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Human papillomavirus genotypes in cervical and other HPV-related anogenital cancer in Rwanda, according to HIV status

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The study aim was to describe human papillomavirus (HPV)-attributable cancer burden in Rwanda, according to anogenital cancer site, HPV type, age and HIV status. Tissue specimens of cervical, vulvar, vaginal, penile and anal cancer diagnosed in 2012–2018 were retrieved from three cancer referral hospitals and tested for high-risk (HR) HPV DNA. Cervical cancer represented the majority of cases (598 of 738), of which 96.0% were HR-HPV positive. HPV-attributable fractions in other cancer sites varied from 53.1% in 81 penile, through 76.7% in 30 vulvar, 83.3% in 24 vaginal, up to 100% in 5 anal cases. HPV16 was the predominant HR-HPV type in cervical cancer (55.0%), followed by HPV18 (16.6%) and HPV45 (13.4%). HPV16 also predominated in other cancer sites (60–80% of HR-HPV-attributable fraction). For cervical cancer, type-specific prevalence varied significantly by histology (higher alpha-9 type prevalence in 509 squamous cell carcinoma *vs.* higher alpha-7 type prevalence in 80 adenocarcinoma), but not between 501 HIV-negative and 97 HIV-positive cases. With respect to types targeted, and/or cross-protected, by HPV vaccines, HPV16/18 accounted

Key words: human papillomavirus, HIV, cancer, epidemiology, attributable fraction

Abbreviations: ADC: adenocarcinoma; ADSC: adenosquamous carcinoma; BCCOE: Butaro Cancer Center of Excellence; CHUB: Butare Teaching Hospital; CI: confidence interval; E7-MPG: E7 PCR bead-based multiplex genotyping assay; FFPE: formalin fixed paraffin embedded; HR HPV: high-risk human papillomavirus; PCR: polymerase chain reaction; PR: prevalence ratio; SCC: squamous cell carcinoma

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: DAMH is minority shareholder of Self-screen B.V., a spin-off company of VU University Medical Center; Self-screen B.V. holds patents related to the work (i.e., high-risk HPV test and methylation markers for cervical screening) and has developed and manufactured CE-IVD assays, which are licensed to Qiagen (QIAscreen® HPV PCR Test and QIAsure® Methylation Test); DAMH has been on the speakers bureau of Qiagen and serves occasionally on the scientific advisory boards of Pfizer and Bristol-Myers Squibb. The other authors declare that they have no conflict of interest.

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for 73%, HPV31/33/45/52/58 for an additional 22% and other HR-HPV types for 5%, of HPV-attributable cancer burden, with no significant difference by HIV status nor age. These data highlight the preventive potential of the ongoing national HPV vaccination program in Rwanda, and in sub-Saharan Africa as a whole. Importantly for this region, the impact of HIV on the distribution of causal HPV types was relatively minor, confirming type-specific relevance of HPV vaccines, irrespective of HIV status.

What's new?

Sub-Saharan Africa suffers the highest cervical-cancer rates in the world, due in part to a lack of cervical-cancer screening programs. In 2011, Rwanda initiated a national HPV-vaccination program, achieving >90% coverage. In this study, the authors characterized the subsequent burden of all forms of anogenital cancer attributable to high-risk (HR) HPV types, including cervical, anal, etc. These results illustrate the preventive potential of a national HPV-vaccination program. They also enhance our understanding of the HPV-attributable fraction of non-cervical anogenital cancers in sub-Saharan Africa overall.

Introduction

Sub-Saharan Africa suffers the highest cervical cancer incidence rates in the world,[1] and accounts for one fifth of the global cervical cancer burden.[2] Rates are driven by high population prevalence of human papillomavirus (HPV), the necessary cause of cervical cancer, combined with lack of cervical cancer screening programs. HPV can also cause vulvar, vaginal, penile and anal cancer,[3] but little is known about the HPV-attributable burden of these rarer anogenital cancers in sub-Saharan Africa.

