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ORIGINAL RESEARCH

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High-risk human papillomavirus testing is superior to visual inspection with acetic acid in cervical cancer screening of Kenyan and Ugandan women living with HIV

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Abstract

Background: The current model of screening by visual inspection with acetic acid (VIA) has not led to a reduction in cervical cancer among sub-Saharan women living with human immunodeficiency virus (HIV)/aquired immunodeficiency syndrome (AIDS) (WLWH), and screening using high-risk human papillomavirus (HR-HPV) testing has not been adequately studied in WLWH. **Methods**: Kenyan women aged 21 to 60 years provided self-collected vaginal swabs for HR-HPV testing (Cobas HPV® Assay), all women then underwent VIA. All WLWH (n = 120) were scheduled for cervical biopsy. Testing parameters were estimated for HR-HPV and VIA for detection of cervical intraepithelial neoplasia (CIN) grades 2 or 3, and CIN grade 3). **Results**: HR-HPV was detected in 49 of 120 (40.8%) WLWH. Cervical biopsy revealed CIN2/3 in 14 WLWH (11.7%) and CIN3 in 6 (5.0%). VIA was abnormal in 17 WLWH (14.2%). The sensitivities of HR-HPV testing for CIN2/3 and CIN3 detection were 78.6% and 100%, respectively, and were superior to VIA (57.1% and 50%, respectively). All 6 cases of CIN3 occurred among WLWH with a positive HR-HPV test; VIA was abnormal in 3 of these women and normal in 3. **Conclusions**: Future cervical cancer screening strategies for WLWH should utilize HR-HPV testing of self-collected swabs. Compared to the high sensitivity of HR-HPV testing, VIA performed poorly for CIN3 detection.

Keywords

HPV testing; Cervical cancer screening; Women living with HIV

1. Introduction

Cervical cancer is the fourth most common cancer among women globally and is responsible for nearly 300,000 deaths annually; 90% of these deaths occur among women living in low- and middle-income countries [1–3]. The highest age-standardized incidence rates worldwide for cervical cancer occur in Eastern Africa, and in sub-Saharan Africa it remains the leading cause of death from cancer [3–6]. The Uganda age-standardized incidence and mortality rates are 56.2 and 41.4 per 100,000 women per year, respectively, and the Kenya age-standardized incidence and mortality rates are 31.3 and 20.6 per 100,000 women per year, respectively [2]. These incidence and mortality rates far exceed those for women living in the United States (4 and 1 per 100,000 women per year, respectively) [7]. Oncogenic types of human papillomaviruses

("high-risk" HPV or HR-HPV) are the causative agents of this malignancy [8]. Persistent detection of HR-HPV over a one or two-year period is associated with a markedly increased risk of cervical cancer [9, 10].

HIV infection accelerates the progression of cervical cancer, and this malignancy is the most frequently detected cancer in women living with HIV (WLWH) [11]. The HIV epidemic continues in sub-Saharan Africa: an estimated 6% of the Kenyan population and 7.5% of the Ugandan population are living with HIV with more than half of these infections occurring in women [12, 13]. Compared to women not living with HIV/AIDS, WLWH have an increased risk of HR-HPV infection and persistence, cervical intraepithelial neoplasia (CIN) including CIN grade 3 (CIN3), and invasive cervical cancer [14, 15]. Early initiation of anti-retroviral therapy (ART) is associated with lower HR-HPV acquisition and persistence, as well as reduced progression of premalignant lesions to invasive cancer [14, 16, 17], but the actual incidence of cervical cancer does not seem to be reduced by use of ART [18].

Cervical cancer is preventable through effective screening. However, the coverage of cervical cancer screening is very limited in sub-Saharan African countries. In Kenya, 5% of women are regularly screened and 14% have ever been screened; most of these women live in urban areas [19]. Likewise, in rural Uganda, there is also a low screening uptake of about 4.8% [20]. Visual inspection with acetic acid (VIA) is the method most often used as a screening technique in low- and middle-income countries, including Kenya and Uganda [21, 22]. However, VIA examination is highly subjective and dependent on the examiner's evaluation [22]. Evidence is inconclusive regarding the effectiveness of VIA in preventing invasive cervical cancer.

