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Evaluation of hpv risk groups among women enrolled in the mulher cervical cancer screening study in Mozambique

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Abstract

Background Limited data are available about the distribution of human papillomavirus (HPV) among women undergoing cervical cancer screening in Mozambique. We describe the prevalence of high-risk HPV risk groups detected in women who participated in the MULHER Study, a prospective trial of Mozambican women undergoing cervical cancer screening with HPV testing.

Methods From January 2020 to January 2023, 9,014 women aged 30–49 years in Maputo City and Gaza Province, Mozambique underwent cervical cancer screening. Cervicovaginal samples were self-collected (97.5%) or provider-collected (2.5%) and primary HPV testing was performed using the GeneXpert HPV testing platform (Cepheid Inc, USA) which provided data on HR-HPV risk groups: HPV16, HPV18/45 and 11 other HR-HPV types in aggregate. Women with a positive HR-HPV test underwent visual assessment using dilute acetic acid applied to the cervix for treatment decisions.

Results Of the 9,014 women enrolled in the MULHER Study, 8,954 (99.3%) had a valid HPV test result. Of those, 2,805 (31.3%) tested positive for at least one HR-HPV group: HPV16 ($n=475$, 16.9%), HPV18/45 ($n=686$, 24.6%) and other HR-HPV ($n=2,150$, 77.1%). A total of 17.8% were positive for multiple HPV HR groups. HR-HPV infection prevalence was higher among women living with HIV (WLWH) than HIV-negative women (39.7% vs. 24.3% respectively; $p<0.001$). WLWH were more likely to test positive for HPV18/45 ($p=0.03$) and for two or more HR-HPV risk groups ($P<0.0001$) compared with HIV-negative women. HPV16 was the most frequently detected HR-HPV group (56.7%) among women diagnosed with invasive cervical cancer.

Conclusions HR-HPV prevalence was high among Mozambican women aged 30–49 years, especially among WLWH, consistent with the high burden of cervical cancer in this population. HPV16 was the most common HR-HPV

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group among women with cervical cancer. Further study is needed to determine the role of HR-HPV genotyping in follow-up and treatment in Mozambique.

Keywords HPV, Cervical cancer, Screening

Background

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide [1]. To date, more than 150 HPV types have been identified. Of those, HPV types that infect the anogenital tract can be classified according to the risk of malignant progression into low-risk HPV (HPV6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, and 89), intermediate-risk HPV (HPV26, 53, 66, 67, and 73), and high-risk HPV (HR-HPV) (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). HPV is implicated as the cause of almost all cervical cancer cases, with HPV16 and 18 being responsible for 70% of cervical cancers [2–4].

Globally, cervical cancer is the fourth most common female cancer with an estimated annual incidence of 604,000 cases and 342,000 related deaths. Approximately 90% of these cases occur in low- and middle-income countries [5]. Mozambique, a country in sub-Saharan Africa, has a high burden of cervical cancer, reporting 47.8 new cervical cancer cases per 100,000 women and 36.9 per 100,000 women related deaths annually [6]. Cervical cancer is the most frequent cancer affecting Mozambican women.

Cervical cancer screening in Mozambique is opportunistic and based on visual inspection with acetic acid (VIA) followed by treatment using ablation. Limited information is available about the distribution of HPV types in Mozambican women undergoing cervical cancer screening [7, 8]. In the current study, we report HR-HPV risk group distribution among women aged 30–49 years who participated in the previously reported MULHER Study, a prospective trial of *M*ozambican women *u*ndergoing cervica*l* cancer screening with *H*PV testing in conjunction with family planning s*er*vices [9].

Methods

Study design and specimen collection

The MULHER study was a prospective cohort study of women aged 30–49 years, conducted from January 2020 through January 2023, in Maputo City and Gaza Province, Mozambique [9]. Women were offered integrated cervical cancer screening with voluntary family planning services as appropriate. The study was approved by the Institutional Review Board from MD Anderson Cancer Center (2020 – 0651) and the Comité Nacional de Bioética para a Saúde, Moçambique (IRB00002657), and all patients provided written informed consent.