Thirteen high-risk (HR) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are classified as human carcinogens (Group 1/2A).[3] Large meta-analyses have confirmed HPV16 and HPV18 to cause a majority of cervical cancer in all world regions, but in sub-Saharan Africa, the attributable fraction of HPV16 in cervical cancer has been reported slightly lower, and HPV45 and HPV35 slightly higher, compared to other regions.[4] In addition, HIV infection increases HPV-related cancer risk[5,6] and may impact type-specific attributable fractions.[7,8]

Rwanda, with a high cervical cancer burden typical of sub-Saharan Africa,[5] initiated a national HPV vaccination program in 2011, achieving >90% coverage.[9] In order to predict the potential impact of this program, as well as to improve the paucity of data on HPV-attributable cancer in sub-Saharan Africa, our aim was to robustly characterize the burden of cervical, vulvar, vaginal, penile and anal cancer attributable to HR-HPV types. To this end, we tested for HPV DNA in tumor specimens of a large and representative series of consecutively diagnosed cancers in three large national referral hospitals across Rwanda between 2012 and 2018. We were particularly interested to investigate HIV infection as a potential modifier of the HPV type-specific attributable cancer burden.

Methods

Study design and population

The study was approved by ethics committees of UR-CMHS-SPH and International Agency for Research on Cancer.

This cross-sectional molecular epidemiological study was based on formalin-fixed paraffin-embedded (FFPE) tissue specimens from patients diagnosed with histologically confirmed cervical, vulvar, vaginal, penile or anal cancer in the histopathology departments of three public cancer referral hospitals in Rwanda: Kigali Teaching Hospital, in the center, Butare Teaching Hospital (CHUB), in the south, and Butaro Cancer Center of Excellence (BCCOE) in the north of the country. Demographic information, cancer anatomical site and histology, year of diagnosis and HIV status were obtained from medical records. Knowledge of HIV status was a prerequisite to inclusion for cervical cancer, but not for the other, rarer, sites. Specimen and data collection were initiated in November 2015 and had both retrospective (archives back to 2012, for CHUB and BCCOE only) and prospective (all three hospitals) components.

The study design was to continue prospective recruitment of all eligible cases until reaching the target of 100 FFPE tissue specimens of HIV-positive cervical cancer. This target was met in January 2018, and a total of 842 eligible de-identified FFPE tissue specimens were sent to VU University Medical Center, Amsterdam, for HPV DNA testing and histological rereview (see below).

After exclusion of 31 cases with invalid results (both negative for beta-globin polymerase chain reaction [PCR] and HPV PCR), 43 cases without any evidence of tumor tissue in the FFPE specimen tested for HPV DNA, and 30 cases of anal adenocarcinoma (ADC; on the basis that they were likely to be misclassified rectal cancers), a total of 738 anogenital cancer cases were included in the analyses.

Histological types of cancers were categorized as squamous cell carcinoma (SCC), ADC, adenosquamous carcinoma (ADSC) or other. ADC and ADSC were grouped in the analyses. In the event of discordance between local and Amsterdam pathologists in histological type of cancer, the final diagnosis (SCC, ADC or other) represents that from Amsterdam.

HPV testing and genotyping

HPV testing was performed on all specimens at the Department of Pathology, VU University Medical Center, Amsterdam. From each cancer case, an H&E-stained slide from the FFPE tissue

block was prepared to select tumor-enriched regions to biopsy from. The H&E section and FFPE tissue block were aligned and a punch biopsy was taken from the FFPE block at a selected tumor region by using a sterile, 1 mm biopsy punch disposable as described before.[10] The punch biopsies were predigested with proteinase K after which DNA was extracted using magnetic beads (NucliSense EasyMag isolation procedure; bioMérieux SA, Marcy l'Étoile, France). Beta-globin PCR analysis was conducted to assess the quality of the DNA to be submitted to HPV PCR.[11] The presence of HPV DNA was determined by conducting a general primer GP5+/6+ -mediated PCR[12] followed by hybridization of PCR products in an enzyme immunoassay with an oligoprobe cocktail that, together, detects the following 13 HR-HPV types classified as Group 1 or Group 2A carcinogens by International Agency for Research on Cancer[3]: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Subsequent HPV genotyping was conducted using a microsphere bead-based assay (Luminex xMAP, Luminex Corp, Austin, TX) as described previously.[13] For all valid cancer cases that were HR-HPV negative by GP5+/6+ PCR, extracted DNAs were retested at International Agency for Research on Cancer, Lyon, with a type-specific E7 PCR bead-based multiplex genotyping assay (E7-MPG), that detects DNA from 21 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82).[14]

