# The Association Between Cervical Human Papillomavirus Infection and Subsequent HIV Acquisition in Tanzanian and Ugandan Women: A Nested Case-Control Study

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***Objective.*** This study was performed to analyze the associations between cervical human papillomavirus (HPV) infection and human immunodeficiency virus (HIV) acquisition, using cervical samples from previous studies in Tanzania and Uganda.

***Methods.*** A total of 161 adult women who acquired HIV infection during follow-up and 464 individually matched HIV-seronegative controls were selected from 5 cohorts of women working in bars and recreational facilities. Stored cervical samples were tested for 37 HPV genotypes, using a polymerase chain reaction assay (Roche Linear Array genotyping assay). Multivariate matched analysis using conditional logistic regression was performed to evaluate HPV infection, persistence, and clearance as predictors of HIV acquisition.

***Results.*** HIV seroconverters were significantly more likely than controls to frequently drink alcohol and to be infected with *Chlamydia trachomatis, Neisseria gonorrhoeae*, or herpes simplex virus type 2. There was no evidence of an association between HIV acquisition and any detectable HPV at the visit prior to HIV seroconversion (adjusted odds ratio, 1.02; 95% confidence interval, .66–1.57) or between HIV acquisition and persistent HPV infection (defined as 2 positive HPV genotype–specific test results at least 6 months apart), cleared HPV infection (defined as a positive HPV test result followed by negative HPV genotype–specific test result), or newly acquired HPV infection, compared with HPV-negative women.

***Conclusions.*** There was no evidence of association between HPV infection status and subsequent HIV acquisition. These results stand in contrast to other observational studies.

***Keywords.*** human papillomavirus; HIV (transmission, prevention); Sub-Saharan Africa.

Human papillomavirus (HPV) is a highly prevalent sexually transmitted virus. There are 40 genotypes that infect the genital mucosa [1], 13 of which are regarded as carcinogenic [2]. Persistent infection with carcinogenic HPV (ie, high-risk HPV [HR-HPV]) genotypes is the cause of almost all cases of cervical cancer [3], the third most common cancer among women worldwide [4]. HPV DNA has also been associated with cancerous lesions of the vagina, vulva, penis, anus, and oropharynx [3, 5]. Bivalent (Cervarix; covering HPV 16 and 18) and quadrivalent (Gardasil; covering HPV 6, 11, 16, and 18) HPV vaccines are available. A nonavalent vaccine (covering HPV 6, 11, 16, 18,

31, 33, 45, 52, and 58) gained Food and Drug Administration approval in December 2014 [6]. More than 80% of men and women contract HPV at least once in their lifetime [3, 7]. However, only a small proportion of these infections persist to cause lesions, which can progress to cancer [8, 9], and 90% of infections are cleared within 2 years [10].

East African women harbor the highest HPV DNA prevalence in the world (31.7%; 95% confidence interval [CI], 29.5–33.8) and experience the highest age-standardized incidence rate of cervical cancer (42.7 cases/100 000 women/year) [4, 11–13]. Among cytologically normal, human immunodeficiency virus (HIV)–negative, young women with a median age of 18 years, 73.5% in Tanzania [14] and 73.2% in Uganda [15] were found to have detectable HPV DNA of any genotype. Detection of HPV DNA increases rapidly after HIV seroconversion in HIV-infected women, compared with detection rates over time in women who remain HIV uninfected [16, 17]. HPV is more likely to persist and to progress to cancer in HIV-positive and immunosuppressed women [18, 19].

There is growing evidence that HPV may be an important cofactor in HIV acquisition. There is a plausible biological mechanism for this association: weakened cell adhesion caused by

HPV infection could expose basal layers of the epithelium in the genital tract and form additional HIV entry points [20, 21]. HPV infection and the mechanisms involved in its clearance may cause an influx of inflammatory cytokines [21], macrophages, and T cells [22], creating a favorable environment for HIV invasion. A meta-analysis found an association between detectable HPV DNA and HIV acquisition in 7 of 8 observational studies of both men and women [23, 24–29]; there was a 2-fold increased risk of HIV acquisition among women with prevalent HPV infection with any HPV genotype (hazard ratio, 2.06; 95% CI, 1.44–2.94). Similar associations were observed for infection with HR HPV and low-risk HPV (LR-HPV) genotypes and in 2 studies analyzing the clearance of any genotype [27, 28], but no association was found with HPV persistence. Since this meta-analysis, 2 further observational studies in South Africa that investigated cervical samples confirmed the association between any HPV infection and HIV acquisition in women [30, 31]. Two studies involving men have shown a positive association between penile HPV clearance and HIV acquisition [32, 33]. Clearance of penile HPV infections was associated with elevated dendritic cell density in the foreskin epidermis [33].

