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Human Papillomavirus Infection and Cervical Abnormalities in Nairobi, Kenya, an Area With a High Prevalence of Human Immunodeficiency Virus Infection

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Human papillomavirus (HPV) infection and cervical abnormalities, and their association with human immunodeficiency virus (HIV) infection were studied in 488 women who visited a health center in Nairobi. PCR-based HPV and cervical cytology tests were carried out on all participants, and peripheral CD4+ T cells and plasma HIV RNA were quantitated in HIV positive women. HIV were positive in 32% (155/488) of the women; 77% of these were untreated, and the others had been treated with anti-retroviral drugs within 6 months. Cervical HPV infection was detected in 17% of HIV negative and 49% of HIV positive women. Low-grade squamous intraepithelial lesions were observed in 6.9% of HIV negative and 21% of HIV positive women, while high-grade squamous intraepithelial lesions and cancer were seen in 0.6% and 5.8%, respectively. Multivariate analysis revealed that HIV and HPV infections were associated with each other. Cervical lesions were significantly associated with high-risk HPVs and with HIV infection, depending on HPV infection. HPV infection increased in accordance with lower CD4+ T cell counts and higher HIV RNA levels, and high-grade lesions were strongly associated with high-risk HPV infection and low CD4+ T cell counts. Immunosuppression as a result of HIV infection appears to be important for malignant progression in the cervix. Nationwide prevention of HIV infection and cervical cancer screening are necessary for the health of women in this area. High-risk HPV infection and low CD4+ T cell counts are the risk factors for cervical cancer. ***J. Med. Virol. 80:847–855, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: HIV; HPV; cervical abnormality; HIV RNA; $CD4^+$ T cell count

INTRODUCTION

Uterine cervical cancer is the second most common cancer and the fifth most common cause of cancer mortality worldwide. Around 468,000 new cases of cervical cancer occur annually, and more than 233,000 women die from the disease worldwide. About 80% of such cases are in resource-poor developing countries [Parkin et al., 2001]. The highest incidence is observed in Latin America, the Caribbean, sub-Saharan Africa, and South and Southeast Asia [Parkin et al., 2001]. Cervical cancer remains a pervasive public health problem in developing countries. Implementation of nationwide cervical cancer screening has been successful in reducing the incidence and mortality of cervical cancer in many developed countries [Peto et al., 2004]. However, the high mortality rate from cervical cancer in women of child-bearing age may be one of the most important social problems in developing countries.

In sub-Saharan Africa, the annual incidence of cervical cancer is more than 93.9 per 100,000 women

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[Parkin et al., 2001]. Although the national incidence of invasive cervical cancer is unknown in Kenya, it has been estimated at 45 per 100,000 women [Gichangi et al., 2002]. In Kenya, 55% of cervical cancer cases are reported to be clinical stage 3 or more advanced, whereas this figure is only 25% in developed countries [Claeys et al., 2003]. Only 6% of women who present with invasive cervical cancer in Kenya have a history of screening [Gichangi et al., 2002]. The lack of a systematic cervical cancer screening program in Kenya appears to be a factor in the high incidence of progressive disease.

Human papillomavirus (HPV) has been identified as an important causative agent of cervical cancer. HPV DNA testing, rather than cytological testing, is recommended for cervical cancer screening in some resource-poor areas [Kuhn et al., 2000] and in those regions with high prevalence of the human immunodeficiency virus [HIV; Womack et al., 2000]. Cervical cancer is a major problem in areas of Africa with a high prevalence of HIV infection. Women with HIV infection are more likely to have a concurrent HPV infection [Temmerman et al., 1999; Womack et al., 2000], and HPV infection is associated with a greater risk for high-grade squamous intraepithelial lesions in HIV positive women than in HIV negative women. HIV-associated attenuation of HPV-specific immune responses may allow for persistent high-grade intraepithelial neoplasia, thus providing sufficient time for the accumulation of genetic changes important in progression to cancer [Palefsky, 2006]. Furthermore, compared to their non-infected counterparts, women infected with HIV and with invasive cervical cancer are more likely to present with advanced clinical disease [Maiman et al., 1997].

In Kenya, 11% of adult women were infected with HIV in 1996, and the incidence decreased to 8.7% in 2003. However, the incidence was higher in women living in large towns such as Nairobi, where it was 12% in 2003 [Ministry of Health, Kenya, 2005]. The high prevalence of HIV infection in Kenya may increase the incidence of cervical cancer and its precursor lesions, although Gichangi et al. [2002] have demonstrated that a two- to threefold increase in the prevalence of HIV in Kenya did not have a proportionate effect on the incidence of cervical cancer. Unlike cervical cancer, the incidence of Kaposi's sarcoma seems to mirror the incidence/prevalence of HIV, being increased significantly in HIV-infected individuals [Goedert et al., 1998]. It has been hypothesized that HIV-infected women die from HIV-related opportunistic infections before they develop invasive cervical cancer [Gichangi et al., 2002]. At the time of this report, the mean survival time for women with HIV infection in Kenya is 5 years, while typically more than 10 years elapse before the development of cervical cancer after HPV infection. Another possibility is that the diagnosis of subclinical cervical cancer may be missed in many women who die from opportunistic infections in AIDS, as many cases of cervical cancer are asymptomatic.