Eligible women were offered cervical cancer screening with primary HPV testing by self-collection of cervicovaginal samples or provider-collection of cervical samples. Nearly all women chose testing with self-collection (97.5%). Specimens were collected using Viba-Brush® (Rovers Medical Devices B. V., Oss, The Netherlands) and stored in a vial with PreservCyt® (ThinPrep System, Hologic Inc., Marlborough, MA, USA).

HPV testing

Specimens were tested for HPV DNA using GeneXpert® (Cepheid Inc., Sunnyvale, CA, USA). The Xpert® HPV test automates the full process including DNA extraction, amplification, and detection of E6/E7 oncogenes of the 14 h-HPV types in a fully integrated cartridge. The test results are reported as HPV16, HPV18/45, and other HR-HPV types (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68) as a pooled result.

Patients testing positive for HR-HPV underwent visual assessment for treatment (VAT) to determine eligibility for ablation. If eligible for ablation, they were treated with thermal ablation or cryotherapy. If ineligible for ablation, they were referred for excision with loop electrosurgical excision procedure (LEEP) or cold-knife conization (CKC). Visual evaluation results were reported as negative; positive and eligible for ablation; positive and ineligible for ablation; and suspicious for cancer.

Statistical analyses

The study data were collected and managed using Research Electronic Data Capture (REDCap), a secure, web-based application [10]. Means, standard deviations, and ranges were used for continuous variables and frequency and percentages for categorical variables. Comparisons by HR-HPV group or human immunodeficiency virus (HIV) status were conducted using the Wilcoxon rank-sum test for continuous measures and Fishers exact test or chi-square for categorical measures. We used logistic regression to calculate adjusted odds ratio (OR) and 95% confidence intervals as a measure of association of age group (30–34, 35–39, 40–44, and 45–49 years), HIV status, and residence with HR-HPV prevalence. In the same model, we treated age groups as a continuous variable to calculate the linear trend. *P* values less than <0.05 were considered significant. All statistical analyses were performed using R software or STATA (Version 17, College Station, TX, USA).

Table 1 HPV positivity according to age, HIV status and residence

	WLWH			HIV Negative			HIV Status Unknown			All			
	N	HPV+	*P**	N	HPV+	*P**	N	HPV+	*P**	*P***	N	HPV+	*P**
All	4,098	39.7%		4,808	24.3%		48	14.6%		< 0.0001	8,954	31.3%	
Age Group (Years)													
30–34	1,165	42.9%	< 0.001	1,897	27.2%	< 0.001	18	11.1%	0.18	< 0.0001	3,080	33.0%	< 0.001
35–39	1,212	40.5%		1,331	23.6%		12	25.0%		< 0.0001	2,555	31.6%	
40–44	1,074	37.7%		977	21.3%		6	33.3%		< 0.0001	2,057	29.9%	
45–49	647	35.8%		603	21.9%		12	0%		< 0.0001	1,262	28.8%	
Residence													
Maputo City	2,048	42.6%	< 0.001	2,370	25.2%	0.001	37	16.2%	0.70	< 0.0001	4,455	33.1%	< 0.001
Gaza Province	1,955	37.1%		2,259	24.3%		0	0%		< 0.0001	4,216	30.3%	
Unknown	95	31.6%		179	12.3%		9	11.1%		0.0004	283	18.7%	

*p** = across categories within sub-population (e.g., differences in HPV positivity by residence among WLWH)

*p*** = across sub-population by HIV status (e.g., HPV positivity by HIV status for women from Maputo City)

Table 2 HR-HPV group by HIV status

	WLWH (*n* = 1,621)	HIV negative (*n* = 1,167)	Total (*n* = 2,788)	*p*-value
HPV16	297 (18.3%)	181 (15.3%)	478 (17.0%)	0.05
HPV18/45	424 (26.1%)	264 (22.5%)	686 (24.6%)	**0.03**
Other HR-HPV**	1253 (77.3%)	911 (77.4%)	2164 (77.6%)	0.60

* The sum of the percentages is higher than 100% due to some participants testing positive for more than one HR-HPV group

** HPV31/33/35/51/5256/58/59/66/68

HPV genotyping results were not available for 7 WLWH and 1 HIV-negative woman

Results

A total of 9,014 women were included in the MULHER study. The median age was 37 years; 4,122 (45.7%) were women living with HIV (WLWH), 4,844 (53.7%) were HIV-negative, and 48 (0.6%) were women whose HIV status was unknown. Of the 8,954 (99.3%) women with valid HR-HPV testing results, 2,805 (31.3%) tested positive for HR-HPV. Stratified by HIV status, 39.7% of WLWH, 24.3% of HIV-negative women, and 14.6% of women whose HIV status was unknown tested positive for HR-HPV (Table 1).