In this article, we present findings from a nested case-control study undertaken to investigate the association between cervical HPV infection and HIV acquisition, using stored cervical samples from 5 previous studies in the Lake Zone and Kilimanjaro regions of Tanzania (4 studies) and Kampala, Uganda (1 study). The extent to which HPV infection, persistence, and clearance appear to be predictors for HIV infection is described.

## METHODS

### Study Participants and Design

Cases (women who acquired HIV infection after enrollment) and controls (women who remained HIV negative) were selected from 5 previously enrolled cohorts of women aged 16–45 years working in bars, guesthouses, and recreational facilities in urban and semiurban areas of Tanzania and Uganda (Table 1). Recruitment methods for these studies have been described previously [34–37]. All studies recruited women from their place of work, followed them for ≥12 months between 2002 and 2011, and measured the incidence of HIV infection and other sexually transmitted infections (STIs) at 3–6-month intervals. There was good retention of participants at 12 months across all studies (range, 68% [36] to 90% [37]). Participants were interviewed at enrollment and follow-up visits on socioeconomic status, education level, alcohol and drug usage, sexual behavior, and family-planning practices.

Across the 5 studies, 178 women were HIV negative at enrollment and seroconverted to HIV during follow-up; of these, 172 attended at least 1 study visit ≤12 months before the first detection of HIV and were selected as cases. Cases were individually matched with 3 randomly selected controls from the same study who attended the same follow-up visits but remained HIV seronegative at the time points selected and at the first visit after the visits selected for analysis. Controls could become HIV seropositive at a later visit. Of the 688 women from the original sample selection list, 664 (166 cases and 498 controls [96.5%]) contributed stored samples for testing; samples from 6 cases could not be located in the laboratory archives. If control samples were missing, substitute controls were selected. Some HPV test

**Table 1.** Summary of 5 Cohort Studies From East Africa That Were Used for the Nested Case-Control Study of the Association Between Human Papillomavirus Infection and Human Immunodeficiency Virus (HIV) Acquisition

| Study (Location) | Population | HIV-Negative Enrollees, No. | Period | Follow-up Duration, mo | Relevant Sample Types | Sample Collection Frequency | Cases (n = 161) | Controls (n = 464) |
|---|---|---|---|---|---|---|---|---|
| HSV2 Suppressive Treatment Trial (Lake Zone, Tanzania) | Women working in settings such as bars and guesthouses; age 16–35 y | 821 | 2003–2007 | 30 | Blood, vaginal swab, cervical swab, CVL | Blood: every 3 mo; cervical-vaginal: 0, 6, 12, 24, 30 mo | 54 | 154 |
| Microbicide Feasibility Cohort (Lake Zone) | Women working in settings such as bars and guesthouses; age 16–54 y | 1156 | 2002–2004 | 12 | Blood, vaginal swab, cervical swab | 0, 6, 12 mo (and at 3 and 9 mo for those with symptoms of HIV infection/STIs) | 19 | 56 |
| Women's Health Project (Lake Zone) | Women working in settings such as bars and guesthouses; age 18–44 y | 966 | 2008–2009 | 12 | Blood, vaginal swab, cervical swab, CVL | Blood: 0, 3, 6, 9, 12; cervical-vaginal: 0, 6, 12 mo | 28 | 82 |
| EDCTP Vaccine Cohort (Kilimanjaro Region, Tanzania) | Women working in settings such as bars and guesthouses; age 18–44 y | 412 | 2009–2011 | 12 | Blood, vaginal swab, cervical swab | 0, 3, 6, 9, 12 mo | 14 | 39 |
| Good Health for Women, (Kampala, Uganda) | Self-reported female sex workers and women working in entertainment; age ≥18 y | 646 | 2008–2011 | 18 | Blood, vaginal swab, cervical swab | 0, 3, 6, 9, 12, 18 mo (blood was also collected at 15 mo) | 46 | 133 |

Abbreviations: CVL, cervico-vaginal lavage; STI, sexually transmitted infection.

results were invalid; therefore, the final sample size comprised 161 cases and 464 controls.

Ethics approval for the 4 studies in Tanzania had been granted by the Medical Research Coordinating Committee of Tanzania. The Ugandan study had received approval from the Science and Ethics Committee of the Ugandan Virus Research Institute and the Uganda National Committee for Science and Technology. All 5 studies were approved by the ethics committee of the London School of Hygiene and Tropical Medicine. All participants provided consent for the storage and potential further analysis of their information and samples.

