BV prevalence among women who become infected with HSV-2, as this might provide insight into the basic mechanisms that underlie vaginal microbial health versus dysbiosis. In addition, it will also be important to determine whether prevention or suppression of HSV-2 infection is associated with less frequent episodes of BV. Primary prevention of HSV-2 has been extensively described under chapter one above. However, for women already infected with HSV-2, it will be important to determine whether HSV-2 suppression reduces the frequency of BV episodes. Importantly, HSV-2 could be an effect modifier in studies assessing interventions to reduce BV. Future trials of treatment and suppression of BV should take HSV-2 status into consideration.

Worth noting are the interactions between HSV-2 and BV, the two genital conditions that appear to drive HIV acquisition risk. Women with BV are more likely to acquire HSV-2 (Table 5.2). There is also growing evidence to support HSV-2 as a risk factor for BV (Table 5.3), and both HSV-2 (Table 5.4) and BV (Table 5.5) increase the risk of acquiring HIV. Thus, there is the potential that interventions to reduce either HSV-2 or BV could influence both risk factors, resulting in a decrease in HIV risk in women.

SUMMARY

Interactions between the STIs have been recognized since the 1980s [148]. Subsequently, STI control efforts were implemented through the 1990s in an effort to reduce HIV incidence. As the trials failing to show a benefit of STI prevention on HIV incidence accumulated, interest and funding for STI control efforts gradually waned [149]. As a result, STI control programs have been neglected and collapsed. From a global perspective, HIV incidence began to decline prior to the antiretroviral therapy rollout, and STI prevention may have been an important contributor to this. In addition, STIs are highly prevalent and are an important source of morbidity and

mortality. In this context, recognizing the high incidence of STIs is key to highlighting the importance of treatment and prevention programs [125, 150]. Understanding the interactions between STIs will help to inform successful prevention strategies.

Table 5.1: Studies of the contribution of sexually transmitted infections to HIV acquisition

Author/Year	Study Design	Population/ Setting	HIV-1 Prevalence (%) or Incidence	STI (Prevalence %)	PAR% for HIV-1 Seroconversion
Gray et al. 1999 [59]	Data from a RCT for HIV-1 prevention	Men and women, Rakai, Uganda	16%	GUD (4.2) Dysuria/Discharge (9.2) Any symptom present	8.8 (3.7–13.8) 3.9 (-2.0-9.5) 9.5 (2.8-15.8)
Brown et al. 2007[151]	Multicenter cohort study	Family planning clinics, Uganda Family planning clinics, Zimbabwe	1.6/100 person-years 4.1/100 person years	HSV-2 (52) HSV-2 (53)	42 65
Van de Wijgert et al. 2009 [48]	Multicenter cohort study	Family planning clinics, Uganda	17% 1.5/100 person years (1.2 – 2.0)	Prevalent HSV-2 (49) *N. gonorrhoeae* (1.8) *C. trachomatis* (2.9) *T. vaginalis* (2.6) *T. pallidum* (1.7) BV by Nugent (21.4) Intermediate flora (10.8) Yeast on wet mount (4.6)	42.5 4.4 -5.3 Undefined Undefined 8.5 9.5 -9.8
		Family planning clinics, Zimbabwe	39% 4.1/100 person years (3.5 – 4.8)	Prevalent HSV-2 (53) *N. gonorrhoeae* (2.2) *C. trachomatis* (3.0) *T. vaginalis* (4.1) *T. pallidum* (1.6) BV by Nugent (28.6) Intermediate flora (22.6) Yeast on wet mount (14.8)	53.1 5.6 2.6 1.4 -0.9 21.3 13.2 6.4

RCT-Randomized controlled trial; GUD-Genital ulcer disease; PAR%-Population attributable risk percent; BV-Bacterial vaginosis