Stratified by 5-year age groups, HR-HPV positivity decreased from 33% in women aged 30–34 years to 28.8% in women aged 45–49 years (ptrend = 0.0017). Similar age trends were observed in WLWH (from 42.9% in aged 30–34 years to 35.8% in aged 45–49 years, ptrend < 0.001) and HIV-negative women (from 27.2% in aged 30–34 years to 21.9% in aged 45–49 years, ptrend < 0.001) (Table 1 and Additional file 1). WLWH from Maputo City (*n* = 2,048), Gaza Province (*n* = 1,955), and unknown location (*n* = 95) had a HR-HPV prevalence of 42.6%, 37.1%, and 31.6%, respectively (Table 1).

In a logistic regression model, WLWH (OR = 2.09, 95%CI = 1.91–2.30) and women from Maputo (OR = 1.15, 95%CI = 1.05–1.26) were more likely to test HR-HPV positive than HIV-negative women and women from Gaza, respectively. Older women were less likely to test positive for HR-HPV than younger women (ptrend < 0.001).

Overall, 478 (17.0%) women tested positive for HPV16, 688 (24.5%) for HPV18/45 and 2,164 (77.1%) for other HR-HPV. A total of 497 women (17.7%) tested positive for 2–3 h-HPV groups. HR-HPV group distribution according to HIV status is shown in Table 2. HPV18/45 was more frequent in WLWH than in HIV-negative women (*p*-value = 0.03).

WLWH were more likely to test positive for multiple HPV types compared with HIV-negative patients (19.9% vs. 14.9%, *p*-value < 0.0001) (Table 3).

In patients who tested positive for more than one HR-HPV group, no difference in any HR-HPV group combination between WLWH and HIV negative was observed (Table 4).

According to cancer risk of the different HR-HPV groups, results negative for HPV16/18/45 and positive only for other HR-HPV were more frequent in HIV-negative women (Table 5).

When stratified by available VAT results (*n* = 2,654) (Table 6), other HR-HPV were the most frequent in all categories, followed by HPV18/45. HPV16 frequency increased with the observed severity of the VAT exam (*p* < 0.001).

Thirty participants were diagnosed with invasive cervical cancer. Of these, one had no VAT result available, two were classified as negative, one as positive and eligible for ablation, five as positive and ineligible for ablation, and 21 as suspicious of cancer. Among the 30 patients diagnosed with cervical cancer, 19 (63.3%) were WLWH. Overall,

Table 3 Number of HR-HPV groups by HIV status

Number of HR-HPV Groups	WLWH (*n* = 1,621)	HIV negative (*n* = 1,167)	Total (2,788)	*p*-value
One	1,298 (80.0%)	993 (85.1%)	2,291	**< 0.001**
Two	293 (18.1%)	159 (13.6%)	452	**0.001**
Three	30 (1.9%)	15 (1.3%)	45	0.28
Two or more	323 (19.9%)	174 (14.9%)	497	< 0.001

* Human immunodeficiency virus status unknown in 9 patients

WLWH: women living with HIV; HPV: human papillomavirus

HPV test results were not available for 7 WLWH and 1 HIV-negative woman

Table 4 Multiple HPV risk group combinations

HR-HPV Group	WLWH (*n* = 323)	HIV-negative (*n* = 174)	Total (*n* = 497)	*p*-value
HPV16 and HPV18/45	39 (12.1%)	16 (9.2%)	55 (11.0%)	0.37
HPV16 and Other HR-HPV**	107 (33.1%)	68 (39.1%)	175 (35.1%)	0.20
HPV18/45 and Other HR-HPV**	147 (45.5%)	75 (43.1%)	222 (44.5%)	0.63
HPV16, HPV18/45, and Other HR-HPV**	30 (9.3%)	15 (8.6%)	45 (9.4%)	0.87

* Human immunodeficiency virus status unknown in 9 patients. **HPV31/33/35/51/5256/58/59/66/68

WLWH: women living with HIV; HPV: human papillomavirus; HR-HPV: high-risk HPV type