### Laboratory Methods

None of the studies had previously tested samples for HPV. Cervical samples from study visits before HIV seroconversion and when HIV was first detected, as well as those from the equivalent visits in controls, were tested for detectable HPV DNA, using the Linear Array HPV genotyping assay (Roche, Pleasanton, California) [38]. This test detects 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 24 LR-HPV types (6, 11, 26, 40, 42, 53, 54, 55 [also known as 44], 61, 62, 64 [also known as 34], 66, 67, 69, 70, 71, 72, 73, 81, 82, IS39, 83, 84, and CP6108 [also known as 89]) [4]. As IS39 is regarded as a subtype of HPV 82, these genotype-specific results were combined during analysis into just 1 variable for HPV 82 [1]. Cervical samples were either extracts of buffer in which cervical swabs were washed (4 studies) or a supernatant aliquot from a cervico-vaginal lavage (CVL) specimen (1 study), depending on the availability of stored samples. HPV genotyping was performed in the laboratory of the National Institute for Medical Research (NIMR), Mwanza, according to the manufacturer's instructions. External quality assurance was performed by the Catalan Institute of Oncology (ICO), Barcelona, Spain. A random selection of 102 samples (5%) was tested by both laboratories. There was good agreement in the detection of any HPV (κ = 0.69; Supplementary Table 1).

Data on HIV infection and STIs were obtained from the original study databases. All studies used >1 test for confirmation of HIV results. The HSV-2 Suppressive Treatment Trial performed parallel enzyme-linked immunosorbent assays (ELISAs; Vironosticka Uni-Form II Ag/Ab [bioMerieux, Marcy l'Etoile, France] and Murex HIV Ag/Ab Combination [Murex Biotech, Dartford, UK]). If results were discordant or indeterminate, HIV-1 p24 antigen enzyme immunoassay (EIA; Biorad Genetic Systems, Hemel Hempstead, UK) was used to detect acute infection. A negative or indeterminate result with the p24 antigen EIA was confirmed by Western blot [34]. The Microbicide Feasibility Study used sequential testing on a gelatin-particle agglutination test (Serodia HIV-1/2; Fujirebio, Tokyo, Japan), followed by a confirmatory ELISA (Vironosticka Uni-Form II) [36]. For both the Mwanza Women's Health cohort and the Moshi Vaccines cohort, parallel testing with rapid diagnostic tests (RDTs) was

performed (SD Bioline HIV-1/2 3.0 [Standard Diagnostics, Gyeonggi-do, Republic of Korea] and Determine HIV-1/2 [Alere Medical, Waltham, Massachusetts]); discordant results were confirmed by ELISA (Vironosticka Uniform II plus O and Murex HIV 1.2); discordant EIA results were confirmed by Western blot (INNO-LIA; Innogenetics, Ghent, Belgium) [37]. The Uganda Good Health for Women project tested women using a single Determine rapid diagnostic test (Abbott Determine HIV-1/2) with confirmation of positive results using parallel ELISAs (Vironosticka Uni-Form II plus O, Murex HIV 1.2.O). Discordant results were confirmed by Western blot (Cambridge Calypte, Portland, Oregon) [35].

Serological tests for syphilis were analyzed by a rapid plasma regain (RPR) test (Biotec, Birdport, UK) and the *Treponema pallidum* hemagglutination (TPHA) test (Biotec). Results from both tests were used to define active syphilis (positive results of both tests), past or treated syphilis (a negative result of the RPR test and a positive result of the TPHA test), and negative for syphilis (negative results of both tests or a positive result of the RPR test and a negative result of the TPHA test) in all but 1 study. One study only performed the TPHA test if participants had a positive result of the RPR test. All studies tested serum samples for antibodies to herpes simplex virus type 2 (HSV-2), using a type-specific immunoglobulin ELISA (Kalon Biologicals, Guildford, UK).

Cervical swabs were tested by polymerase chain reaction analysis for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (Amplicor; Roche). Vaginal swabs were used to culture *Trichomonas vaginalis*, using In-Pouch (Biomed Diagnostics, White City, Oregon), and to prepare a Gram-stained slide for the detection of bacterial vaginosis, using the Nugent score.

### Statistical Methods

Conditional logistic regression was used to analyze associations between HIV acquisition and (1) the presence of an HPV genotype–specific infection at the visit prior to the HIV seroconversion visit (the s − 1 visit), or at the equivalent visit in controls, (2) evidence of any HPV clearance, persistent HPV infection only, or HPV acquisition only. Clearance was defined as a genotype-specific positive test result followed by a negative test result for the same genotype, regardless of the status of other HPV coinfections. Persistence was defined as detectable infection with the same genotype for ≥6 months with no evidence of clearance of other genotypes. Acquisition was defined as a negative HPV test result followed by a positive HPV test result for the same genotype and no evidence of clearance or persistence of other genotypes. The possible combinations of visits attended by the cases and controls selected for this study and visit classifications are detailed in Figure 1. Owing to variations in questionnaires, a number of variables identified a priori as potential confounders were recoded to be consistent across studies (Table 2).