Table 5.2: Studies of BV as a risk factor for HSV-2

Author	*Population/Setting*	*Exposure definition*	*HSV-2 Risk (95% CI)*
Cherpes et al. [10]	Health care clinic attendees, Pittsburgh, USA	Abnormal flora on gram stain (Nugent's score 4-6)	aOR 1.7 (1.1 – 2.7)
		Abnormal flora on gram stain (Nugent's score ≥7)	aOR: 2.2 (1.5 – 3.2)
Cherpes et al. [11]	Health care clinic attendees, Pittsburgh, USA	Abnormal flora on gram stain (Nugent's score 4-6)	aHR 1.2 (0.4 – 3.4)
		Abnormal flora on gram stain (Nugent's score ≥7)	aHR 2.1 (1.0 – 4.5)
Gottlieb et al. [13]	Public STI clinics Multiple sites, USA	BV by Amsel criteria	aHR 1.9 (1.1 – 3.5)
Allsworth et al. [8]	General population, NHANES USA	Abnormal flora on gram stain (Nugent's score ≥7)	aRR 1.32 (1.11 – 1.56)
Gallo et al. [12]	STI clinic attendees, Alabama, USA	BV by Amsel criteria	aHR 2.4 (1.1 – 5.5)

aOR - Adjusted Odds Ratio; aHR – adjusted Hazard Ratio; aRR – adjusted Relative Risk

Table 5.3: Prospective studies of the association between HSV-2 and BV

Author	*Population/Setting*	*Exposure definition*	*BV Risk (95% CI)*
Kaul et al. [14]	FSWs, Nairobi	HSV-2 by Elisa	aIRR 1.4 (1.1 – 1.8)
Nagot et al. [15]	Family Planning Clinic attendees, Burkina Faso	HSV-2 by Elisa	aRR 1.73 (1.12 – 2.65)
Cherpes et al. [9]	Health care clinic attendees, Pittsburgh, USA	HSV-2 by Elisa	aHR 1.7 (1.3 – 2.3)

aIRR – adjusted Incident Rate Ratio; aRR - Adjusted Relative Risk; aHR – adjusted Hazard Ratio

Table 5.4: Prospective studies of the association between HSV-2 infection and HIV acquisition

Author/Year	Study Design	Population/Setting	Study size	Adjustment	*Adjusted Estimate (95% CI)*
Sobngwi-Tambekou et al. 2009 [152]	Cohort	Men enrolled in a male circumcision trial, Orange Farm, South Africa	3274	Age, circumcision status, sexual risk behavior, marital status, religion	*Prevalent HSV-2 : IRR 3.3 (1.5–7.4)* *Incident HSV-2: IRR 7.0 (3.9–12.4)*
Van de Wijgert et al. 2009 [48]	Cohort	Family planning clinics, Uganda & Zimbabwe	4439	Sexual risk behavior and other STIs	*Prevalent HSV-2: HR 3.69 (2.45-5.55)* *Incident HSV-2: HR 5.35 (3.06-9.36)*
Tobian et al. 2009 [153]	Cohort	Men enrolled in a male circumcision trial, Rakai, Uganda	6396	Age, education, marital status, sexual risk behavior	*Prevalent HSV-2: IRR 2.78 (1.64 – 5.68)* *Incidence HSV-2: IRR 5.28 (2.79–9.98)*
Baeten et al. 2007 [39]	Cohort	FSWs, Mombasa Kenya	1206	Age, education, sexual risk behavior, other STIs	*Prevalent HSV-2: HR 3.58 (1.64–7.82)* *Recent Incident HSV-2: HR 4.94 (1.91-12.80)*
Kapiga et al. 2007 [154]	Cohort	Female bar/hotel workers, Moshi, Tanzania	845	Other STIs, sexual risk behavior	*Prevalent HSV-2: HR 4.3 (1.5–12.4)* *Incident HSV-2: HR 5.5 (1.2–25.4)*
Brown et al. 2007[151]	Cohort	Uganda	2235	Age, sexual risk behavior, other STIs, HC	*Prevalent HSV-2: HR 2.8 (1.5-5.3)* *Prevalent HSV-2: HR 4.6 (1.6-13.1)*
		Zimbabwe	2296	Age, sexual risk behavior, other STIs, HC	*Prevalent HSV-2: HR 4.4 (2.7 – 7.2)* *Incident HSV-2: HR: 8.6 (4.3-17.1)*
Brown et al. 2006 [155]	Cohort	Men who have sex with men, USA	4295	Age, sexual risk behavior, other STIs	*Prevalent HSV-2: HR 1.5 (1.1 – 2.1)* *Remote incident HSV-2: HR 1.7 (0.8 – 3.3)* *Recent incident HSV-2: HR 3.6 (1.7 – 7.8)*
Todd et al. 2005 [156]	Nested case-control	General population, Tanzania	1287	Age, marital status	*Women-prevalent HSV-2: HR 2.88 (0.92 – 8.96)* *Women-incident HSV-2: HR 4.76 (1.21 – 18.8)* *Men-prevalent HSV-2: HR 3.66 (1.28 – 10.4)* *Men-incident HSV-2: HR 5.60 (1.67 – 18.8)*