HR-HPV testing using clinically validated assays is an alternative screening method for cervical cancer [23]. In studies of women not infected with HIV, HR-HPV testing of cervical or self-collected vaginal swabs has higher sensitivity for detection of cervical precancers than cytological screening or VIA, requires less infrastructure than cytological screening programs, and can be utilized in community settings [24–29]. HR-HPV testing provides reassurance for women who test negative, permitting a safe extension of screening intervals [30, 31]. The benefits and limitations of HR-HPV testing in WLWH are not completely understood. Because a high percentage of WLWH may have a HR-HPV type detected at any time, the specificity of HR-HPV testing may be suboptimal, increasing the number of women with positive HR-HPV tests but no cervical lesions.

The World Health Organization (WHO) and other groups have recommended that VIA may be used to triage HR-HPV-positive women, considering the low specificity of HPV testing [32–34].

This could prevent having to perform colposcopy and cervical biopsy on all women with a positive HR-HPV test [33, 34]. However, the utility of HR-HPV testing followed by VIA in screening for cervical cancer needs to be further determined, especially in WLWH [35, 36]. The primary aim of this study was to compare HR-HPV testing and VIA examination for detection of precancerous lesions of the cervix in a cohort of WLWH in Uganda or Kenya. A second goal was to assess the utility of screening in which only those WLWH with a positive HR-HPV test would undergo VIA (a "triage" strategy).

2. Methods

2.1 Overall design

This study is a cross-sectional analysis of enrollment data from a longitudinal three-year study being conducted to evaluate cervical cancer screening strategies among WLWH in Eastern Africa [37]. The study is being conducted at two sites: the Academic Model Providing Access to Healthcare (AMPATH) Cervical Cancer Screening Program at Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya and the Uganda Cancer Institute (UCI)—Kawempe Division, Mulago, Uganda.

2.2 Study participants

Women aged 21 to 60 years were eligible to participate if they presented to receive cervical cancer screening services at MTRH or UCI. Additional inclusion criteria included no prior hysterectomy, no history of invasive cervical cancer, and the ability to understand and provide written, informed consent to participate in the study. Exclusion criteria included the presence of suspicious cervical lesions, pregnancy or being ineligible for VIA screening due to no identifiable squamo-columnar junction. A balanced cohort of women was recruited: 1:1 ratio of enrollment in Kenya *vs.* Uganda, and 1:1 ratio of WLWH and a control group of women not living with HIV, as previously described [37]. The analytical cohort for the current study included only WLWH.

2.3 Interview and sample collection

A structured face-to-face interview was conducted at enrollment with trained research assistants to capture medical, social, behavioral and biological information. Blood samples were collected for CD4 cell count and HIV viral load assessment if recent measures could not be determined from medical records. Participants were given instructions for self-collection of vaginal specimens; each woman then received a self-collection kit containing a cervical-vaginal brush and a capped vial containing PreservCyt® medium.

2.4 HPV testing

Self-collected swabs were delivered by the study staff to either the Lancet Laboratory in Nairobi or the UCI Laboratory in Kampala for HR-HPV DNA testing using the Cobas HPV® Assay (Alameda, CA, USA). Type-specific detections of HPV 16, HPV 18, as well as any of 12 additional oncogenic types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, as a group, described in this report as "Non-16/18 HR-HPV") were provided by the assay [38, 39].

2.5 Gynecological examination and VIA

A gynecological examination was performed on all women consisting of inspection of the vulva, speculum examination and bimanual palpation of the pelvic organs. No cytological studies were performed, as this test was not generally available for women attending the clinics. No studies to examine specimens for P16ink4A, were performed, as this test is not

routinely available in Kenya or Uganda [40]. Women with obviously suspicious cancer of the cervix, non-visualized cervix or non-visualized squamo-columnar junction were excluded from the study, and the standard of care was provided for them.

VIA was performed on all study participants. Cotton swabs soaked in freshly prepared 5% acetic acid were applied to the cervix for 1 minute, then the cervix was inspected after an additional 1 minute to detect acetowhite changes. In case of unclear findings on the VIA examination, another research nurse viewed the cervix, and where there was discrepancy, a gynecologist viewed the cervix, and the result was determined by the gynecologist. All women with abnormal VIA examinations were treated according to established local algorithms and were counseled on follow-up screenings.