First, age-adjusted analyses, with age as a categorical variable, were performed on all available covariates identified a priori as
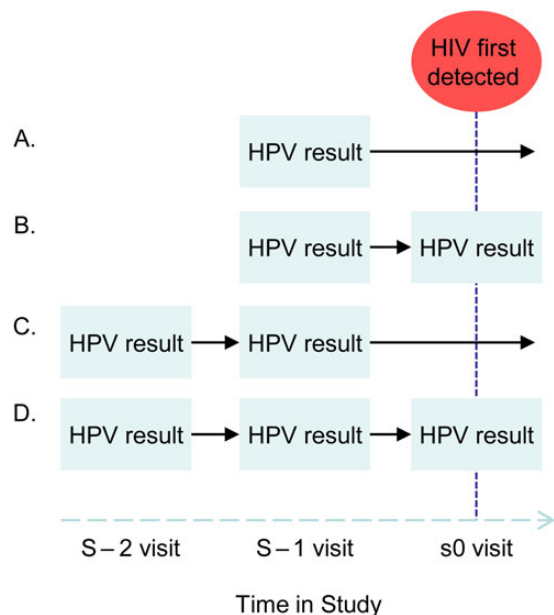
**Figure 1.** Combinations of original study visits and available human papillomavirus (HPV) test results, relative to the visit at which human immunodeficiency virus (HIV) was first detected, attended by cases and controls and selected for analysis. The s − 1 visit denotes the visit preceding the visit during which HIV seroconversion was detected, and the s − 2 visit denotes the visit preceding the s − 1 visit.

potential confounders and common to all study questionnaires, to assess their association with HIV acquisition (Table 2). Information on sociodemographic and behavioral covariates was either from baseline (the enrollment questionnaire) or time-updated to values at the time of first detection of HIV seroconversion (the index visit; s0). Since STI data were not gathered at every visit, time-updated STI results used data from both the s0 visit and the s − 1 visit; women were classified as positive for the STI if they had a positive test result at either time point.

Next, age-adjusted analyses of the association of HPV with HIV acquisition were performed. Multivariable models to control for potential confounding were then constructed. Variables describing mobility, vaginal cleansing practices, *Trichomonas* infection, and presence of genital warts were not included in the multivariable analysis because of missing data. The remaining covariates were assessed iteratively for evidence of a change in the effect estimate for the association of HPV and HIV, starting with covariates with the strongest association with HIV in age-adjusted analyses. To minimize the number of parameters estimated in the model, only covariates that influenced the size of the effect estimate for HPV remained in the multivariate model. Multicollinearity was assessed against a model adjusted only for age. All P values were generated by likelihood ratio tests. Assuming an HPV prevalence of 30%–50% in control subjects, 150 HIV-positive cases and 450 HIV-negative controls, we calculated that we would have ≥80% power to detect an odds ratio (OR) of ≥1.75 for the association between

HIV acquisition and HPV infection at the visit before HIV seroconversion.

## RESULTS

Across all time points, 90 of the 1258 samples (7%) returned an invalid/inhibited HPV result on first testing. Sixty-six of these samples had sufficient volume to be retested; 41 gave a valid HPV result. Valid HPV results were therefore available for 1209 samples (96%). At the s − 1 time point, 625 of 664 women (94%; 161 cases and 464 controls) returned valid HPV test results and therefore contributed to the main analysis of the association between HIV status and HPV prevalence.

The mean age of cases and controls at enrollment was 25 years (range 16–45 years; Table 2). One fifth of women (22%) were married or living as married. Over 50% of women stated that their main source of income came from restaurant or bar or guesthouse work and 24% identified themselves as local brew or street food sellers. A proportion of those whose income source was categorized as "other" may have been sex workers, as this was only determined as a separate category in the Ugandan study. Although 49% had completed primary education, <18% had some secondary or higher education. In time-updated data from the visit of first detection of HIV (s0) approximately one third of women reported not currently using any form of contraception, whereas 57% reported having used a condom at last sex.

Younger age was strongly associated with HIV seroconversion ($P_{trend} < .001$; Table 2). In age-adjusted analysis, women reporting a higher number of lifetime partners, having been paid for sex in the past 3 months, and those drinking alcohol more frequently, had a significantly increased odds of HIV seroconversion. Women who had completed primary education had a higher risk than those with incomplete primary education. Women who experienced forced sex during the 3 months prior to enrollment had a higher risk of HIV seroconversion, but this effect was not statistically significant. At the s0 or the s − 1 visit, 10% of cases and controls had *C. trachomatis* infection, 11% had *N. gonorrhoeae* infection, and 86% had evidence of exposure to HSV-2. These infections significantly increased the odds of HIV seroconversion by 2.8-, 2.6-, and 2.0-fold, respectively (Table 2).

### HPV Prevalence Before HIV Acquisition
The median interval between the s − 1 visit and the first visit at which HIV was detected (the s0 visit) was 3 months; 87% of cases and controls had a cervical sample at or <6 months prior to the s0 visit.

At the s − 1 visit, 49% of women (51.6% of cases and 48.5% of controls) across groups A–D in Figure 1 had detectable HPV infection of any genotype. In adjusted analyses, there was no evidence that prevalent infection with any HPV genotype at the visit prior to the first detection of HIV was associated with HIV acquisition (adjusted OR for any HPV type infection, 1.02; 95% CI, .66–1.57; Table 3).