HPV genotyping results were not available for 7 WLWH and 1 HIV-negative woman

Table 5 HR-HPV groups ranked hierarchically according to cancer risk

HPV Risk Group*	WLWH (*n* = 1,621)	HIV negative (*n* = 1,167)	Total (*n* = 2,788)	*p*-value
HPV16	297 (18.3%)	181 (15.5%)	478 (17.1%)	0.05
HPV18/45	355 (21.9%)	233 (20.0%)	588 (21.1%)	0.22
Other HR-HPV	969 (59.8%)	753 (64.5%)	1,722 (61.8)	**0.01**

HPV genotyping results were not available for 7 WLWH and 1 HIV negative

Table 6 HPV risk groups according to the VAT result

	VAT Result				
	Negative (*n* = 1,397)	Positive Eligible for ablation (*n* = 979)	Positive Ineligible for ablation (*n* = 229)	Suspicious for Cancer (*n* = 49)	*p*-value
HPV16	189 (13.5%)	185 (18.9%)	58 (25.3%)	19 (38.8%)	**< 0.001**
HPV18/45	329 (23.5%)	228 (23.3%)	77 (33.6%)	12 (24.5%)	0.008
Other HR-HPV types	1,094 (*n* = 78.3%)	755 (77.1%)	173 (75.5%)	26 (53.1%)	0.05

* VAT results were not available for 151 patients. The sum of the percentages exceeds 100% due to participants testing positive for more than one HR-HPV group

HPV16 was most commonly detected (*n* = 12; 40.0%), followed by HPV18/45 (*n* = 8; 26.6%), multiple HR-HPV groups (HPV16 plus other HR-HPV (*n* = 5; 16.7%), and other HR-HPV (*n* = 5; 16.7%) (Table 7). In the subset of 30 patients diagnosed with cervical cancer, 17 (56.7%) were positive for HPV16 and 25 (83.3%) were positive for either HPV 16 or HPV 18/45 alone or in combination with other HR-HPV.

Discussion

To the best of our knowledge, this is the largest study of HPV risk group prevalence and screening outcomes in Mozambican women. We found a high prevalence of HR-HPV in Mozambican women, especially WLWH, consistent with the high burden of cervical cancer in this population. WLWH had a HR-HPV prevalence that was more 50% greater than HIV-negative women, similar to previous studies conducted in Maputo [11, 12], Tanzania, Kenya, Uganda and Nigeria. Our study showed that HPV prevalence decreased with older age, as previously observed [13–17].

With regards to the HR-HPV groups observed in the study, other HR-HPV was the most frequent, followed by HPV16 and then HPV18/45. HPV16 was the most frequent HPV risk group detected among women diagnosed with invasive cervical cancer (56.7%) similar to the findings from Castellsagué et al. [18] and Tawe et al. [19].

Previous studies conducted in Maputo observed other HR-HPV as the most frequent HR-HPV group similar to what we found in our study [7, 8, 12]. Bule and

Table 7 HPV risk group among participants diagnosed with cervical cancer according to HIV status

	All (*N* = 30)	WLWH (*N* = 19)	HIV negative (*N* = 11)	*p*-value*
HPV16	12 (40.0%)	9 (47.4%)	3 (27.2%)	0.44
HPV18/45	8 (26.7%)	3 (15.8%)	5 (45.4%)	0.10
Other HR-HPV	5 (16.7%)	3 (15.8%)	2 (18.2%)	1.00
HPV16 & Other HR-HPV	5 (16.7%)	4 (21.0%)	1 (9.2%)	0.62
Any HPV16	17 (56.7%)	13 (68.4%)	4 (36.4%)	0.13

WLWH: women living with HIV; HIV: human immunodeficiency virus; HPV: human papillomavirus

colleagues, in 2017, tested 504 female university students, aged 18–30 years, using Anyplex™ II HPV 28 Detection kit (Seegene) and observed HPV16 as the most frequent HPV risk group (7.5%), followed by other HR-HPV risk group [7]. Another study in Mozambique conducted between 2009 and 2011 in 236 young women, aged 18–24 years, found that the most frequent HR HPV group was other HR-HPV, followed by HPV16, using CLART Human Papillomavirus 2 (Genomica) [8]. A third study conducted between 2018 and 2019 that enrolled 233 women with gynaecological symptoms, aged 14–45 years, found that other HR-HPV group was the most prevalent type, using HPV Direct Flow CHIP kit (Vitro Master Diagnóstica) [12].