HSV-2-Herpes simplex virus type 2; IRR-Incident rate ratio; HR-Hazard ratio; OR-Odds ratio; FSWs-Female sex workers; HC-Hormonal contraceptive use

Table 5.5: Prospective studies of the association between BV and HIV acquisition

Author	***Population/Setting***	***Exposure definition***	HIV-1 Risk (95% CI)
Martin et al. [18]	FSWs, Kenya	Abnormal flora on Gram stain (Nugent's score ≥4)	aHR 1.9 (1.1-3.1)
Martin et al. [38]	FSWs, Kenya	BV by Gram stain (Nugent's score ≥7)	aHR 1.4 (1.0-2.1)
Taha et al. [36]	Antenatal clinics, Malawi	Criteria: pH>4.5, abnormal discharge, clue cells, positive amine (whiff) test ≥3 criteria (BV)	Antepartum 3.68* Postpartum aRR 1.84 (0.67-5.09)
Myer et al. [37]	Cervical cancer screening study, South Africa	BV by Gram stain (Nugent's score ≥7)	aOR 2.0 (1.1-3.6)
Van de Wijgert et al. [49]	Family planning clinics, Uganda and Zimbabwe	BV by Gram stain (Nugent's score ≥7)	HR 2.2 (1.5-3.0)
Van de Wijgert et al. [48]	Family planning clinics, Uganda and Zimbabwe	BV by Gram stain (Nugent's score ≥7)	HR 2.12 (1.50-3.01)

aHR-Adjusted hazard ratio; aOR-Adjusted odds ratio

* P-value for trend = 0.04

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152. Sobngwi-Tambekou, J., et al., *Effect of HSV-2 serostatus on acquisition of HIV by young men: results of a longitudinal study in Orange Farm, South Africa.* J Infect Dis, 2009. **199**(7): p. 958-64.

153. Tobian, A.A., et al., *Incident HIV and herpes simplex virus type 2 infection among men in Rakai, Uganda.* Aids, 2009. **23**(12): p. 1589-94.

154. Kapiga, S.H., et al., *The role of herpes simplex virus type 2 and other genital infections in the acquisition of HIV-1 among high-risk women in northern Tanzania.* J Infect Dis, 2007. **195**(9): p. 1260-9.

155. Brown, E.L., et al., *High risk of human immunodeficiency virus in men who have sex with men with herpes simplex virus type 2 in the EXPLORE study.* Am J Epidemiol, 2006. **164**(8): p. 733-41.

156. Todd, J., et al., *Risk factors influencing HIV infection incidence in a rural African population: a nested case-control study.* J Infect Dis, 2006. **193**(3): p. 458-66.

CURRICULUM VITAE

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PERSONAL DATA

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EDUCATION

1996-1997	Integrated Management of Information Systems (IMIS) Higher Diploma, Strathmore College
1997-2003	Bachelor of Medicine and Surgery (MBChB), University of Nairobi
2006-2008	Masters in Public Health (MPH), University of Washington
2009-2014	PhD Epidemiology, University of Washington

POSITIONS AND EMPLOYMENT

Sept. 2003 - Aug 2004	Medical Officer Intern Rift Valley Provincial General Hospital, Nakuru
Aug 2004 – Dec 2004	Medical Officer Rift Valley Provincial General Hospital, Nakuru
Jan 2005 – Mar 2008	Project Physician Mombasa HIV/STD Project
Apr 2008 -	Clinic Section Head Mombasa HIV/STD Project
2009 -	Abstract Mentor International AIDS Society

HONORS/AWARDS

1997	Best Student, IMIS part III, Strathmore College
1997	Best Student, IMIS part IV, Strathmore College
2006	Fogarty Scholar through the International AIDS Research Training Program (IARTP) at the University of Washington