Statistical analysis

Unless otherwise specified, HPV type-specific prevalence is reported as a proportion of all cases tested by GP5+/6+ HPV PCR. Prevalence ratios (PRs) and corresponding 95% confidence intervals (CIs) were used to compare HPV positivity for individual, or groups of, HPV types, calculated using binomial regression models with a log link. For attributable fractions of groups of HPV types, a hierarchical classification to account for (the few) multiple infections was used: (*i*) HPV16/18, (*ii*) HPV31/33/45/52/58 (in the absence of multiple infection with HPV16/18) and (*iii*) other HR HPV (in the absence of multiple infection with any of the aforementioned HPV types), and the prevalence of these groups of types was compared among HR-HPV-positive cases only. All statistical analyses were two-sided and were done in STATA version 14.

Results

A final total of 738 cancer cases were included in the study, representing cancers of the cervix (n = 598), penis (n = 81), vulva (n = 30), vagina (n = 24) and anus (n = 5). The major characteristics of the study population are shown in Table 1, according to cancer site. The majority of cervical cancer cases came from BCCOE, whereas other anogenital cancers were more equally distributed across the three hospitals. The mean age was 54.4 years

Table 1. Characteristics of the study population, according to cancer site

	Cervix		Penis		Vulva		Vagina		Anus[1]	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Total	598	100	81	100	30	100	24	100	5	100
Hospital										
CHUK (Kigali)	147	24.8	32	39.5	7	23.3	12	50.0	2	40.0
CHUB (Butare)	83	13.8	20	24.7	9	30.0	7	29.2	1	20.0
BCCOE (Butaro)	368	61.4	29	35.8	14	46.7	5	20.8	2	40.0
Age at diagnosis										
<45 years	130	21.7	22	27.2	7	23.3	8	33.0	1	20.0
45–54 years	179	29.9	13	16.0	7	23.0	7	29.0	1	20.0
≥55 years	289	48.3	46	56.8	16	53.0	9	37.5	3	60.0
Mean age (SD)	54.4 (11.3)		56.3 (16.9)		54.7(11.9)		51.2 (18.2)		51.8 (10.8)	
Year of diagnosis										
2012–2015	262	43.8	39	48.2	12	40.0	13	54.2	3	60.0
2016–2018	336	56.2	42	51.8	18	60.0	11	45.8	2	40.0
Histology subtype										
SCC	509	85.1	80	98.8	29	96.7	17	70.8	5	100
ADC/ADSC	80	13.4	1	1.2	0	0.0	6	25.0	0	0.0
Others	9	1.5	0	0.0	1	3.3	1	3.3	0	0.0
HIV status[2]										
Negative	501	83.8	50	83.3	14	56.0	16	84.2	2	50.0
Positive	97	16.2	10	16.7	11	44.0	3	15.8	2	50.0
Unknown	0		21		5		5		1	

[1]Including two male and three female cases.
[2]Percentages shown among subjects of known HIV status only. Knowledge of HIV status was an inclusion criteria for cervical cancer.
Abbreviations: ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; BCCOE, Butaro Cancer Center of Excellence; CHUB, Butare Teaching Hospital; CHUK, Kigali Teaching Hospital; SCC, squamous cell carcinoma.

for cervical cancer and varied from 51.2 years for vaginal cancer up to 56.3 years for penile cancer. Cancers were diagnosed approximately equally between the periods 2012–2015 and 2016–2018. SCC was the predominant histological subtype in all cancer sites, but 13.4% of cervical cancer and 25.0% of vaginal cancer were ADC/ADSC. Penile and vulvar cancer were almost entirely SCC. Among cases with known HIV status, HIV positivity was 16.2% in cervical, 16.7% in penile, 44.0% in vulvar, 15.8% in vaginal and 50.0% in anal cancer (Table 1). In cervical cancer (other cancers were too few to allow such a comparison), HIV positivity was higher in younger women, 31.1% in 119 <45 years, 21.1% in 190 45–54 years and 6.9% in 289 ≥55 years, and did not differ between ADC and SCC (data not shown).