**Table 2.** Characteristics of the Matched Cases and Controls and Age-Adjusted Associations With Case-Control Status, Using Conditional Logistic Regression

| Characteristic | Cases, No. (%) (n = 161) | Controls, No. (%) (n = 464) | Age-Adjusted OR (95% CI) | P Value |
|---|---|---|---|---|
| Enrollment data | | | | |
| Age group, y | | | | |
|   16–24 | 79 (49.0) | 178 (38.4) | 1 | <.001[a] |
|   25–34 | 70 (43.5) | 208 (44.8) | 0.64 (.41–.99) | |
|   ≥35 | 12 (7.5) | 78 (16.8) | 0.28 (.13–.57) | |
| Marital status | | | | |
|   Married/living as married | 27 (16.8) | 109 (23.5) | 1 | .221 |
|   Separated/divorced/widowed | 94 (58.4) | 252 (54.3) | 1.54 (.92–2.57) | |
|   Single | 40 (24.8) | 103 (22.2) | 1.22 (.66–2.25) | |
| Religion | | | | |
|   Christian | 121 (75.2) | 326 (70.3) | 1 | .495 |
|   Muslim | 34 (21.1) | 121 (26.1) | 0.78 (.49–1.23) | |
|   Other/no religion | 6 (3.7) | 17 (3.6) | 1.21 (.46–3.19) | |
| Main occupation | | | | |
|   Restaurant/bar worker/cleaner | 85 (52.8) | 247 (53.2) | 1 | .916 |
|   Local brew or street food vendor | 40 (24.8) | 113 (24.4) | 1.05 (.64–1.74) | |
|   Manager/owner/office worker | 8 (5.0) | 34 (7.3) | 0.79 (.33–1.89) | |
|   Sex worker[b] | 18 (11.2) | 46 (9.9) | 1.22 (.59–2.53) | |
|   None/other | 10 (6.2) | 24 (5.2) | 1.26 (.56–2.80) | |
| Maximum education level | | | | |
|   None/incomplete primary | 48 (29.8) | 162 (34.9) | 1 | .008 |
|   Complete primary | 92 (57.1) | 214 (46.1) | 1.61 (1.04–2.51) | |
|   Some secondary/higher | 21 (13.0) | 88 (19.0) | 0.66 (.36–1.23) | |
| Crowding[c] | | | | |
|   0–2 | 95 (56.9) | 248 (50.5) | 1 | .116[a] |
|   3–4 | 45 (27.0) | 129 (26.3) | 0.64 (.41–.99) | |
|   ≥5 | 27 (16.2) | 114 (23.2) | 0.29 (.14–.59) | |
| Lifetime no. of sex partners[d] | | | | |
|   <5 | 50 (31.9) | 179 (39.5) | 1 | .024[a] |
|   5–9 | 39 (24.8) | 92 (20.3) | 1.71 (1.02–2.87) | |
|   ≥10 | 24 (15.3) | 80 (17.7) | 1.49 (.81–2.75) | |
|   Do not remember | 44 (28.1) | 102 (22.5) | 2.78 (1.33–5.82) | |
| Forced sex[e] | | | | |
|   No | 121 (77.1) | 378 (83.1) | 1 | .058 |
|   Yes | 36 (22.9) | 77 (16.9) | 1.79 (.98–3.23) | |
| Alcohol consumption | | | | |
|   Never | 53 (32.9) | 193 (41.6) | 1 | .003[a] |
|   1 time/wk or less | 9 (5.6) | 52 (11.2) | 0.60 (.28–1.32) | |
|   2–3 times/wk | 47 (29.2) | 114 (24.6) | 1.75 (1.06–2.91) | |
|   ≥4 times/wk | 52 (32.3) | 105 (22.6) | 2.02 (1.23–3.33) | |
| Time-updated data | | | | |
| Current method of family planning[f] | | | | |
|   Nothing | 50 (31.1) | 143 (31.4) | 1 | .650 |
|   Oral pill | 29 (18.0) | 84 (18.5) | 1.04 (.58–1.86) | |
|   Injection | 45 (28.0) | 100 (22.0) | 1.30 (.77–2.17) | |
|   Condom | 30 (18.6) | 103 (22.6) | 0.83 (.48–1.43) | |
|   Other (eg, traditional/calendar) | 7 (4.4) | 25 (5.5) | 0.93 (.34–2.57) | |
| Condom use at last sex[g] | | | | |
|   No | 87 (55.4) | 268 (60.1) | 1 | .439 |
|   Yes | 70 (44.6) | 178 (39.9) | 1.16 (.79–1.71) | |
| Transactional sex[h] | | | | |
|   No | 85 (53.1) | 290 (63.7) | 1 | .002 |
|   Yes | 75 (46.9) | 165 (36.3) | 2.18 (.37–.98) | |
| Partners in the last 3 mo | | | | |
|   None/1 | 94 (58.8) | 298 (65.5) | 1 | .148[a] |
|   2–9 | 39 (24.4) | 89 (19.6) | 1.50 (.91–2.47) | |
|   ≥10 | 18 (11.3) | 54 (11.9) | 1.30 (.58–2.93) | |
|   Do not remember | 9 (5.6) | 14 (3.1) | 2.52 (.90–7.00) | |