2.6 Cervical biopsy

All WLWH were scheduled for cervical biopsy regardless of VIA findings. Biopsy tissue from 2 cervical sites was placed in labelled tubes containing buffered formalin and transported to the pathology laboratory for paraffin embedding, preparation of sections, staining and pathological assessment at the local institution.

2.7 Statistical analysis

Demographic and behavioral participant characteristics including age, marital status, years of education completed, homeownership, travel time to healthcare center and number of lifetime sex partners were summarized at enrollment by descriptive statistics. In addition, participant characteristics were compared by country. HIV-related factors including ART use, CD4 count, and HIV viral load were reported and compared by country. Detection of HR-HPV (any HR-HPV, HPV 16, HPV 18 and non-16/18 HR-HPV), VIA abnormality, and biopsy-proven CIN2 and 3 combined (CIN2/3) or CIN3 were compared for WLWH by country. Chi-square tests or Fisher's exact tests were used for comparison of categorical variables, and *t*-tests or Wilcoxon rank sum tests were used for continuous variables as appropriate. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (95% CI) were calculated for HR-HPV and VIA for detection of CIN2/3 and CIN3. McNemar's tests were used to compare sensitivity or specificity of HR-HPV compared to VIA in detecting CIN2/3 and CIN3. Relative sensitivity or specificity of HR-HPV compared to VIA was presented as the relative risk of sensitivity or specificity of the two screening tests in detection of a specific CIN outcome. In addition, sensitivity, specificity, PPV and NPV were calculated for VIA in detection of CIN2/3 or CIN3 for the subset of WLWH who had a positive HR-HPV test result. Analyses were performed using SAS (Statistical Analysis System) 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Participant characteristics

A total of 122 WLWH were enrolled between November 2021 and March 2022. Two women (1 from each country) refused cervical biopsy and were excluded from this analysis. The analytical cohort therefore consisted of 120 WLWH equally divided between Kenya and Uganda.

The median age at study enrollment was 38.2 years (Interquartile range (IQR) 32.3, 42.7) (Table 1). Compared to participants from Kenya, those from Uganda were younger, were more likely to be married or living with a partner and were more likely to live >60 minutes from health care services (Table 1). All women were receiving ART; the median CD4 cell count was 773 cells/μL for all participants and was marginally higher for women from Uganda compared to those from Kenya (820.5 cells/μL *vs.* 719.0 cells/μL, $p = 0.056$). Most WLWH had an undetectable HIV viral load; 13 (10.8%) women had a detectable HIV viral load including 8 women (13.3%) from Kenya and 5 (8.3%) from Uganda (not significant).

3.2 HR-HPV testing

All WLWH provided self-collected vaginal swabs; all swabs were adequate based on positivity of the internal control used in the Cobas HPV® Assay. Overall, HR-HPV of any type (Any HR-HPV) was detected in 49 of 120 (40.8%) women (Table 2). The median age for the 49 women with any positive HR-HPV results was 36.6 years (IQR 32.1–40.0 years), compared to a median age of 39.1 years (IQR 33.0–43.5 years) for the 71 women with negative HR-HPV results ($p = 0.048$).

3.3 VIA examination

All WLWH underwent VIA; the VIA examination was abnormal in 17 of 120 women (14.2%) (Table 2). A higher percentage of women from Kenya had an abnormal VIA examination than did women from Uganda (20.0% *vs.* 8.3%), this difference approached significance ($p = 0.067$).

3.4 Cervical biopsy

For WLWH, cases of CIN2 and CIN3 combined (CIN2/3) were identified in 14 of 120 (11.7%); of these, 8 cases were CIN2 (6.7% of women) and 6 cases were CIN3 (5.0% of women) (Table 2). CIN2/3 was detected in more WLWH from Kenya than in those from Uganda, with marginal significance (16.7% *vs.* 6.7%, $p = 0.088$). No cases of invasive cancer were identified among the 120 women.

The age, VIA result, and HR-HPV result for each WLWH with biopsy-proven CIN2 or CIN3 are shown in Table 3 below. Of the 8 women with CIN2, 5 had HR-HPV detected: 1 woman had HPV 18 detected and 5 had a non-16/18 HR-HPV detected. Three women with CIN2 did not have HR-HPV detected. All 6 women with CIN3 had HR-HPV detected: specific types or groups of types detected are shown in Table 3; HPV 16 and/or HPV 18 were detected in 3 of the 6 women with CIN3 identified in cervical biopsies.