The prevalence of HR-HPV types in each of the anogenital cancer sites is shown in Table 2. Overall prevalence of HR HPV varied from 53.1% in penile cancer up to 96.0% in cervical cancer. Multiple HR-HPV infection was relatively rare in all cancer types, so that type-specific prevalences represent that almost entirely of single infections. HPV16 was the predominant HR-HPV type in all cancer sites, but the relative prevalence of different HR-HPV types varied somewhat by cancer site. In the cervix, for which the sample size, and hence robustness of estimates, was much higher than for other sites, HPV16 (55.0%) was followed by HPV18 (16.6%), HPV45 (13.4%) and HPV33 (4.5%), with other HR-HPV types being detected in 0.3–2.8% of cases. In penile cancer, HPV18 was also the second most common HR type (8.6%) after HPV16 (32.1%). In vulvar cancer, HPV16 (46.7%) was followed by HPV33 and HPV31 (10.0% each), and in vaginal cancer, HPV16 (50.0%) was followed by HPV45 (16.7%). Anal cancers were very few, but HPV16 predominated (4/5 cases).

HR-HPV prevalence is compared between 501 HIV-negative and 97 HIV-positive cervical cancers in Table 3. Overall HR-HPV prevalence did not differ significantly by HIV status (96.2% and 94.8%, respectively). HPV16 prevalence was slightly lower in HIV positive (50.5%) than HIV negative (55.9%), but this difference was not significant. The only borderline statistically significant difference was an overrepresentation of HPV31 in HIV-positive (5.1%) *vs.* HIV-negative (1.8%) cervical cancer (PR: 2.9, 95% CI: 1.0–8.4).

Other anogenital cancer sites were too rare to allow comparison of HR-HPV prevalence according to HIV status. However, upon combination of penile, vulvar, vaginal and anal cancer together, HR-HPV prevalence was not significantly different between 26 HIV-positive (76.9%) and 82 HIV-negative cases (62.2%), nor that of HPV 16 (30.8% and 39.0%, respectively) (data not shown).

Table 4 compares HR-HPV prevalence in cervical cancer according to histology type, namely 509 SCC *vs.* 80 ADC. Overall prevalence of any HR HPV was borderline significantly lower in ADC (87.5%) than in SCC (97.4%) (PR: 0.9, 95% CI: 0.8–1.0). With respect to individual types, HPV16 was significantly underrepresented in ADC (26.3%) *vs.* SCC (59.5%) (PR: 0.4, 95% CI: 0.3–0.6), as was HPV33 (0.0% *vs.* 5.3%, PR: 0.0, 95% CI: 0.0–0.9). Whereas, HPV18 was significantly overrepresented in ADC (38.8%) *vs.* SCC (13.2%) (PR: 2.9, 95% CI: 2.1–4.2) as was HPV45 (27.5% *vs.* 11.0%, PR: 2.5, 95% CI: 1.6–3.9). Taken as a group, alpha 7 HPV types (HPV18, 39, 45, 59 and 68) were significantly overrepresented in ADC *vs.* SCC (66.3% *vs.* 25.3%, PR: 2.6, 95% CI: 2.1–3.2), whereas alpha-9 types (HPV16, 31, 33, 35, 52 and 58) were significantly underrepresented (27.5% *vs.* 74.1%, PR: 0.4, 95% CI:

Table 2. Prevalence of HR-HPV types among 738 cases of cancers, by anogenital cancer site

	Cervix (*n* = 598)		Penis (*n* = 81)		Vulva (*n* = 30)		Vagina (*n* = 24)		Anus (*n* = 5)	
HPV type	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Total	598	100	81	100	30	100	24	100	5	100
Any HR HPV	574	96.0	43	53.1	23	76.7	20	83.3	5	100
Multiple HR HPV	28	4.7	1	1.2	0	0.0	2	8.3	0	0.0
HPV16	329	55.0	26	32.1	14	46.7	12	50.0	4	80.0
HPV18	99	16.6	7	8.6	1	3.3	3	12.5	0	0.0
HPV45	80	13.4	2	2.8	2	6.8	4	16.7	1	20.0
HPV33	27	4.5	0	0.0	3	10.0	1	4.2	0	0.0
HPV35	17	2.8	3	3.7	0	0.0	1	4.2	0	0.0
HPV52	16	2.7	0	0.0	0	0.0	0	0.0	0	0.0
HPV31	14	2.3	1	1.2	3	10.0	1	4.2	0	0.0
HPV58	10	1.8	2	2.5	0	0.0	0	0.0	0	0.0
HPV51	5	0.7	1	1.2	0	0.0	0	0.0	0	0.0
HPV68	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0
HPV39	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0
HPV56	3	0.5	1	1.2	0	0.0	0	0.0	0	0.0
HPV59	2	0.3	1	1.2	0	0.0	0	0.0	0	0.0

Abbreviation: HR HPV, high-risk human papillomavirus.