2008	Advanced In Country Scholar; IARTP
2009-2011	Fogarty scholar through IARTP (PhD Epidemiology Student)
2009 July	IAS Young Investigator Award: Women, Girls, HIV
2010 February	CROI Young Investigator Award
2010 February	CFAR Travel Award
2010 October	CFAR Young Investigator Award
2011 July	ISSTDR Young Investigator Award
2012 July	CFAR Young Investigator Award
2014 March	CROI Young Investigator Award

PROFESSIONAL MEMBERSHIPS

2003–present	Kenya Medical Association
2009-present	Kenya Medical Women Association
2009–present	International AIDS Society
2009-present	Center for AIDS Research, University of Washington

TEACHING RESPONSIBLITIES

Teaching Assistant and Facilitator Positions

1. Teaching Assistant, Vaccines (Epi 527). Fall 2010
2. Facilitator, University of Washington Principles of HIV/STI Course. Jan 2011, Jan 2012 (Nairobi)
3. Facilitator, University of Washington Clinical Management of HIV Course Jan-Mar 2011, Jan-Mar 2012 (Mombasa).

Course Lecturer

1. Clinical Management of HIV and STDs (CONJ 553). Winter 2007
2. AIDS and STDs in Women and Children (OBGYN/EPI 590). Spring 2010

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Peer-reviewed publications

1. Day S, Graham SM, **Masese LN**, Richardson BA, Kiarie JN, Jaoko W, Mandaliya K,Chohan V, Overbaugh J, Scott McClelland R. A Prospective Cohort Study of the Effect of Depot Medroxyprogesterone Acetate on Detection of Plasma and Cervical HIV-1 in Women Initiating and Continuing Antiretroviral Therapy. J Acquir Immune Defic Syndr. 2014 May 4. [Epub ahead of print] PubMed PMID: 24798764.

2. **Masese L**, Baeten JM, Richardson BA, Bukusi E, John-Stewart G, Jaoko W, Shafi J, Kiarie J, McClelland RS. Incident herpes simplex virus type 2 infection increases the risk

of subsequent episodes of bacterial vaginosis. J Infect Dis. 2014 Apr 1;209(7):1023-7. doi: 10.1093/infdis/jit634. Epub 2013 Nov 22. PubMed PMID: 24273042; PubMed Central PMCID: PMC3952675.

3. Wilson KS, Odem-Davis K, Shafi J, Kashonga F, Wanje G, **Masese L**, Mandaliya K, Jaoko W, McClelland RS. Association Between Alcohol Use and Sexually Transmitted Infection Incidence Among Kenyan Women Engaged in Transactional Sex. AIDS Behav. 2013 Nov 1. [Epub ahead of print] PubMed PMID: 24179037.

4. Balkus JE, Richardson BA, Mochache V, Chohan V, Chan JD, **Masese L**, Shafi J, Marrazzo J, Farquhar C, McClelland RS. A Prospective Cohort Study Comparing the Effect of Single-Dose 2 g Metronidazole on Trichomonas vaginalis Infection in HIV-Seropositive Versus HIV-Seronegative Women. Sex Transm Dis. 2013 Jun;40(6):499-505.

5. Day SL, Odem-Davis K, Mandaliya KN, Jerome KR, Cook L, **Masese LN**, Scott J, Kim HN, Graham SM, McClelland RS. Prevalence, clinical and virologic outcomes of hepatitis B virus co-infection in HIV-1 positive Kenyan women on antiretroviral therapy. PLoS One. 2013;8(3):e59346.

6. **Masese L**, Baeten JM, Richardson BA, Deya R, Kabare E, Bukusi E, John-Stewart G, Jaoko W, McClelland RS. Incidence and correlates of Chlamydia trachomatis infection in a high-risk cohort of Kenyan women. Sex Transm Dis. 2013 Mar;40(3):221-5.

7. Kavanaugh BE, Odem-Davis K, Jaoko W, Estambale B, Kiarie JN, **Masese LN**, Deya R, Manhart LE, Graham SM, McClelland RS. Prevalence and correlates of genital warts in Kenyan female sex workers. Sex Transm Dis. 2012 Nov;39(11):902-5.

8. **Masese L**, McClelland RS, Gitau R, Wanje G, Shafi J, Kashonga F, Ndinya-Achola JO, Lester R, Richardson BA, Kurth A. A pilot study of the feasibility of a vaginal washing cessation intervention among Kenyan female sex workers. Sex Transm Infect. 2012 Sep 21.