In the present study, a nested cross-sectional study was undertaken within an ongoing prospective study of HPV/HIV infection and cervical abnormality in Nairobi, Kenya, to establish their prevalence and any association between these conditions.

SUBJECTS AND METHODS

Study Design

This cross-sectional study was part of an ongoing cohort study conducted in Nairobi, Kenya, from November 2004 to August 2009. The subjects were sexually active women, aged 16–61 years old, who attended the Riruta Health Center in Nairobi from November 2004 to June 2005 for pregnancy, family planning, and gynecological concerns. The health center is located near a large slum town. After providing written informed consent, all of the women were invited to participate in this study. The recruitment criteria included women who were willing to undergo voluntary counseling and testing for HIV infection, a cervical Pap test, and an HPV DNA test. Ethics committee in Kenya Medical Research Institute (KEMRI) approved this study. More than 650 women volunteered, and 488 eligible women, including 83 pregnant women who were within 30 weeks of the gestation period, were evaluated in the present study. The remaining subjects were not eligible mainly because some information and data necessary for analysis were not available.

An educational talk about cervical cancer and its risk factors, screening methods, and management was presented to all of the women who visited the health center. The women who agreed to participate in this study were invited for a detailed explanation of the procedures involved. After an informed consent was signed, a structured questionnaire was administered, and a pelvic examination, Pap test, and HPV DNA test were performed in all participants. The questionnaire sought employment status, education, past and current sexually transmitted infection, and current pregnancy status. The first of two cervical scraped-cell samples was placed on a glass slide and fixed immediately in 95% ethanol for the Pap test, and the second was placed into a tube containing DNA extraction solution (10 mM Tris, pH 8.0, 1 mM EDTA, 2% SDS) for the HPV DNA test. After cytological screening, some abnormal and borderline cases ($n = 45$) were recalled and subjected to further examination by colposcopy and pathological diagnosis using punch biopsy specimens. HPV test samples were stored at -20°C. Blood samples were collected for screening of HIV. The samples were separated into serum and blood using a particle agglutination kit and were stored at -20°C.

HPV DNA Detection and Typing

DNA was extracted from cervical cell samples using a DNA extraction kit (SMI test). HPV L1 genes were amplified by PCR using modified GP5+ and GP6+ [de Roda Husman et al., 1995] multiprimers, designed to avoid mismatches between primer sequences and complement target HPV L1 genes. The modified GP

primer sequences were (5′ → 3′): GP5 + M1, TTTRTTACTGTTGTWGATACTAC, GP5 + M2, TGTWACTGTTGTWGATACCAC, GP5 + M3, GTWACTGTTGTRGACACCAC GP6 + M1, AATTGAAAWATAAACTGTAAWTCATATTC, GP6 + M2, GAAACATAAAYTGTAAATCAWATTC, and GP6 + M3, GAAAAATAAAYTGCAAATCAWACTC.

DNA quality was confirmed by detecting the beta-globin gene by PCR. Samples exhibiting a band of ~140 bp on agarose gels stained with ethidium bromide were defined as positive for HPV infection. HPV typing was determined by a slit blot hybridization method using type-specific FITC-labeled oligoprobes. The probe sequences were as follows (5′ → 3′): low-risk HPV probe to detect HPV6, 11, 13, and 44, ACATGGCGCATGTATTGTTTATA; HPV16, TCTGAAGTAGATATGGCAGC; HPV18, CCCAGGTACAGGAGACTG; HPV26, GGATGCAGATGCTGCAG; HPV30, CTTGAATTATATGTGGATAACGTTTG; HPV31, TCACTGTTTGCAATTGCAG; HPV33, ACTGTCACTAGTTACTTGTGT; HPV35, TCACTAGAAGACACAGCAGA; HPV39, ATGTAGAAGGTATGGAAGACTC; HPV45, GTACTTGGCACAGGATTTTG; HPV51, GGAAACCGCAGCAGTG; HPV52, GCTTTCCTTTTTAACCTCAGC; HPV53, ATGTAGACATAGACTGTGTGG; HPV56, TCATATTTACTTAACTGTTCTGTAGC; HPV58, CCTTCCTTAGTTACTTCAGTGC; HPV59, AGAAGAAGTAGTAGAAGCACAC; HPV66, GTTAATGTGCTTTTAGCTGCA; HPV67, GCCTCTGATTTTTCCTCAGA; HPV68, AAATATTTGGTACAGCTGATTCA; HPV70, CAGGTATGGCCGTTTCG; and HPV82, TTGTGCAACAGATGGAGTA.

