Comparison of HPV Infection and Cervical Disease Between HIV-infected and HIV-uninfected Adolescent Females in Cape Town, South Africa

Purpose of Study

The purpose of this study is to investigate the difference in HPV genotype distribution between HIV-infected and HIV-uninfected adolescents in Cape Town, South Africa, and to assess the corresponding difference in HPV-related cervical lesions.

Background

HIV infection has a significant impact on HPV infection and the progression of HPV-related cervical disease. The CDC designated invasive cervical cancer as an AIDS defining illness in 1993 [1]. Compared to their HIV-uninfected counterparts, HIV-infected women have:

1. Increased overall HPV prevalence and multiple infections

In a 2006 meta-analysis of HPV genotypes among HIV-infected women that included over 5,500 subjects from around the globe, HIV-infected women were found not only to have a high prevalence of HPV infection (36.3% among those without any cervical cytological abnormalities), but also to be more likely infected with multiple HPV genotypes concurrently [2]. This increased likelihood of multiple coexistent HPV genotype infections among HIV-infected women has been observed in a number of other studies as well [3-6].

2. Increased prevalence of high-risk HPV

Not only are HIV-infected women more likely to be infected with HPV overall, they are also more likely to be infected with one of the 15-18 HPV genotypes that are “high-risk” or “probable high-risk” for progressing to cervical cancer (HR-HPV). In a study of over 2000 women, HIV-infected women were found to have an odds ratio of 5.07 for infection with HR-HPV when compared to HIV-uninfected women [4].

3. A Distinct pattern of HR-HPV infection

Importantly, the greater overall prevalence of HR-HPV among HIV-infected women occurs in a genotype distribution that is distinct from that in HIV-uninfected women. In a meta-analysis that included data from over 5,500 HIV-infected women, several non-vaccine oncogenic genotypes were found to be more prevalent than HPV 16 among HIV-infected women with high-grade squamous intra-epithelial lesions (HSIL), including genotypes 51, 52, and 58 [2]. Luque et al reported a similar findings in a study of HPV genotype distribution among HIV-infected women in which HPV16 and HPV18 were found to be only the 3rd and 9th most prevalent HR-HPV genotypes among HIV-infected women, respectively [7]. These data have significant implications for the effectiveness of the current HPV vaccines among HIV-infected women.

4. Increased risk of persistence and progression of HPV related cervical lesions
HIV infection has a significant impact on the natural history of HPV infection. Regardless of the level of HIV associated immunosuppression, regression of cervical dysplasia is reduced among HIV-infected women [8]. While most HPV infections are transient, among HIV-infected women, rates of persistent infection are increased multifold [9]. In contrast to other HIV associated malignancies such as Kaposi's sarcoma and non-Hodgkin's lymphoma, extreme immunosuppression is not required to observe an increase in the incidence of invasive cervical cancer among HIV-infected women – in fact the majority of cervical cancer cases in HIV-infected women occur in those with CD4 counts greater than 200 [10].

5. A relationship between level of immunosuppression and HPV disease
More advanced HIV disease (lower CD4 and higher viral load) is strongly associated with increased cumulative HPV prevalence, more advanced cervical dysplasia, and increased rate of progression of cervical disease [4, 6, 8, 9, 11]. Not surprisingly, HIV-infected women receiving anti-retroviral therapy have less cervical disease than those who are not [12]. Ironically, however, as survival among HIV-infected women increases, the overall burden of cervical cancer among this group is likely to increase as longevity allows time for disease progression [13].

In summary, compared to their HIV-uninfected counterparts, HIV-uninfected women have:

- Increased prevalence of HPV overall
- Increased prevalence of HR-HPV
- More multiple infections
- Higher relative prevalence of non-vaccine oncogenic HPV genotypes
- Increased persistence of HPV infections
- Increased progression of cervical disease caused by HPV
- Increased risk of invasive cervical cancer

The vast majority of global prevalence data for HPV genotypes is based on studies of women from Europe and North America. It is in the developing world, however, where most deaths from cervical cancer occur [14, 15]. Africa in particular has been underrepresented in available data. Although African women only accounted for 6% of cases in a meta-analysis of worldwide distribution of HPV genotypes among women with cervical cancer, they were found to have the lowest proportion of cervical cancer cases attributable to HPV16/18 [16]. The combined impact of HIV on HPV infection and regional differences in HPV genotype distribution can be appreciated when specifically looking at HPV infections among HIV-infected women in Africa. The general findings that HIV is associated with increased prevalence of HR-HPV, increased progression of cervical disease, increased persistence of HPV infection, and a distribution of HR-HPV that demonstrates a decreased relative prevalence of vaccine types has

been confirmed in numerous African studies [17-24]. Studying HPV in African women provides an opportunity to assess the additive impact of HIV and regional differences in HPV genotype distributions.