*Table 2    continued.*

| Characteristic | Cases, No. (%) (n = 161) | Controls, No. (%) (n = 464) | Age-Adjusted OR (95% CI) | P Value |
|---|---|---|---|---|
| STI test results at the s0 and s − 1 visits | | | | |
| *C. trachomatis* | | | | |
| Negative at both visits | 133 (82.6) | 431 (92.9) | 1 | <.001 |
| Positive at ≥1 visit | 28 (17.4) | 33 (7.1) | 2.76 (1.57–4.87) | |
| *N. gonorrhoeae* | | | | |
| Negative at both visits | 130 (80.8) | 426 (91.8) | 1 | <.001 |
| Positive at ≥1 visit | 31 (19.3) | 38 (8.2) | 2.58 (1.51–4.41) | |
| HSV-2[i] | | | | |
| Negative at both visits | 15 (9.4) | 70 (15.2) | 1 | .022 |
| Positive at ≥1 visit | 144 (90.6) | 392 (84.9) | 2.00 (1.08–3.75) | |

Abbreviations: CI, confidence interval; *C. trachomatis, Chlamydia trachomatis*; HSV-2, herpes simplex virus type 2; *N. gonorrhoeae, Neisseria gonorrhoeae*; OR, odds ratio; s0 visit, the visit during which HIV seroconversion was detected; s − 1 visit, the visit preceding the visit during which HIV seroconversion was detected STI, sexually transmitted infection.

[a] For linear trend. In the association with number of partners, we assume that women who stated "do not remember" had the highest number of partners.

[b] Only the Ugandan study included sex work as a category in answer to the question "What is your main source of income?"

[c] Data are no. of people living in the participant's residence.

[d] Data for 15 women (11 [2.4%] of controls and 4 [2.5%] of cases) were missing.

[e] Data are for experience of forced sex during the 3 mo before enrollment. Data for 13 women (9 [1.9%] of controls and 4 [2.5%] of cases) were missing.

[f] Data for 9 women (1.9% controls) were missing.

[g] Data for 22 women (18 [3.8%] of controls and 4 [2.5%] of cases) were missing.

[h] Data are for experience of transactional sex during the 3 mo before enrollment. Data for 10 women (1 case [<1%] and 9 controls [2%]) were missing.

[i] Data for 4 women (2 cases [1%] and 2 controls [<1%]) were missing.

Additional analyses of HR-HPV and LR-HPV types, analyses of nonavalent vaccine types (Table 3), and analyses restricted to the 87% of participants with s − 1 samples collected ≤6 months before HIV seroconversion (Supplementary Table 2) did not show notably different results.

### HPV Clearance or Persistence and HIV Acquisition

A total of 119 cases and 323 controls had data from at least 2 time points (groups B–D in Figure 1) and were included in the analysis of the association of HPV clearance and/or persistence with HIV acquisition. Among these women, 42% had evidence of clearance of an HPV genotype irrespective of the status of other genotype-specific infections (the "any clearance" group), 10% had evidence of 6-month persistence with an HPV genotype only (with no evidence of clearance, with/without acquisition of a different genotype), and 13% had evidence of acquisition of an HPV genotype only (with

**Table 3.    Associations Between Human Papillomavirus (HPV) Infection at the Visit Preceding the First Detection of Human Immunodeficiency Virus (HIV) Seroconversion (the s − 1 Visit) and Subsequent HIV Acquisition Among Women With a Valid HPV Test Result at the s − 1 Visit**

| HPV Infection Status at s − 1 | Cases, No. (%) (n = 161) | Controls, No. (%) (n = 464) | Age-Adjusted Analysis OR (95% CI) | P Value | Adjusted Analysis 1[a] OR (95% CI) | P Value | Adjusted Analysis 2[b] OR (95% CI) | P Value |
|---|---|---|---|---|---|---|---|---|
| Any type | | | | | | | | |
| No infection | 78 (48.5) | 239 (51.5) | 1 | .783 | 1 | .923 | 1 | .926 |
| Any HPV infection | 83 (51.6) | 225 (48.5) | 1.06 (.72–1.56) | | 1.02 (.68–1.53) | | 1.02 (.66–1.57) | |
| Nonavalent vaccine types | | | | | | | | |
| No HPV infection | 78 (48.5) | 239 (51.5) | 1 | .867 | 1 | .875 | 1 | .901 |
| Nonavalent vaccine–type HPV infection | 38 (23.6) | 103 (22.2) | 0.99 (.61–1.60) | | 0.95 (.57–1.56) | | 0.95 (.56–1.61) | |
| Other HPV infection | 45 (28.0) | 122 (26.3) | 1.12 (.71–1.75) | | 1.09 (.68–1.75) | | 1.08 (.66–1.78) | |
| HR/LR-HPV infection | | | | | | | | |
| No HPV infection | 78 (48.5) | 239 (51.5) | 1 | .667 | 1 | .738 | 1 | .658 |
| HR-HPV infection only | 27 (16.8) | 70 (15.1) | 1.08 (.63–1.85) | | 1.07 (.60–1.89) | | 1.15 (.63–2.08) | |
| LR-HPV infection only | 33 (20.5) | 83 (17.9) | 1.26 (.75–2.12) | | 1.19 (.69–2.06) | | 1.17 (.66–2.08) | |
| HR/LR-HPV coinfection | 23 (14.3) | 72 (15.5) | 0.85 (.48–1.48) | | 0.82 (.45–1.47) | | 0.78 (.43–1.44) | |