The slit blot hybridization method was similar to a dot blot hybridization. For the amplified PCR product (25 μl) of each sample, 5 μl were used to confirm HPV DNA amplification, and the remaining 20 μl were used for HPV typing. The amplified DNA was denatured by the addition of 200 μl of 0.4 M NaOH colored with blue ink and incubation at 95°C for 1 min, followed by cooling on ice. After denaturing, each sample was loaded onto a nylon membrane (Hydrobond N+; GE Healthcare, Tokyo, Japan) in one of 21 slit windows of a slit blot apparatus (Life Technologies, Rockville, MD) and aspirated under negative pressure. After aspiration, the membrane was lightly washed in 1× sodium salt citrate buffer (SSC) and semi-dried in air. The membrane was cut vertically between the slits, creating 21 strips for hybridization with different HPV probes. The membrane strips were hybridized with different HPV probes in plastic bags for 3 hr at 40°C in a hybridization oven. The membranes were washed in 1× SSC at room temperature for 20 min, and then in 1× SSC at 40°C for 20 min. A chemiluminescence detection system (CDP-Star; Amersham) was used to visualize the DNA hybridized with each HPV probe, and images were captured using a charge-coupled device camera.

The samples were classified into three risk groups based on HPV type: high-risk (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 67, 68, and 82), low-risk (types 6, 11, 13, and 44), and undetermined-risk (types 26, 30, 53, 66, and 70). Some samples contained an unknown type of HPV, which was detected as an HPV band on agarose gels for PCR, but gave no signal in the slit blot hybridization.

Definition of Cervical Abnormalities

Pap smears were classified according to the 2001 Bethesda System [Solomon et al., 2002] as unsatisfactory, negative, atypical squamous cells of undetermined significance, atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, squamous cell carcinoma, adenocarcinoma in situ, or adenocarcinoma. The slides were initially screened by two cytotechnologists, and samples that were borderline abnormal (atypical squamous cells of undetermined significance/atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions) and abnormal (low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions, and cancer) were re-evaluated by a surgical pathologist. The final diagnosis of the cytological results was agreed upon by a cytotechnologist and a surgical pathologist. Cases showing abnormal cytology (n = 45), including atypical squamous cells of undetermined significance and atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, in the initial screening were histologically evaluated using biopsy specimens obtained by colposcopy. Discrepant results that showed a difference of more than two grades between cytology and histology were observed in four cases. The remaining abnormal cases were diagnosed by cytology alone, and thus only cytological diagnosis was used in the present study. Histologically confirmed cervical intraepithelial neoplasia grade 1 was counted as low-grade squamous intraepithelial lesion, and cervical intraepithelial neoplasia grade 2 or 3, as high-grade squamous intraepithelial lesion. One invasive squamous cell carcinoma was found in one HIV positive woman.

HIV Serological Testing, Lymphocyte Subset Counting, and Plasma HIV RNA Determination

Serological testing for HIV was performed using a kit (UniGold HIV-1/2, Trinity Biotech; HIV-1/2/0, Abbot Laboratories, Chicago, IL). HIV positive samples were confirmed with a particle agglutination test kit (KEMRI HIV PA kit; Kenya Medical Research Institute).

Lymphocyte subset counts were performed by standard flow cytometry, according to the method described by Maurer et al. [1990]. The details of the procedure were performed in accordance with the manufacturer's instructions (Tritest; Becton-Dickinson, Franklin Lakes, NJ). Briefly, 50 μl of whole blood with EDTA were incubated with three-color fluorochrome-labeled monoclonal antibodies. After incubation, flow cytometric analysis was performed on a Facscalibur cytometer using an automatic acquisition and analysis program (Multiset; Becton-Dickinson). The $CD4^+$ T cell count was used in the present study.

To quantify HIV-1 RNA, a NucliSens EasyQ assay (Biomerieux, Lyon, France) was used. This assay employs three methods: the Boom method for nucleic acid release and isolation, nucleic acid sequence-based amplification (NASBA) to amplify RNA, and real-time detection of amplicons using fluorescent molecular beacons. The measurement and interpretation were performed using an EasyQ analyzer. Kinetic analysis of the fluorescence signals revealed the respective amplification efficiency of the wild-type target and calibrator RNA, and thus the quantity of HIV-1 RNA in the original sample. The RNA level is presented as the estimated copy number of HIV RNA transcripts.