The proposed study is unique in its focus on the HIV-infected, South African, adolescent population, one that is growing rapidly due to both behavioral and perinatal transmission. Newly infected adolescents (particularly young women) are the most significant driver of the epidemic in South Africa, with HIV incidence rates in South African women peaking in the 15-24 age group. Modeling estimates suggest that in South Africa an estimated 36% of all heterosexual transmission of HIV occurs in the 15-24 age group, and that this proportion is substantially higher (up to 45%) among women. In addition, HIV-positive infants born to women with HIV infection prior to the availability of PMTCT in South Africa are now surviving into adolescence. The increasing availability of ART has afforded them longer survival, and modeling predictions indicate a substantial and prolonged emergent epidemic of HIV/AIDS in older children and adolescents [25, 26]. This population is understudied regarding HPV acquisition and persistence. Evaluation for HPV-related cervical disease in a young population provides an opportunity for prompt early intervention.

Study Objectives

The primary hypothesis of the proposed study is that HPV genotype distributions differ between HIV-infected and HIV-uninfected adolescents in Cape Town, South Africa. The secondary hypothesis is that HPV-related cervical disease has increased incidence, prevalence, persistence, and severity among HIV-infected adolescents.

The specific aims of the proposed study are:
1. To compare the HPV genotype distribution and persistence between HIV-infected and HIV-uninfected adolescent females in Cape Town, South Africa.
2. To compare the incidence, prevalence, and progression/regression of HPV-related cervical lesions among HIV-infected and HIV-uninfected adolescent females in Cape Town, South Africa.

Study Design

A four-year long prospective cohort study is proposed in which HPV genotype distribution and cervical disease will be assessed serially in HIV-infected and HIV-uninfected adolescent females.

Upon enrollment, study participants will have baseline Pap smear testing and genital HPV DNA analysis. Repeat HPV DNA analysis will be conducted at six-month intervals until the end of the study. A second Pap smear will be obtained at 24 months, and a final Pap smear at the end of the study (48 months).

Clinically and epidemiologically relevant baseline data will be collected including HAART regimen, most recent CD4 count and viral load (among the HIV-infected group), STI history, smoking history, detailed sexual history, pregnancy history, and family planning needs
. Additional CD4 and viral load data will be acquired as available per routine testing throughout the study period. A repeat sexual history will be obtained at the end of the study (48 months) in order to assess for the possible impact of interval changes in sexual behavior and exposure.

Study Population
All study participants will be recruited through the Masiphumelele Youth Centre where the Desmond Tutu HIV Foundation (DTHF) has been working since 1999. Recruitment will be conducted with the assistance and supervision of Dr. Linda-Gail Bekker. Masiphumelele is approximately 20km south of Cape Town. Several cross-sectional and cohort studies focusing on adolescents have been successfully conducted in this severely under resourced community of 15 000 people. In 2005, DTHF researchers showed that the mean HIV prevalence in the 11-19 yr old was 11%; and the HIV prevalence was 5% in the local high school (the adult HIV prevalence is 26%). Sexual risk assessment suggests that young people in this community practice a range of high-risk HIV behaviors. Based on the demography of this population we expect all research participants to be ethnically African.

Inclusion Criteria: female, age 16-21, sexually active, able and willing to provide consent (ages 18 and older), willing to provide assent (ages 17 and under), parent or legal guardian willing to provide written consent (ages 17 and under).

Exclusion Criteria: history of HPV vaccination, history of cervical surgery.

Study Procedures
Specimens for Pap smear will be obtained by adequately credentialed providers and processed by BARC laboratories using the Bethesda system of reporting. Although we expect extremely few or no findings of high-grade squamous epithelial lesions (HSIL) or cancer in-situ on cervical smear, in the event that HSIL or worse is identified, the affected participant will be referred to False Bay Hospital for colposcopy and additional intervention. Participants with two consecutive smears with LSIL will also be referred for colposcopy. Participants that are referred for colposcopy will be retained in the study.

Specimens for HPV DNA analysis will be obtained via self-collected vaginal swabs (using Digene swab 5123-1220). These specimens will be tested for HPV DNA in the laboratories of Dr. Anna-Lise Williamson at the University of Cape Town using Roche's Linear Array HPV Genotyping Test. This detection kit amplifies target DNA utilizing the polymerase chain reaction (PCR) and is designed to detect 37 human genital HPV genotypes in cervical cells [27]. These

37 types include all 15 “high-risk” oncogenic HPV types that have been identified by the International Agency for Research on Cancer [28] as well as the three HPV types identified as “probable high-risk” [28].

For the HIV-uninfected group, HIV testing and counseling will be performed at each six-month visit. Although very few conversions are expected during the follow-up period, any HIV sero-converters will be retained within the study but censored from the survival analysis portion of data analysis. HIV sero-converters will be referred to the nearby Masiphumelele Clinic for further evaluation, treatment, and counseling support.