3.5 Parameters of HR-HPV and VIA for detection of CIN2/3 and CIN3 among WLWH

For detection of CIN2/3, HR-HPV testing had a sensitivity and specificity of 78.6% (95% CI: 57.1–100.0) and 64.2% (95% CI: 55.0–73.3), respectively (Table 4). The PPV and NPV of HR-HPV testing were 22.5% (95% CI: 10.8–34.1) and

TABLE 1. Demographic characteristics and HIV-related factors among 120 women living with HIV (WLWH).

Variables	WLWH			
	All (n = 120)	Kenya (n = 60)	Uganda (n = 60)	*p*
Demographics				
Age at study enrollment, Median (IQR)	38.2 (32.3, 42.7)	40.2 (36.8, 43.9)	34.9 (30.8, 39.9)	<0.001[3]
Married or living with partner, n (%)	53 (44.2%)	21 (35.0%)	32 (53.3%)	0.043[4]
Years of education, Median (IQR)	9.0 (7.0, 12.0)	10.0 (8.0, 12.0)	9.0 (6.0, 11.0)	0.218[5]
Home ownership, n (%)	32 (26.7%)	19 (31.7%)	13 (21.7%)	0.215[4]
>60 min to health clinic, n (%)	10 (8.3%)	1 (1.7%)	9 (15.0%)	0.008[6]
Number of lifetime sex partners, Median (IQR)	4.0 (2.5, 5.0)	4.0 (2.5, 5.0)	3.5 (2.5, 5.0)	0.730[5]
HIV-related factors				
CD4 cell count (cells/μL), Median (IQR), (Range)	773.0 (543.5, 971.0) (92.0, 1915.0)	719.0 (472.5, 921.0) (92.0, 1915.0)	820.5 (603.5, 1061.5) (123.0, 1755.0)	0.056[5]
Detectable HIV viral load, n (%)[1]	13 (10.8%)	8 (13.3%)	5 (8.3%)	0.378[4]
HIV viral load (copies/mL), Median (IQR), (Range)[2]	0.0 (0.0, 0.0) (0.0, 409,038.0)	0.0 (0.0, 0.0) (0.0, 409, 038.0)	0.0 (0.0, 0.0) (0.0, 34,453.0)	0.371[5]

[1]*Detectable HIV viral load defined as >40 copies/mL.*
[2]*HIV viral load ≤40 copies/mL was recorded as 0 copies/mL.*
[3]*p-value calculated from t-test.*
[4]*p-value calculated from Chi-Square test.*
[5]*p-value calculated from Wilcoxon signed ranks test.*
[6]*p-value calculated from Fisher's exact test.*
WLWH: women living with HIV; IQR: Interquartile range; HIV: human immunodeficiency virus.

TABLE 2. HR-HPV, VIA, and cervical biopsy results among 120 women living with HIV (WLWH).

Detection, n (%)	WLWH			
	All (n = 120)	Kenya (n = 60)	Uganda (n = 60)	*p*
Any HR-HPV[1]	49 (40.8%)	20 (33.3%)	29 (48.3%)	0.095[5]
HPV 16	8 (6.7%)	3 (5.0%)	5 (8.3%)	0.717[6]
HPV 18	11 (9.2%)	7 (11.7%)	4 (6.7%)	0.343[5]
HPV 16/18	17 (14.2%)	9 (15.0%)	8 (13.3%)	0.794[5]
Non-16/18 HR-HPV[2]	46 (38.3%)	19 (31.7%)	27 (45.0%)	0.133[5]
Abnormal VIA	17 (14.2%)	12 (20.0%)	5 (8.3%)	0.067[5]
CIN2/3[3]	14 (11.7%)	10 (16.7%)	4 (6.7%)	0.088[5]
CIN3[4]	6 (5.0%)	5 (8.3%)	1 (1.7%)	0.207[6]

[1]*HR-HPV: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.*
[2]*Non-16/18 HR-HPV: HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.*
[3]*Cervical intraepithelial lesion grade 2 or grade 3, combined.*
[4]*Cervical intraepithelial lesion grade 3.*
[5]*p-value calculated from Chi-Square test.*
[6]*p-value calculated from Fisher's exact test.*
WLWH: women living with HIV; HR-HPV: high-risk human papillomavirus; VIA: visual inspection with acetic acid; CIN: cervical intraepithelial neoplasia.