Statistical Analysis and Drawing Figure

The Chi-squared test or Fisher's test was used to compare the positive rate or prevalence between two groups. The number of $CD4^+$ T lymphocytes and the HIV RNA copy number were compared between two groups using the Mann–Whitney test. The Chi-squared test for trends was used to demonstrate a change in positive rates according to increased age. Before the multivariate analysis using an unconditional logistic regression model, the forward and backward regression analyses were performed to select possible factors associated with HIV infection or abnormal cytology. The multivariate analysis was performed using JMP ver 5.1.1(SAS Institute, Inc., Cary, NC), and the Chi-squared test for trends and other analyses, and drawing figure were conducted using Prism version 4 (GraphPad Software, San Diego, CA).

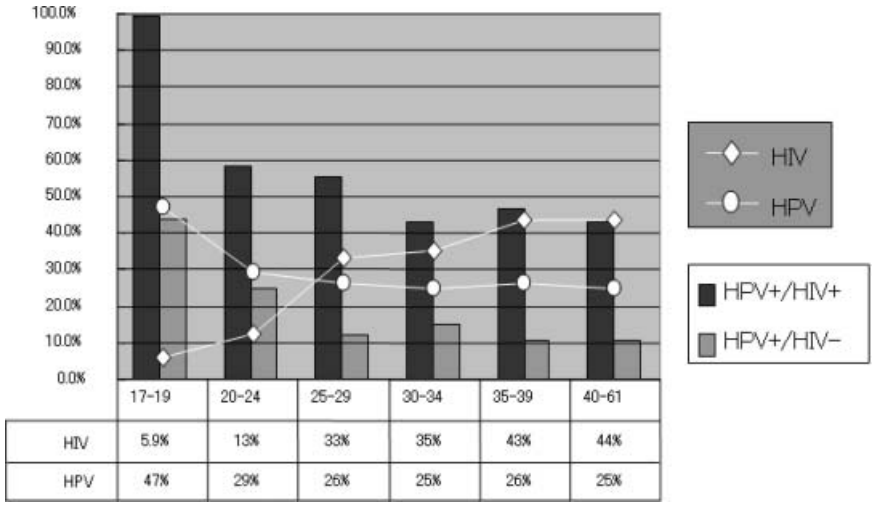
RESULTS

Prevalence of HIV and HPV Infections and Abnormal Cervical Cytology

The HIV positive rate was 32% (155/488) in the eligible women. The average ages were 31.2 years for all subjects, 33.9 years for HIV positive women, and 29.9 years for HIV negative women. HIV infection increased according to the age of the women ($P < 0.001$, Chi-squared test for trends; Fig. 1). Among HIV positive women, 23% were treated with antiretroviral drugs within 6 months, and the remaining 77% were not treated.

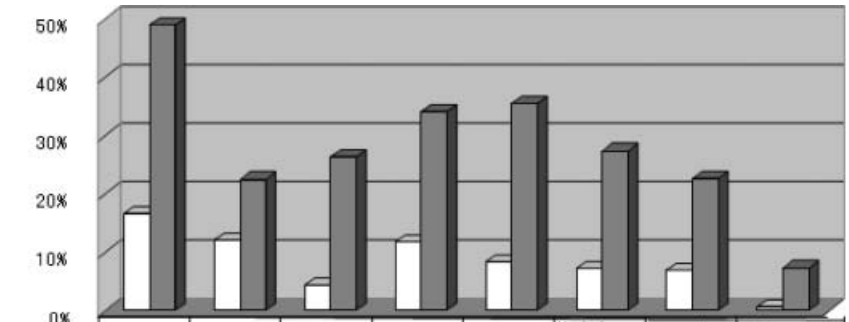
The overall prevalence of HPV infection in the uterine cervix was 27% (132/488) (Fig. 1). HPV was detected in 100% in HIV positive women aged 17–19 years, 58% of HIV positive women aged 20–24 years, and 43–56% of HIV positive women aged >25 years, while it was in 44% in HIV negative women aged 17–19 years, 25% of HIV negative women aged 20–24 years, and 10–15% of HIV negative women aged >25 years (Fig. 1). HPV infection decreased according to age in HIV negative ($P = 0.002$, Chi-squared test for trends), but no such tendency was seen in HIV positive women (Fig. 1).