Table 3. Prevalence of HPV types among 598 cervical cancers, according to HIV status

	HIV negative		HIV positive		HIV positive *vs.* HIV negative	
HPV type	***n***	**%**	***n***	**%**	**PR**	**95% CI**
Total	501		97			
Any HR HPV	482	96.2	92	94.8	1.0	0.9–1.0
Multiple HR HPV	21	4.2	7	7.2	1.7	0.8–3.9
HPV16	280	55.9	49	50.5	0.9	0.7–1.1
HPV18	83	16.6	16	16.5	1.0	0.6–1.6
HPV45	65	13.0	15	15.5	1.2	0.7–2.0
HPV33	24	4.8	3	3.1	0.6	0.2–2.1
HPV35	16	3.2	1	1.0	0.3	0.0–2.4
HPV52	13	2.6	3	3.1	1.2	0.3–4.1
HPV31	9	1.8	5	5.1	2.9	1.0–8.4
HPV58	8	1.6	2	2.1	1.3	0.3–6.0
HPV39	2	0.4	1	1.0	2.6	0.2–28.2
HPV68	2	0.4	2	2.1	5.2	0.7–36.2
HPV56	2	0.4	1	1.0	2.6	0.2–28.2
HPV59	2	0.4	0	0.0	0.0	0.0–27.4
HPV51	1	0.6	1	1.0	1.7	0.2–16.4
Any alpha 7	151	30.1	34	35.1	1.2	0.9–1.6
Any alpha 9	342	68.3	62	63.9	0.9	0.8–1.1

Abbreviations: CI, confidence interval; HR HPV, high-risk human papillomavirus; PR, prevalence ratio.

0.3–0.5). Of note, HIV positivity was similar in SCC and ADC (16.3% in both) (data not shown).

The burden of cancers attributable to groups of HPV types (16/18; 31/33/45/52/58; other HR) is shown in Figure 1, both as absolute numbers and as a fraction of all HR-HPV-positive cancers. Overall, HPV16/18 accounted for 73% of the HPV-related cancer burden, HPV31/33/45/52/58 for an additional 22% and other HR HPV for the remaining 5%. This was driven

Table 4. Prevalence of HPV types among 589 cervical cancers, according to histological type

	SCC		ADC/ADSC			
HPV type	***n***	**%**	***n***	**%**	**PR**	**95% CI**
Total	509		80			
Any HR HPV	496	97.4	70	87.5	0.9	0.8–1.0
Multiple HR HPV	22	4.3	6	7.5	1.7	0.7–4.1
HPV16	303	59.5	21	26.3	0.4	0.3–0.6
HPV18	67	13.2	31	38.8	2.9	2.1–4.2
HPV45	56	11.0	22	27.5	2.5	1.6–3.9
HPV33	27	5.3	0	0.0	0.0	0–0.9
HPV31	14	2.8	0	0.0	0.0	0–1.9
HPV35	15	2.9	2	2.5	0.8	0.2–3.6
HPV52	14	2.8	2	2.5	0.9	0.2–3.9
HPV58	10	2.0	0	0.0	0.0	0–2.8
HPV39	3	0.6	0	0.0	0.0	0–15
HPV68	4	0.8	0	0.0	0.0	0–9.6
HPV56	3	0.6	0	0.0	0.0	0–15
HPV59	2	0.4	0	0.0	0.0	0–34
HPV51	4	0.8	0	0.0	0.0	0–9.6
Any alpha 7	129	25.3	53	66.3	2.6	2.1–3.2
Any alpha 9	377	74.1	22	27.5	0.4	0.3–0.5

Abbreviations: ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; CI, confidence interval; HR HPV, high-risk human papillomavirus; PR, prevalence ratio; SCC, squamous cell carcinoma.