TABLE 3. Age, VIA result (normal indicated by "−" and abnormal indicated by "+") and HR-HPV (Cobas HPV® Assay) result (negative indicated by "−" and positive indicated by "+") among 120 women living with HIV who had biopsy-proven cervical intraepithelial lesion grade 2 (CIN2) or grade 3 (CIN3).

CIN2 (n = 8)					
Age (yr)	VIA	HR-HPV[1]	HPV 16	HPV 18	Non-16/18 HR-HPV[2]
27	−	+	−	−	+
28	+	−	−	−	−
30	+	−	−	−	−
37	+	+	−	−	+
37	+	+	−	+	+
38	−	+	−	−	+
43	+	+	−	−	+
49	−	−	−	−	−
CIN3 (n = 6)					
Age (yr)	VIA	HR-HPV[1]	HPV 16	HPV 18	Non-16/18 HR-HPV[2]
29	+	+	+	+	+
38	−	+	−	−	+
40	+	+	−	+	−
45	+	+	−	−	+
48	−	+	−	+	+
50	−	+	−	−	+

[1]*HR-HPV: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.*
[2]*Non-16/18 HR-HPV: HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.*
CIN: cervical intraepithelial neoplasia; VIA: visual inspection with acetic acid; HR-HPV: high-risk human papillomavirus.

95.8% (95% CI: 91.1–100.0), respectively. VIA examination had a sensitivity and specificity for detection of CIN2/3 of 57.1% (95% CI: 31.2–83.1) and 91.5% (95% CI: 86.2–96.8), respectively. The PPV and NPV of VIA were 47.1% (95% CI: 23.3–70.8) and 94.2% (95% CI: 89.7–98.7), respectively. The relative sensitivity of HR-HPV *vs.* VIA for detection of CIN2/3, was 1.4 (95% CI: 0.8–2.3), $p = 0.257$ and the relative specificity was 0.7 (95% CI: 0.6–0.8), $p < 0.001$.

For detection of CIN3, HR-HPV testing had a sensitivity and specificity of 100% (95% CI: 100.0–100.0) and 62.3% (95% CI: 53.4–71.2), respectively (Table 4). The PPV and NPV of HR-HPV testing were 12.2% (95% CI: 3.1–21.4) and 100% (95% CI: 100.0–100.0), respectively. VIA examination had a sensitivity and specificity for detection of CIN3 of 50.0% (95% CI: 10.0–90.0) and 87.7% (95% CI: 81.7–93.7), respectively, and a PPV and NPV of 17.7% (95% CI: 0.0–35.8) and 97.1% (95% CI: 93.8–100.0), respectively. The relative sensitivity of HR-HPV *vs.* VIA for detection of CIN3 was 2.0 (95% CI: 0.9–4.5), $p = 0.083$, and the relative specificity was 0.7 (95% CI: 0.6–0.8), $p < 0.001$.

3.6 The possible utility of VIA as a triage for women with a positive HR-HPV test

A second analysis was performed to examine the utility of using HR-HPV testing as a triage step to VIA examination for detection of CIN2/3 or CIN3. Of all 120 WLWH, 49 had a positive Cobas HPV® Assay result for HR-HPV. As indicated in Table 5, these 49 women included 11 women with biopsy-proven CIN2/3. Among the 11 women with CIN2/3, 5 had a normal VIA examination and 6 had an abnormal VIA examination. Thus, for detection of CIN2/3 in the subset of WLWH who had a positive HR-HPV test, the sensitivity and specificity of VIA were 54.6% (95% CI: 25.1–84.0) and 86.8% (95% CI: 76.1–97.6), respectively; the PPV and NPV of VIA were 54.6% (95% CI: 25.1–84.0) and 86.8% (95% CI: 76.1–97.6), respectively.