Among HIV positive women, 49% (76/155) were positive for HPV, while 17% (56/333) of HIV negative women were HPV-positive (Fig. 2). Single- and multiple-type HPV infections were seen in 23% and 26% of HIV positive women, respectively, and in 12% and 4.5% of HIV negative women, respectively. The low- and high-risk HPV infection frequencies were 34% and 35%, respectively, in HIV positive women and 12% and 8.4%, respectively, in HIV negative women. The age-adjusted odds ratios for any HPV, single-type HPV, high-risk HPV, and multiple-type HPV infections in HIV positive women compared to HIV negative women were 2.8, 2.5, 8.6, and 11.0, respectively. In cytological tests, 47% of HIV positive women had an abnormal cervix (atypical squamous cells undetermined significance/atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions, 20%; low-grade squamous intraepithelial lesion, 21%; and high-grade squamous intraepithelial lesion/cancer, 5.8%), whereas 14% of HIV negative women had an abnormal cervix (atypical squamous cells undetermined significance/atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, 6.6%; low-grade squamous intraepithelial lesion, 6.9%; and high-grade



	17–19	20–24	25–29	30–34	35–39	40–61
HIV	5.9%	13%	33%	35%	43%	44%
HPV	47%	29%	26%	25%	26%	25%

Fig. 1. Prevalence of HIV and HPV infections according to age. The lines indicate the prevalence of HIV and HPV infections, and the bars represent the HPV prevalence in HIV positive and HIV negative women.



	Any Type HPV	Single Type HPV	Multiple Type HPV	Low-risk /other HPV	High-risk HPV	Atypical squamous cells of undetermined significance	Low-grade squamous intraepithelial lesions	High-grade squamous intraepithelial lesions/cancer
HIV-	17%	12%	4.5%	12%	8.4%	6.6%	6.9%	0.6%
HIV+	49%	23%	26%	34%	35%	20%	21%	5.8%
Odds ratio (HIV+ vs HIV-)								
crude	2.4	2.1	7.6	3.9	6.0	4.9	5.0	15.7
age adjusted	2.8	2.5	11.0	5.1	8.6	5.1	5.6	16.6

Fig. 2. HPV types and infection patterns, and cervical abnormalities in HIV negative/HIV positive women.

squamous intraepithelial lesion, 0.6%). The age-adjusted odds ratios for atypical squamous cells undetermined significance/atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, low-grade squamous intraepithelial lesion, and high-grade squamous intraepithelial lesion in HIV positive women compared to HIV negative women were 5.1, 5.6, and 16.6, respectively. These results suggest that HIV infection increases the susceptibility to HPV infection and increases the risk of cervical abnormalities.

Association Between HIV and Cervical HPV Infections and Abnormal Cervical Lesions

To determine the risk factors for HIV infection, the multivariate analysis using a logistic regression model was carried out. Forward and backward stepwise analyses showed that age >25 years, taking medication, age <20 years at the beginning of sexual activity, more than two life-time sex partners, a past history of condyloma or sexually transmitted diseases (STDs), cervical HPV infection, and cervical abnormalities were independently associated with HIV infection. In contrast, subjects who were visiting family planning clinics were at lower risk for HIV infection (Table I). History of education and present employment were not associated with the risk of HIV infection.

The same analysis to determine the risk of cervical abnormalities, including low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions, and cancer (Table II), revealed that HPV infection was a factor, although past history of STDs or HIV infection were also associated with it in the univariate analysis. The latter factors were associated with cervical abnormalities depending on HPV infection, because they became significant in the analysis after excluding the factor "HPV infection."

Relationships Between HIV Immunosuppression, HPV Infection, and Development of Cervical Lesions

The relationship between immunosuppression and HPV infection was evaluated. Among HIV positive women, those who were infected with HPV had a significantly lower number of $CD4^+$ T leukocytes in the blood ($P < 0.0001$, Mann–Whitney test), and a significantly higher HIV RNA copy number (HIV load; $P < 0.05$, Mann–Whitney test) than those who were HPV-negative. Women with low-risk, high-risk, and multiple-type HPV infections had a significantly lower $CD4^+$ T cell count and higher HIV RNA copy number (Fig. 3).

An analysis of cervical abnormalities showed that the $CD4^+$ T cell count was significantly lower ($P = 0.021$) in women with high-grade squamous intraepithelial lesion than in those with a normal cervix, whereas the $CD4^+$ T cell count did not differ significantly between women with low-grade squamous intraepithelial lesion and those with a normal cervix ($P = 0.094$; Fig. 4). The HIV RNA copy number was not significantly higher in women with either high- or low-grade squamous intraepithelial lesion compared to women with a normal cervix ($P > 0.1$; Fig. 4). Susceptibility to cervical HPV infection was related to immunosuppressive status, as represented by a low $CD4^+$ T cell count and high HIV load. Development of abnormal cervical lesions was also related to immunosuppressive status, as represented by a low $CD4^+$ T cell count.