Abbreviations: CI, confidence interval; HR, high risk; LR, low risk; OR, odds ratio.

[a] Data are from 160 cases and 455 controls with available data and were adjusted for variables that influenced the effect estimate of the association between any HPV at s1 and HIV (ie, age group, alcohol consumption at enrollment, and transactional sex in the 3 months prior to first detection of HIV [time-updated variable]).

[b] Data are from 158 cases and 454 controls with available data and were adjusted for the same variables as for adjusted analysis 1, as well as for the time-updated sexually transmitted infection variables *Chlamydia trachomatis* detection, *Neisseria gonorrhea* detection, and herpes simplex virus type 2 (HSV-2) detection. Women were classified as positive for *C. trachomatis*, *N. gonorrhea*, or HSV-2 if they had results for tests performed at the s0 and/or s − 1 visits and if the result from at least 1 visit was positive.

**Table 4. Associations of Human Papillomavirus (HPV) Persistence, Clearance, and Acquisition With Subsequent Human Immunodeficiency Virus (HIV) Infection Among Women Who Had Valid HPV Test Results From at Least 2 Time Points**

| HPV Persistence, Clearance, Acquisition Prior to HIV Seroconversion[a] | Cases, No. (%) (n = 119) | Controls, No. (%) (n = 323) | Age-Adjusted Analysis | | Adjusted Analysis 1[b] | | Adjusted Analysis 2[c] | |
|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P Value | OR (95% CI) | P Value | OR (95% CI) | P Value |
| Among all women with >1 valid result (at s0, s − 1, or s − 2) | | | | | | | | |
| Uninfected | 37 (31.1) | 118 (36.5) | 1 | .771 | 1 | 0.765 | 1 | .685 |
| Any HPV clearance | 53 (44.5) | 134 (41.5) | 1.17 (.68–2.00) | | 1.08 (.61–1.89) | | 0.85 (.46–1.55) | |
| HPV persistence for 6 mo | 10 (8.4) | 33 (10.2) | 0.97 (.41–2.28) | | 0.94 (.39–2.27) | | 0.93 (.37–2.29) | |
| HPV acquisition only | 19 (16.0) | 38 (11.8) | 1.42 (.68–2.95) | | 1.44 (.68–3.04) | | 1.33 (.61–2.90) | |

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Women who remained HPV negative at all available time points were classified as uninfected. Women with evidence of any HPV clearance, defined as a genotype-specific positive test result followed by a genotype-specific negative result, regardless of concurrent HPV persistence or acquisition, were classified as having any HPV clearance. Women with at least 2 genotype-specific positive test results at least 6 mo apart, with or without evidence of acquisition of other genotypes, were classified as having HPV persistence for 6 mo. Women who only had a genotype-specific negative test result followed by a positive result for the same genotype at a later time point, with no evidence of persistence or clearance of other genotypes, were classified as having HPV acquisition only.

[b] Data are for 118 cases and 319 controls with available data and are adjusted for age group, alcohol consumption at enrollment as a linear variable, and transactional sex in the 3 months before the visit when HIV was first detected (ie, the same model as used for the analysis of the effect of prevalent HPV in Table 3; all other covariates were checked, but no other residual confounding was found).

[c] Data are for 117 cases and 319 controls with available data and are adjusted for age, alcohol consumption at enrollment as a linear variable, transactional sex, and Chlamydia trachomatis and herpes simplex virus type 2 detection at time of or the visit before the first visit when HIV was detected. Neisseria gonorrhea detection was removed from the model to reduce the number of parameters, as it did not affect the adjusted OR.

no concurrent clearance or persistence; Table 4). Among the 237 women with HPV data from an s − 2 visit, 94% had a s − 2 cervical sample collected at or within 12 months prior to the s0 visit.