A meta-analysis conducted in sub-Saharan African countries identified HPV16 (18%) as the most prevalent, followed by HPV35 (10.1%), HPV52 (9.9%), HPV18 (9.7%), and HPV45 (6.8%). Altogether, other HR-HPV group risk were the most prevalent [20]. These findings were also observed in studies from Tanzania, Kenya, Uganda and Nigeria [15, 21].

In our study, infection with multiple HR-HPV groups was observed in almost 18% of the participants who tested positive for HPV. This is similar to previous studies conducted in Mozambique [7] and Ghana [22], but less common than what was observed in Ethiopia (23.9%) [23] and a previous study in Mozambique (32%) [12]. In the studies conducted in Ethiopia [23] and Mozambique [12], women recruited had gynecological symptoms and the multiple HPV types were more frequently detected in WLWH than in HIV-negative women. Co-infection with HPV18/45 and other HR-HPV was more frequent in WLWH when compared with HIV-negative women.

We observed that HPV16 positivity increased with the severity of VAT results. This is similar to findings by Clifford et al. who performed a meta-analysis of HR-HPV types encompassing data from 19,883 WLWH worldwide. They observed that in Africa, HPV16 positivity increased with severity of cytologic and pathologic abnormalities [24]. Studies performed by Guan et al. [25]. and by Castle et al.. also showed HPV positivity increased with the severity of VAT results [17].

Among the 30 patients diagnosed with invasive cervical cancer in our study, HPV16 was the most frequent HPV risk group identified (56.7%). This is consistent with other studies performed in Ghana [22], Brazil [26], Mali, Senegal [27] and the United States [28]. More than 80% of the cancer cases were associated with HPV16 or 18/45, indicating the importance of HPV vaccination for cervical cancer prevention in Mozambique.

A limitation of our study was that cytology or histopathologic diagnosis was not obtained for the majority of HPV-positive women. Patients testing positive for HPV underwent VAT followed by immediate treatment with ablation without biopsy.

Our study had a disproportionate percentage of WLWH. This may be because the HIV clinics and cervical cancer screening clinics are co-located and a large proportion of women attending HIV clinics were referred for screening within the study.

Conclusion

Our study demonstrates that HPV16 was the most frequent HPV risk group in invasive cervical cancer cases in Mozambique for the MULHER Study population. However, other HR-HPV risk groups have a high frequency in the screened population and were also observed in cervical cancer cases.

Abbreviations

HPV	Human papillomavirus
HR-HPV	High-risk HPV
VIA	Visual inspection with acetic acid
VAT	Visual assessment for treatment
LEEP	Excision with loop electrosurgical excision procedure
CKC	Cold-knife conization
REDCap	Research electronic data capture
HIV	Human immunodeficiency virus
OR	Odds ratio
WLWH	Women living with HIV

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13027-025-00655-1.

Supplementary Material 1: **Additional file 1**: Age-group HR-HPV prevalence for Mozambican women living with HIV (WLWH) and HIV negative women.

Author contributions

Conception and design: CMO, MPS, EL, CL and KMS; Administrative support: MPS, EB, EL, HH, CL and KMS; Provision of study material or patients: NO, AN,

RR, AANM, JC, CL and GT. Collection and assembly of data: CMO, MPS, VA, MC, and KMS. Data analysis and interpretation: CMO, MPS, EC, MC, PEC, and KMS. Manuscript writing: all authors. All authors had final responsibility for the decision to submit for publication.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Institutional Review Board from MD Anderson Cancer Center (2020 – 0651) and the Comité Nacional de Bioética para a Saúde, Moçambique (IRB00002657), and all patients provided written informed consent.

Consent for publication
Not applicable.

Competing interests
Dr. Castle has received HPV tests and assays for research at a reduced or no cost from Cepheid and Atila BioSystems. The other authors have no relevant financial or non-financial interests to disclose.

Disclaimer
Opinions expressed by the authors are their own and this material should not be interpreted as representing the official viewpoint of the U.S. Department of Health and Human Services, the National Institutes of Health, or the National Cancer Institute.

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