There were 6 cases of CIN3 among the 49 women with a positive Cobas HPV® Assay. The VIA examinations were normal in 3 of these women and abnormal in 3 women (Table 5). Therefore, the sensitivity and specificity of VIA for CIN3 detection in the subset of WLWH with a positive HR-HPV test were 50.0% (95% CI: 10.0–90.0) and 81.4% (95% CI: 69.8–93.0), respectively; the PPV and NPV of VIA were 27.3% (95% CI: 1.0–53.6) and 92.1% (95% CI: 83.5–100.0), respectively.

4. Discussion

Women in sub-Saharan Africa suffer from cervical cancer at a much higher rate than women living in wealthy countries. Complete elimination of cervical cancer in Africa is theoretically possible, including in those women living with HIV, through a combination of effective screening of adult women to detect treatable precancerous lesions, combined with widespread vaccination of girls and young women against

TABLE 4. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HR-HPV and VIA for detection of cervical intraepithelial lesion grade 2 and grade 3 combined (CIN2/3), or grade 3 (CIN3) alone among 120 women living with HIV.

	Biopsy outcome (n = 120)		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
	CIN2/3					
	Yes	No				
Any HR-HPV						
Positive	11	38	78.6 (57.1–100.0)	64.2 (55.0–73.3)	22.5 (10.8–34.1)	95.8 (91.1–100.0)
Negative	3	68				
VIA						
Abnormal	8	9	57.1 (31.2–83.1)	91.5 (86.2–96.8)	47.1 (23.3–70.8)	94.2 (89.7–98.7)
Normal	6	97				
Relative Risk HR-HPV *vs.* VIA			1.4 (0.8–2.3)	0.7 (0.6–0.8)		
p-value, Relative Risk[1]			0.257	<0.001		
	CIN3					
	Yes	No				
Any HR-HPV						
Positive	6	43	100.0 (100.0–100.0)	62.3 (53.4–71.2)	12.2 (3.1–21.4)	100.0 (100.0–100.0)
Negative	0	71				
VIA						
Abnormal	3	14	50.0 (10.0–90.0)	87.7 (81.7–93.7)	17.7 (0.0–35.8)	97.1 (93.8–100.0)
Normal	3	100				
Relative Risk HR-HPV *vs.* VIA			2.0 (0.9–4.5)	0.7 (0.6–0.8)		
p-value, Relative Risk[1]			0.083	<0.001		

[1]*p-value was calculated based on McNemar's test.*
CI: confidence intervals; PPV: positive predictive value; NPV: negative predictive value; CIN: cervical intraepithelial neoplasia; HR-HPV: high-risk human papillomavirus; VIA: visual inspection with acetic acid.

TABLE 5. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of VIA for detection of cervical intraepithelial lesion grade 2 (CIN2) and grade 3 (CIN3) combined (CIN2/3), or CIN3 alone among 49 women living with HIV who had a positive HR-HPV test result.

Biopsy outcome		VIA		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Abnormal	Normal				
CIN2/3							
	Yes	6	5	54.6 (25.1–84.0)	86.8 (76.1–97.6)	54.6 (25.1–84.0)	86.8 (76.1–97.6)
	No	5	33				
CIN3							
	Yes	3	3	50.0 (10.0–90.0)	81.4 (69.8–93.0)	27.3 (1.0–53.6)	92.1 (83.5–100.0)
	No	8	35				

VIA: visual inspection with acetic acid; CI: confidence intervals; PPV: positive predictive value; NPV: negative predictive value; CIN: cervical intraepithelial neoplasia.

HPV, the causative agent of this malignancy [41, 42]. Current screening programs in Uganda, Kenya, and other sub-Saharan countries rely on the use of VIA, but the evidence is inconclusive that programs using VIA are effective in reducing the burden of cervical cancer. It is critical that new ways to detect precancerous cervical lesions are tested and made available. Our study compared HR-HPV testing and VIA examination for detection of precancerous lesions of the cervix in a cohort of WLWH. We found a high prevalence of HR-HPV infection, VIA positivity and CIN2/3 among these women. The sensitivity of VIA was poor for detection of CIN2/3 and CIN3, but specificity was good. HR-HPV testing was

sensitive, but not specific for detection of CIN2/3 and CIN3.