TABLE I. Demographic Factors Associated With HIV Infection

Demographic factors	No. of cases	No. of subjects	%	Univariate analysis ORs	Univariate analysis 95% CI	Multivariate analysis ORs	Multivariate analysis 95% CI	*P*-value
Age								
17–24 years	13	113	12	1		1		
25–61 years	142	375	38	3.29	1.94–5.58	5.89	2.60–14.7	<0.0001*
Taking medication								
No	70	391	18	1		1		
Yes	85	97	88	4.90	3.91–6.13	21.1	10.1–47.5	<0.0001*
Age at first sexual intercourse								
Older than 20 years	23	134	17	1		1		
10–19 years	132	354	37	2.17	1.46–3.23	2.29	1.12–4.81	0.025*
No. of lifetime sexual partners								
0–1	57	247	23	1		1		
More than 2	98	241	41	1.76	1.34–2.32	2.46	1.31–4.68	0.0053*
Present pregnancy								
No	143	403	35	1		1		
Yes	12	85	14	0.39	0.23–0.68	0.46	0.19–1.05	0.073
Visitor to family planning								
No	109	284	38	1		1		
Yes	46	204	23	0.59	0.44–0.79	0.23	0.11–0.45	<0.0001*
History of condyloma acuminata								
No	134	444	30	1		1		
Yes	21	44	48	1.58	1.13–2.22	3.29	1.18–9.14	0.022*
History of STD								
No	76	323	24	1		1		
Yes	79	165	48	2.04	1.58–2.62	2.16	1.12–3.82	0.0083*
Cytology								
Normal	82	368	22	1		1		
Abnormal including ASCUS	73	120	61	2.73	2.15–3.47	2.88	1.50–5.54	0.0014*
HPV infection								
Negative	79	357	22	1		1		
Positive (any types)	76	131	58	2.62	2.06–3.34	3.08	1.61–5.91	0.0007*

*Statistically significant.

TABLE II. Demographic Factors Associated With Cytological Abnormality

Demographic factors	Cervical abnormality No. of cases	Cervical abnormality No. of subjects	%	Univariate analysis ORs	Univariate analysis 95% CI	Multivriate analysis ORs	Multivriate analysis 95% CI
Age							
17–24 years	13	113	12	1		1	
25–61 years	54	375	14	1.25	0.71–2.21	0.88	0.38–2.03
No. of lifetime sexual partners							
0–5	62	465	13	1		1	
More than 6	5	23	22	1.63	0.73–3.67	0.66	0.18–2.42
Present pregnancy							
No	62	403	15	1		1	
Yes	5	85	6	0.38	0.16–0.92*	0.47	0.15–1.48
Visitor to family planning							
No	40	284	14	1		1	
Yes	27	204	13	0.94	0.60–1.48	1.29	0.64–2.57
History of STD							
No	32	323	10	1		1	
Yes	35	165	21	2.14	1.38–3.33*	1.83	0.94–3.53
HIV							
Negative	25	333	8	1		1	
Positive	42	155	27	3.61	2.29–5.70*	1.81	0.86–3.81
HPV infection							
Negative	79	357		1		1	
Positive (any types)	76	131		9.5	6.07–14.8*	16.1	8.21–31.5*

*Statistically significant.