9. Balkus JE, Jaoko W, Mandaliya K, Richardson BA, **Masese L**, Gitau R, Kiarie J, Marrazzo J, Farquhar C, McClelland RS. The posttrial effect of oral periodic presumptive treatment for vaginal infections on the incidence of bacterial vaginosis and Lactobacillus colonization. Sex Transm Dis. 2012 May;39(5):361-5.

10. **Masese LN**, Graham SM, Gitau R, Peshu N, Jaoko W, Ndinya-Achola JO, Mandaliya K, Richardson BA, Overbaugh J, McClelland RS. A prospective study of vaginal trichomoniasis and HIV-1 shedding in women on antiretroviral therapy. BMC Infect Dis. 2011 Nov 3;11:307.

11. Graham SM, **Masese L**, Gitau R, Richardson BA, Mandaliya K, Peshu N, Jaoko W,Ndinya-Achola J, Overbaugh J, McClelland RS. Genital ulceration does not increase HIV-1 shedding in cervical or vaginal secretions of women taking antiretroviral therapy. Sex Transm Infect. 2011 Mar;87(2):114-7.

12. Graham SM, **Masese L**, Gitau R, Jalalian-Lechak Z, Richardson BA, Peshu N, Mandaliya K, Kiarie JN, Jaoko W, Ndinya-Achola J, Overbaugh J, McClelland RS.Antiretroviral adherence and development of drug resistance are the strongest

predictors of genital HIV-1 shedding among women initiating treatment. J Infect Dis. 2010 Nov 15;202(10):1538-42.

13. Gitau RW, Graham SM, **Masese LN**, Overbaugh J, Chohan V, Peshu N, Richardson BA,Jaoko W, Ndinya-Achola JO, McClelland RS. Effect of acquisition and treatment of cervical infections on HIV-1 shedding in women on antiretroviral therapy. AIDS. 2010 Nov 13;24(17):2733-7.

14. McClelland RS, Graham SM, Richardson BA, Peshu N, **Masese LN**, Wanje GH,Mandaliya KN, Kurth AE, Jaoko W, Ndinya-Achola JO. Treatment with antiretroviral therapy is not associated with increased sexual risk behavior in Kenyan female sex workers. AIDS. 2010 Mar 27;24(6):891-7.

15. Graham SM, **Masese L**, Gitau R, Mwakangalu D, Jaoko W, Ndinya-Achola J,Mandaliya K, Peshu N, Baeten JM, McClelland RS. Increased risk of genital ulcer disease in women during the first month after initiating antiretroviral therapy. J Acquir Immune Defic Syndr. 2009 Dec;52(5):600-3.

16. McClelland RS, Richardson BA, Graham SM, **Masese LN**, Gitau R, Lavreys L, Mandaliya K, Jaoko W, Baeten JM, Ndinya-Achola JO. A prospective study of risk factors for bacterial vaginosis in HIV-1-seronegative African women. Sex Transm Dis. 2008 Jun;35(6):617-23.

Presentations and Abstracts

1. **Masese L**, Baeten JM, Richardson BA Graham SM, Bukusi E, Shafi J, Jaoko W, Kiarie J, McClelland RS. Changes in the Contribution (PAR%) of STIs To HIV Acquisition Among Kenyan FSWs From 1993 To 2012. 21st Conference on Retroviruses and Opportunistic Infections (CROI 2014), 3 - 6th March, 2014, Boston, USA: poster 944

2. **Masese L**, McClelland RS, Gitau R, Wanje G, Shafi J, Kashonga F, Ndinya-Achola J, Richardson BA, Lester R, Kurth A. A pilot study of the effectiveness of a vaginal washing cessation intervention among Kenyan female sex workers and the effects of cessation of vaginal washing on biological markers. Oral Presentation, 2010 CFAR Joint Symposium on HIV Research in Women 27th-28th October, 2010, Chicago, USA

3. **Masese LN**, Graham SM, Gitau R, Jaoko W, Peshu N, Ndinya-Achola JO, Mandaliya K, Richardson BA, Overbaugh J, McClelland RS. A Prospective Study of Vaginal Trichomoniasis and HIV-1 Shedding in Women on Antiretroviral Therapy: 17th Conference on Retroviruses and Opportunistic Infections (CROI 2010), 16 - 19th February, 2010, San Francisco, USA: poster 1023