HIV infection is associated with an increased presence and persistence of oncogenic HPV infection, development of precancerous cervical lesions, and acceleration of the progression of precancerous lesions to invasive cancer [14, 43, 44]. The prevalence of HIV Infection remains high in Uganda and Kenya despite programs to provide antiretroviral treatment for an increasing percentage of HIV-infected people living in these countries. The current analysis utilized enrollment data that was part of a longitudinal study to determine strategies to detect precancerous cervical lesions in HIV-infected women living in Uganda or Kenya. Data from this analysis indicates that VIA lacks sensitivity for detection of CIN3, the immediate precursor lesion to invasive cancer. While all cases of CIN3 occurred in women who had HR-HPV detected in self-collected vaginal swabs, CIN3 cases were equally distributed between WLWH with normal or abnormal VIA examinations. VIA examination was more specific than HR-HPV testing for CIN2/3 and CIN3 in WLWH, due to the high prevalence of HR-HPV detection in this population.

Other studies have indicated that HR-HPV testing may be superior to VIA in detection of cervical dysplastic lesions, and while many focused on WLWH in sub-Saharan Africa, not all studies included cervical biopsies for all women, and some utilized sub-optimal HR-HPV detection methods. A meta-analysis performed by Kelly *et al*. [45], summarized the diagnostic accuracy of cervical cancer screening strategies for CIN2/3 among WLWH. Of studies that included cervical biopsies for all or nearly all women, the sensitivity of HR-HPV testing was greater than 90% for CIN2/3 and approximately 95% for CIN3 and specificities were between 60% and 65%, consistent with the results presented in our study.

The current study differs from these prior studies in some important ways. First, our cohort of WLWH is well characterized. Second, all WLWH included in the analytical cohort underwent cervical biopsy regardless of HR-HPV and VIA results, making calculations of sensitivity and specificity of these tests possible, using pathology results as the gold standard. Third, VIA examinations were performed in the best of conditions; university-associated clinics that care for large numbers of WLWH and have a great deal of experience with VIA. Lastly, the HR-HPV test utilized was the Cobas HPV® Assay, a PCR-based test that is approved as a primary screening test for cervical cancer in the United States and elsewhere.

The findings of this study offer significant implications for public health policy in sub-Saharan Africa. It has been proposed that HR-HPV testing could serve as a triage to determine the subset of women who would benefit from a second test, such as cytology, a second molecular test, or VIA [33, 34, 45]. This in theory could prevent having to perform colposcopy and cervical biopsy on all women with a positive HR-HPV test, Luckett *et al*. [46], conducted a study in Botswana that included a cohort of WLWH. A primary outcome of the study was to evaluate performance of HR-HPV testing followed by a triage step with VIA for detection of CIN2/3 in these women. Those with positive HPV results returned for a triage visit where all underwent VIA, colposcopy, and biopsy if a visible cervical lesion was present, or if no lesion was visible, collection of a small endocervical sample. The sensitivity of this strategy was 56.15% (95% CI: 47.18–64.84) for detection of CIN2/3. In the current study, all WLWH underwent cervical biopsy, revealing 6 cases of CIN3. If HR-HPV positivity had been used as a triage step for subsequent testing by VIA, then the 49 with a positive HR-HPV test (of the entire group of 120 WLWH) would have been "triaged" to undergo a VIA examination. All 6 cases of CIN3 occurred among these 49 women, and the 6 cases were equally distributed between those with normal VIA examinations (N = 3 cases) and those with abnormal VIA examinations (N = 3 cases). Thus, there appeared to be little or no utility in such a strategy that utilizes a triage strategy of having women with a positive HPV result return for VIA [40, 47].