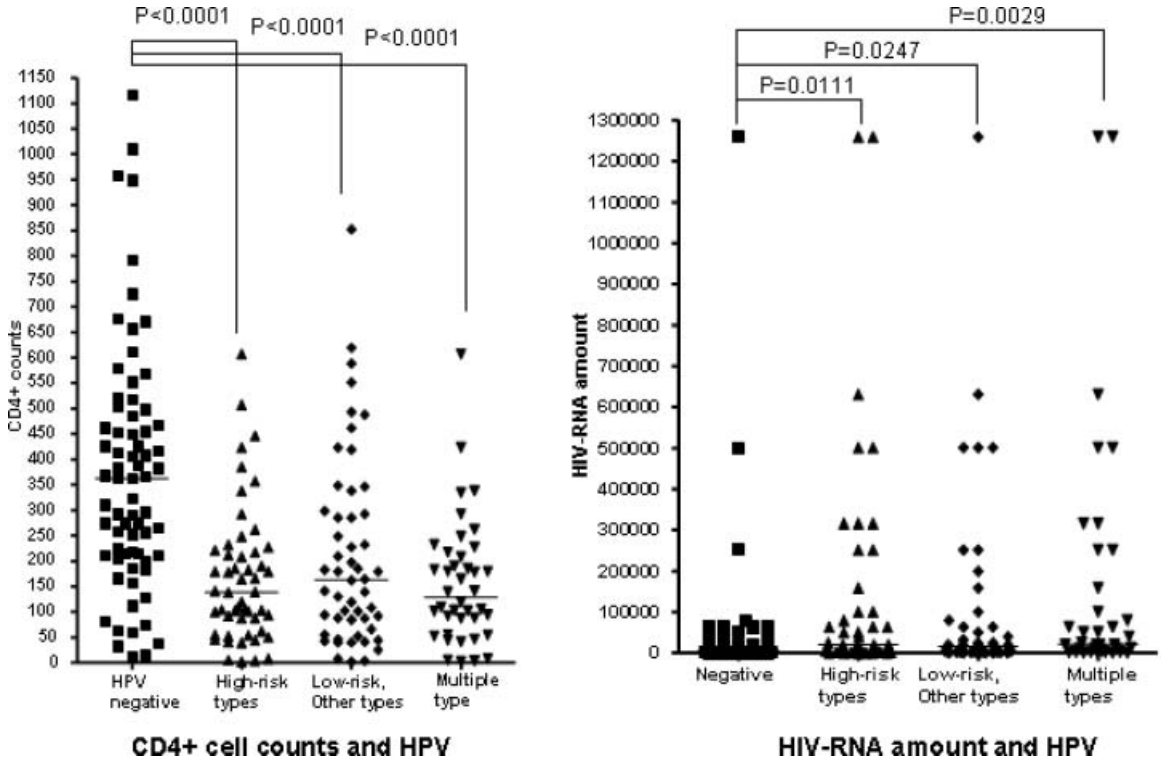


Fig. 3. $CD4^+$ T cell counts and plasma HIV-RNA amount in HPV^-/HPV^+ women. Each dot represents the $CD4^+$ T cell count and plasma RNA amount in each subject. Differences in $CD4^+$ T cell counts between the groups were evaluated by the Mann–Whitney test.

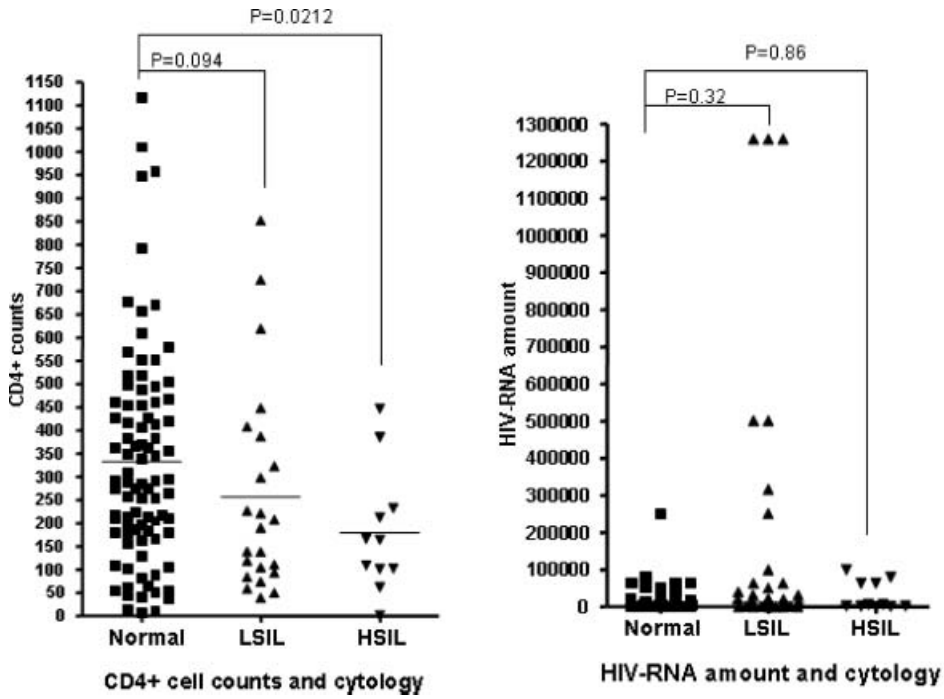


Fig. 4. $CD4^+$ T cell count and plasma HIV-RNA amount in abnormal cytology. Each dot represents the $CD4^+$ T cell count and plasma RNA amount in each subject. Differences in $CD4^+$ T cell counts between the groups were evaluated by the Mann–Whitney test.

DISCUSSION

In the present study, 77% of HIV positive women received no treatment and 23% received treatment within 6 months, suggesting poor control of HIV infection in this population. The relationship between HIV and HPV infections and the development of cervical abnormalities was evaluated.