Table 1: Study Procedures by Visit

	0 months	6	12	18	24	30	36	42	48
Pap smear	X				X				X
Self-collected vaginal swab for HPV DNA	X	X	X	X	X	X	X	X	X
HIV counseling and testing (HIV-uninfected group only)	X	X	X	X	X	X	X	X	X
Baseline CRF	X								
Follow-up sexual history									X
Recording of updated CD4 and viral load data if available (HIV-infected group only)		X	X	X	X	X	X	X	X

Participant Retention
Study participants will receive R100 in reimbursement for time and travel for each visit. In addition, a R150 incentive will be offered to participants who complete all 9 study visits. In order to maximize participant retention reminder telephone calls will be made during the intervals between study visits.

Statistical Considerations
A total of 50 HIV-infected and 50 HIV-uninfected adolescent females will be recruited for this study. Data will be analyzed to assess differences between the two groups in:

- Baseline HPV genotype distribution
- Cumulative HPV genotype distribution
- HPV persistence
- Baseline prevalence of cervical dysplasia
- Cumulative prevalence of cervical dysplasia
- Risk of incident cervical dysplasia
- Risk of progression/regression of cervical lesions

Estimates of the prevalence of HR-HPV genotypes from a study of South African women are reported in Allan et al [29]. In the healthy control group these estimates are consistently in the 0.5% to 2% per genotype.
Estimates of the prevalence of HR-HPV genotypes among HIV+ women are reported in several sources. In Strickler et al [30] prevalence estimates from studies of HIV+ American women (WIHS, n = 2058, HERS, n = 871) are

reported. They are shown to be significantly negatively correlated with CD4 count. At least 1/2 of those used in the proposed study are in the range of 5% to 15%. If we conservatively assume a prevalence profile vector with 6 prevalence values of (0.15, 0.10, 0.05, 0.05, 0.05, 0.05) and 12 at 0.02 for the HIV+ group, and a constant prevalence value of 0.02 for the HIV- group, then a sample size of 50 per group can detect the aggregate difference using a chi-squared statistic with a power of 85% (5% significance level).

Very large odds ratios for abnormal pap smears given the presence of HR-HPV are reported in Luque et al [31] (OR = 4.22, single HPV vs. no HPV, P = 0.09; OR =22.5, multiple HPV vs. no HPV, P = 0.02). Similar estimates are reported in Luque et al [7]. Among the control group studied in Allan [29] pap smear grades were observed in frequencies 84.8%, 7.9%, 3.8% and 3.6% (normal, ASCUS, LSIL, HSIL). If we assume a frequency for abnormal positive pap-smears of 25% and 5% for the HIV+ and HIV- groups (OR = 6.3), then the difference can be detected with power 80% using 50 subjects per group (5% significance level).

A chi-squared statistic will be used to test for group differences in aggregate as well as single genotype frequencies. Chi-squared tests will also be used to test for group differences in abnormal cytology. In addition to cross-sectional analysis, repeated measures will be analyzed in various ways. The data may be expressed as binned time-to-event measures. Life tables, and other survival analysis methods, will then be used to estimate rates of infection and of abnormal cytology progression, and to estimate rate difference between groups, thus providing greater modeling flexibility. This will be especially important in determining associations between HPV infection and abnormal cytology. Additionally, repeated measures will permit the distinction between persistent (observed more than once consecutively) and transient infections. Rates for each will be separately estimated and compared.

Human Subjects Considerations

- IRB approvals will be obtained from the University of Cape Town Faculty of Health Sciences Research Ethics Committee and the University of Rochester Research Subjects Review Board.
- Approved consent forms will be used for participants aged 18 and older.
- Approved parental consent and child assent forms will be used for participants aged 17 and younger.
- Consent and assent forms will be available in English and Xhosa
- Participants will be clearly informed that they may withdraw from the study at any time and that choosing not to participate or to withdraw from this study will in no way affect access to care.
- Study participants will be assigned study participant numbers and identifying data will not be obtained. Study records will therefore be de-identified. Paper records will be stored in locked cabinets and electronic data will be stored in password protected computers.

- This study poses minimal risks to patients. The Pap smear procedure may cause scant cervical bleeding.
- HPV DNA testing does not provide any benefit to participants. There is no treatment for HPV infection per se.
- Study participants found to have cervical disease on Pap smear will be managed per protocol within the University of Cape Town system.
- HIV-uninfected study participants who seroconvert during the study period will be referred to the Hanan Cruisaid Clinic in Gugulethu for further evaluation and treatment.
- Parental consent will be obtained for all study participants age 17 and under. Still, all study participants will retain privacy with regards to test results, including HIV test results. In the case of a positive test result counselors will encourage participants under age 18 to disclose these results to a trusted adult.

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