The Cobas HPV® Assay provides genotyping results for HPV 16 and 18 and tests for 12 additional HR-HPV types as a single, combined result. This test is utilized for women who are not HIV-infected as a triage to further screening by colposcopy if they are specifically positive for HPV 16 or 18, but not for those women with a positive test solely for the group of non-16/18 HR-HPV types. In the current study, 4 WLWH with CIN2 and 3 WLWH with CIN3 were negative for HPV 16 and HPV 18, but positive for non-16/18 HR-HPV. If only those WLWH with HPV 16/18 positivity were referred to colposcopy, cases of CIN2/3 caused by non-16/18 HR-HPV types would be missed. Other groups have found increased detection of non 16/18 HR-HPV types (such as HPV 52) in WLWH with highly dysplastic cervical lesions [48]. It is possible that the optimal strategy for WLWH is to triage all to colposcopy who have a positive HR-HPV result for any type: HPV 16, HPV 18 or non-16/18 HR-HPV. This approach would miss very few women with underlying CIN2/3 lesions but would require many additional colposcopic examinations and biopsies. Perhaps a triage strategy that would be much better than using the low-sensitivity VIA method would be to have those WLWH with a positive HR-HPV test undergo testing for P16ink4A, then biopsy those with a positive test [40]. This strategy could take advantage of the high sensitivity of P16ink4A testing for underlying CIN2/3, if actual studies in WLWH have been performed that support this approach. Other triage-based approaches may utilize novel biomarkers to triage those women with positive HR-HPV tests, but these approaches need to be tested in HIV-infected African women [47].

A limitation of the current study is the small sample size that was justified by power calculations that were performed to support the main hypotheses of the overall longitudinal, prospective study. Despite the modest size of our study, statistically significant results were found that demonstrate the clear superiority of HR-HPV testing compared to VIA as a screening method for cervical cancer in WLWH. A second limitation is that the study participants were enrolled at academic institutions in urban environments, and the results may not be generalizable to rural Kenyan or Ugandan WLWH. However, because the study was performed at these academic institutions, the nurses performing the VIA examinations had undergone extensive training and had many years of experience performing VIA examinations.

5. Conclusions

In summary, a cohort of WLWH living in Kenya or Uganda underwent cervical biopsy following HR-HPV testing and VIA. HR-HPV testing using the Cobas HPV® Assay was sensitive for detection of biopsy-proven CIN2/3 and CIN3, while sensitivity of VIA was poor for detection of these endpoints. In addition, our study indicates that HR-HPV testing as a *triage step* for VIA is unlikely to be of high value due to the poor sensitivity of VIA, even among the women with a positive HR-HPV test. Future screening strategies for WLWH should utilize HR-HPV testing of self-collected swabs for CIN3 detection.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author (Darron Brown) upon reasonable request.

AUTHOR CONTRIBUTIONS

MN—contributed to funding acquisition, project administration, methodology, resources, data curation, formal analysis, writing (original draft, review and editing). YT—contributed to funding acquisition, conceptualization, methodology, data curation, formal analysis, validation, writing (original draft, review and editing). PT—contributed to study supervision, investigation, project administration, writing (review and editing). OO—contributed to funding acquisition, project administration and writing (review and editing). PI—contributed to project administration and writing (editing). KM—contributed to study supervision, writing (review and editing). KP—contributed to project administration, writing (editing). CM—contributed to project administration, study supervision. JN—contributed to study supervision, writing (review and editing). CN—contributed to study supervision. BM—contributed to project administration, data management and distribution, methodology, writing (original draft, review and editing). CY—contributed to funding acquisition, project administration, conceptualization, methodology, study supervision, writing (original draft, review and editing). AE—contributed to funding acquisition, project administration, conceptualization, methodology, study supervision, writing (original draft, review and editing). PJL—contributed to funding acquisition, project administration, conceptualization, methodology, writing (review and editing). DRB—contributed to funding acquisition, project administration, conceptualization, methodology, study supervision, writing (original draft, review and editing).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Study approval was granted from the local review boards at Moi Teaching and Referral Hospital (MTRH) and Moi University, Eldoret, Kenya, the Kenya Medical Research Institute's Scientific and Ethics Review Unit (KEMRI-SERU), the Infectious Diseases Institute, Uganda Cancer Institute, Makerere University, Kampala, Uganda and the Institutional Review Board of Indiana University (Numbers IU-11449 and UG-REC-019). All participants provided written informed consent for participation in the study and for use of clinical specimens, either in Swahili, Luganda or English. All study procedures were performed in accordance with relevant guidelines and regulations outlined by the Ethics Review Boards indicated above. All authors give consent for publication.

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CONFLICT OF INTEREST

Dr. Brown receives research funding and has received royalties and consulting fees in the past from Merck and Co., Inc. Dr. Brown serves on the Scientific Advisory Board for PDS, Inc. The other authors do not report any potential conflicts of interest.

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