Adjusted analyses based on data from all available time points did not show evidence of an association between the clearance, persistence, or acquisition of HPV infection and HIV acquisition (Table 4). Additional analyses restricted to s − 2 and s − 1 time points and excluding data from the s0 visit, when HIV infection could have influenced HPV infection, yielded somewhat larger effect estimates for all associations between HPV and HIV. However, CIs were wide, and none of the results were statistically significant (Supplementary Table 3).

## DISCUSSION

In these cohorts of Tanzanian and Ugandan women, there was no evidence of an association between HPV infection, clearance, persistence, or acquisition and the odds of HIV acquisition. These results stand in contrast to other observational studies in high-risk women, which found a 2-fold increased risk of HIV acquisition in those who were HPV positive [23]. There is a plausible mechanism by which HPV infection could increase the risk of HIV acquisition through mechanical or immunological changes in the cervix [20–22]. There is strong evidence of cofactor effects of other STIs on HIV transmission [39–46]. We sought to confirm the findings of previous studies and add to limited data on HPV clearance and subsequent HIV acquisition, but we were unable to provide evidence in our study populations for such association.

Strengths of our study include its size; with 161 cases and 464 controls, our study is the largest to date. In previous studies, the number of HIV seroconverters ranged from 4 to 145 women

[24–26, 28, 47]. The original cohort studies used for our investigation had high retention rates, and the characteristics of cases and controls were very similar. Cases and controls were selected from the same populations and matched to account for different study sites and designs and for individuals' adherence to study procedures. A sensitivity analysis showed that results did not differ between studies using cervical swab specimens and the one study using CVL specimens to diagnose HPV infection. The risk of misclassification of HIV infection status was low, as all studies used confirmatory HIV tests.

There are a number of possible reasons why our results differ from those reported in the literature. Apart from the Ugandan women, who included self-identified sex workers, it could be considered that the remainder of the participants had a lower risk of HIV acquisition than women recruited in previous studies, some of which only recruited sex workers [24, 26]. However, >80% of both cases and controls in our study were HSV-2 seropositive. Although cases in previous studies had a similarly high HSV-2 prevalence, the prevalence in controls was significantly lower in previous studies [27, 47]. Interactions between HPV, other sexually transmitted pathogens, and the cervical-vaginal microbiome are unclear; however, the effect of STIs on HIV acquisition may diminish the relative effect of HPV. External quality control results from the ICO laboratory indicated fair agreement in the detection of HPV genotypes; nondifferential misclassification of HPV infection may have diluted the measured effect of HPV on HIV.

Across all exposure variables investigated, there was little evidence of confounding. No information on the relative risk status of the participants' sex partners was available and missing data precluded controlling for T. vaginalis infection, mobility, vaginal cleansing practices, and the presence of genital warts.

However, when analyzing the available data on these variables, there were no significant associations with HIV status. Cases and controls were well balanced with regard to baseline STIs; however, residual confounding related to differences in sexual behavior cannot be excluded. The numbers of participants with clearance and 6-month persistence were small, and CIs were wide; the existence of a moderate effect of HPV on HIV acquisition cannot be ruled out.

Our study used samples that had been stored in freezers for 3–9 years. Samples that have been embedded in paraffin show good reproducibility [48, 49]; however, the durability of DNA in frozen swab aliquots or CVL specimens after long-term storage is undocumented. There is some evidence that detection of β-globin decreases over time, leading to a greater number of faint/invalid results and, therefore, potential misclassification of HPV infection status. The inclusion of s0 samples in our analysis could limit the temporal relevance of the analysis of clearance and persistence. Acquisition of HIV between the $s-1$ and s0 time points may have increased the probability of HPV persistence and/or new acquisition of HPV, which was then detectable at the s0 time point [17]. However, these time points have been used in the 2 previous studies analyzing the effect of HPV clearance and persistence on HIV, with a similar prevalence of clearance (around 40%) [27, 28]. When restricting our analysis to the $s-2$ and $s-1$ time points, the effect estimates were larger; however, CIs were wide, and $P$ values preclude any clear conclusions. All studies of the association between HPV and HIV infection potentially suffer from inadequate methods of defining HPV infection status. More research is needed to find biological markers of newly acquired infection, latent infection, clearance, and persistence.

In conclusion, the HIV epidemic remains a serious problem in sub-Saharan Africa, which simultaneously harbors the highest burden of HPV infection in the world [4]. If previous reports are accurate and HPV infection leads to a 2-fold increase in risk of HIV acquisition, the highly effective HPV vaccines could substantially influence rates of HIV acquisition in areas of high HPV incidence. Whereas previous studies suggested that HPV infection enhances the risk of HIV acquisition, our study, based on a large data set from 5 cohorts in East Africa, could not confirm this association; more research is needed to clarify these contrasting results. Given the contradictory evidence of an association between HPV and HIV, a cluster randomized controlled trial of phased delivery of HPV vaccine with monitored HIV infection incidence may be the only way of concluding whether there is a causal relationship between HPV infection and HIV acquisition.

## Supplementary Material

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

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