The prevalence of HIV in this study population was 32%. This is higher than the rate in the general population (12%) of Nairobi [Ministry of Health, Kenya, 2005], suggesting that the subjects may belong to a group at higher risk of HIV infection. This might have been expected. Because this health center has sponsored an AIDS prevention campaign, high-risk subjects would be more likely to have attended the center for voluntary counseling and HIV testing. Further, because the health center is located near a slum town in Nairobi, many of the subjects may have been disadvantaged economically. A multivariate analysis revealed that risk factors for HIV infection included older age, taking medication, <20 years old at sexual debut, having more than two life-time sex partners, a history of condyloma or STDs, cervical HPV infection, and cervical abnormalities, including atypical squamous cells of undetermined significance. Employment and education status were not significant factors. HIV infection cannot be cleared; thus, the number of HIV positive women is likely to increase with age. Sexual contact with a male partner appears to be the most important means of HIV transmission, and thus the number of sex partners and past history of condyloma or STDs were associated with the risk for infection. In the present study, 5% of the subjects had more than six sex partners, and 73% were 10–19 years old at sexual debut. In our previous study in Hokuriku, Japan [Sasagawa et al., 2005], 31% of the subjects had more than six life-time sex partners, and 67% had their sexual debut at 12–19 years of age. In the present study, the number of life-time sex partners was less than that in Japan, but the percentage of those <20 years old at sexual debut did not differ between the two study populations. Thus, the present subjects did not practice sexual behaviors as risky as those of the young Japanese women in the previous study. This suggests that HIV is transmitted readily to women from men in an area where HIV is highly prevalent. Cervical HPV infection and cervical abnormalities were independent risk factors for HIV infection in this study (Table I). Among HIV negative women, cervical HPV infection was more prevalent in younger women (Fig. 1), while the HIV positive rate in women increased with age (Fig. 1), suggesting that cervical HPV infection preceded HIV infection in some HIV positive women. HPV infection in the cervix may increase the risk of HIV transmission by sexual contact, although this hypothesis should be clarified in a future prospective study.

The prevalence of HPV was 49% in HIV positive women and 17% in HIV negative women in this study. This is similar to previous findings of HPV prevalence of 41% in HIV positive women and 14% in HIV negative women in Nairobi [Temmerman et al., 1999]. In Zimbabwe, the HPV prevalence was 54% in HIV positive women and 27.6% in HIV negative women [Baay et al., 2004]. Our multivariate analysis demonstrated that HIV infection was the most important risk factor for cervical HPV infection (data not shown). This relationship might be different in a population with lower prevalence of HIV infection. Many life-time sex partners, younger age at sexual debut, and history of STD are risk factors for HPV infection [Ho et al., 1998; Sasagawa et al., 2005]. The impact of sexual behavior on HPV infection may be attenuated by a high prevalence of HIV infection. High-risk and multiple-type HPV infections were significantly increased in HIV positive women in the present study, and this was similar to previous findings [Ahdieh et al., 2000]. It has been reported that immunosuppression induced by HIV not only allows cervical HPV infection but also leads to reactivation of HPV infection [Strickler et al., 2005]. Although it is not known whether either mechanism is responsible for an increased prevalence of HPV infection, it was found that a low $CD4^+$ T cell count and high HIV load were strongly associated with cervical HPV infection and that a low $CD4^+$ T cell count was associated with the development of cervical lesions. These findings were similar to those of previous studies [e.g., Clark et al., 1993]. The prevalence of oral, anal, and cervical HPV infection in HIV positive compared to HIV negative individuals increases with progressively lower $CD4^+$ T counts, as does the incidence of high-grade intraepithelial neoplasia [Palefsky, 2006]. It is possible that attenuated HPV-specific immune responses induced by HIV infection allow for persistent HPV infection and premalignant lesions, thus providing sufficient time for the accumulation of genetic changes important for progression to cancer. However, late-stage cancer invasion is not influenced greatly by the immune status [Frisch et al., 2000]. In the present study, the $CD4^+$ T cell count, rather than HIV load, was associated strongly with the presence of abnormal cervical cytology, in contrast to the findings of previous studies [e.g., Massad et al., 1999]. A low $CD4^+$ T cell count may be associated more strongly with established immunosuppression rather than a high HIV RNA copy number (viral load).

Temmerman et al. [1999] reported a 3.6-fold increased risk for high-grade squamous intraepithelial lesions associated with HIV-1 in women who attended a family planning clinic in Kenya. We found low-grade squamous intraepithelial lesions in 6.9% and 21% of HIV negative and HIV positive women, respectively, and high-grade squamous intraepithelial lesions in 0.6% and 5.8%, respectively. One case of cervical cancer was observed in a HIV positive woman. The incidence of cervical intraepithelial neoplasia and cervical cancer is considered to be very high in Nairobi. The age-adjusted odds ratio for high-grade squamous intraepithelial lesions in HIV positive versus HIV negative women was 16.6, and there was a strong association between HIV infection and the presence of high-grade squamous intraepithelial

lesions. Some cervical cancer and high-grade squamous intraepithelial lesion cases may have been missed in the previous study in Nairobi, because in many cases, the conditions are asymptomatic and would not likely be detected without cervical cancer screening or an HPV DNA test.

The implementation of nationwide cervical cancer screening, and HIV screening, is required urgently for the promotion of health in women of reproductive age in Nairobi. The introduction of a HPV DNA test as a first screening for cervical cancer may be cost-effective in this area [Kuhn et al., 2000]. High-risk HPV infection and low $CD4^+$ T cell count may be good markers for predicting women at high-risk of cervical cancer or precursor lesions.

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