4. Gitau R, Graham SM, Masese LN, Overbaugh J, Chohan V, Richardson BA, Peshu N, Jaoko W, Ndinya-Achola JO, McClelland RS. Effect of Acquisition and Treatment of Cervical Infections on Genital HIV-1 Shedding in Women on Antiretroviral Therapy: A Prospective Cohort Study. 17th Conference on Retroviruses and Opportunistic Infections (CROI 2010), 16 - 19th February, 2010, San Francisco USA: poster 482

5. **Masese LN**, Graham SM, Richardson BA, Peshu N, Wanje GH, Mandaliya KN, Kurth AE Jaoko W, Ndinya-Achola JO, McClelland RS. A Prospective Cohort Study of the

Effect of Antiretroviral Therapy on Sexual Risk Behavior in a High-risk Cohort of Kenyan Women. 5th IAS Conference, 19th -22nd July, 2009, Cape Town, South Africa

6. Graham SM, **Masese L**, Gitau R, Richardson B, Peshu N, Mandaliya K, Jaoko W, Ndinya-Achola J, Overbaugh McClelland RS. Correlates of Genital HIV-1 Shedding among ARV-naïve Women Initiating Therapy. Conference on Retroviruses and Opportunistic Infections (CROI 2009), 8th – 11thFebruary, Montreal, Canada: poster 971

7. Gitau R, Richardson BA, Graham SM, **Masese LN**, Lavreys L, Mandaliya K, Jaoko W, Baeten JM, Ndinya-Achola JO, McClelland RS. A Prospective Study of Risk Factors for Bacterial Vaginosis in African Women. International Congress on Infectious Diseases 2008; Kuala Lumpur, Malaysia: Abstract 40.026

8. **Masese LN**, Graham SM, Gitau R, Mwakangalu DM, Jaoko W, Ndinya-Achola JO, Peshu N, Mandaliya K, McClelland RS. Extending the durability of first line antiretroviral regimens: A pilot study of directly administered antiretroviral therapy. International Congress on Infectious Diseases 2008; Kuala Lumpur, Malaysia: Abstract 25.006

9. Graham SM, **Masese L**, Gitau R, Mwakangalu D, Jaoko W, Ndinya-Achola J, Mandaliya K, Peshu N, Baeten JM, McClelland RS. Increased risk for genital ulcer disease among Kenyan women during the first month after initiation of antiretroviral therapy. XVII International AIDS Conference, Mexico City, Mexico, August 2008. Poster THPE0194.

10. Kariuki M, Gitau R, **Masese L**, Mbuvi J, Njeru M - University of Nairobi. VCT and Infection Control Survey among Medical Students in East Africa; Abstract Presented in ICASA Conference 2003: Nairobi Kenya

ONGOING RESEARCH SUPPORT

09/2009 – 06/2014 P01 HD64915 Project 2; McClelland (Study Leader)
Vaginal microbiota and the risk of HIV acquisition in women.
The goal of this nested case-control study is to examine the association between vaginal bacterial flora and HIV-1 acquisition.
Role: Clinic Section Head

06/2012– 05/2017 RO1 HD72617; McClelland (PI)
Women's lifecourse events & HIV transmission potential: A multidisciplinary study
This study aims to understand how the effect of HIV-positive women's reproductive decisions effect their HIV transmission potential by looking at two effective methods of prevention 1) condom use and 2) ART adherence with viral suppression
Role: Clinic Section Head

04/2013 – 03/2014 Global WACH Grant; Masese (PI)
Exploring the feasibility of screening for sexually transmitted infections among female adolescents and young women in Kenya
This multidisciplinary research seeks to advance our understanding of the feasibility of providing screening for STIs to adolescent and young adult women in a resource-limited setting in Africa.

Role: PI

09/2014 – 08/2015 North Pacific Global Health Grant; Masese (PI)
HPV Vaccine Preparedness among Youth in the Coast Region of Kenya
This pilot study will utilize capture-recapture method to assess the potential number of girls 9-13 years who will be missed if vaccination is rolled out through schools.
Role: PI