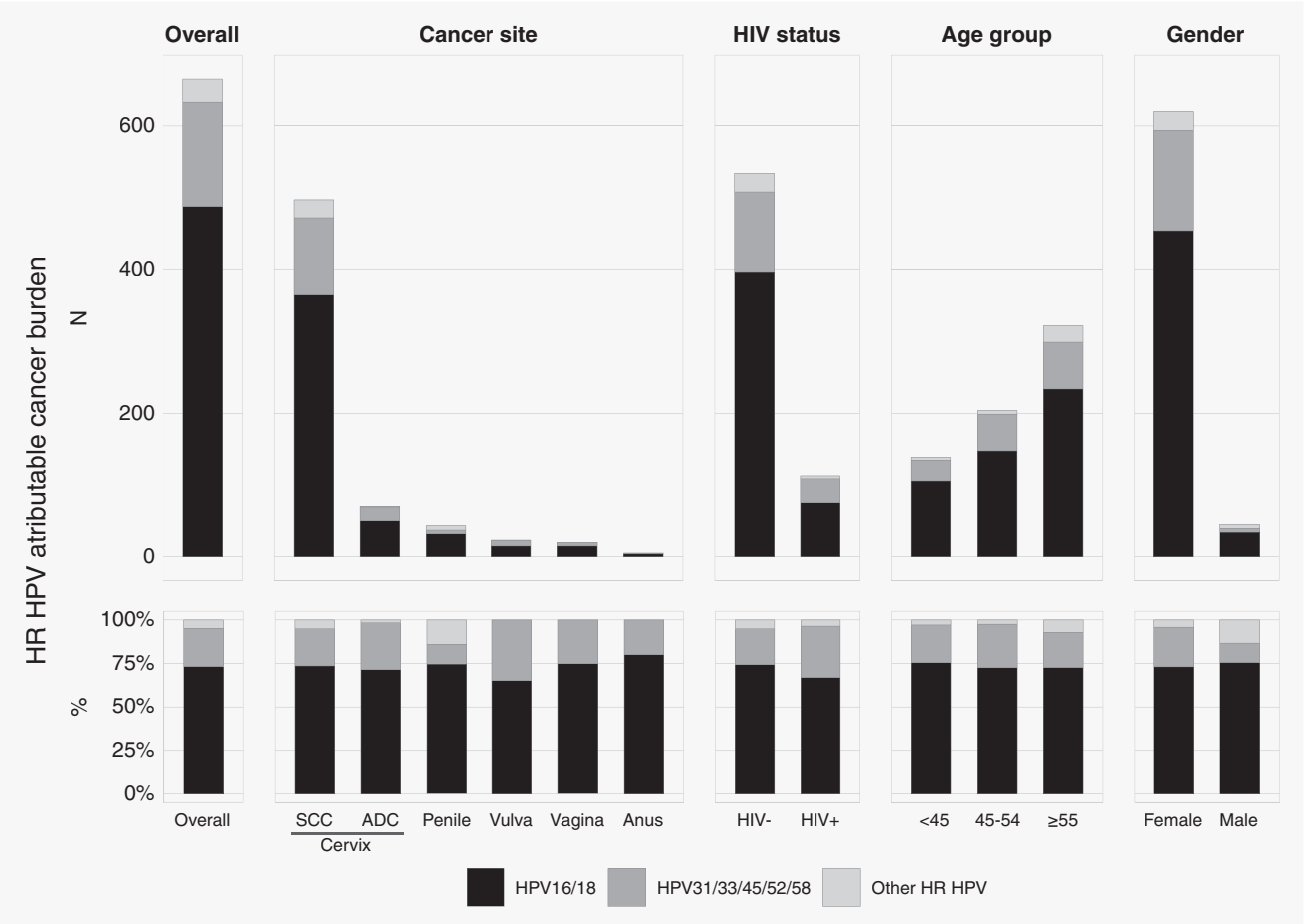

Figure 1. Human papillomavirus (HPV) attributable burden of cancer in Rwanda, according to groups of HPV types.

by SCC of the cervix, which was by far the most commonly diagnosed cancer type. The HPV16/18 fraction was lowest in HR-HPV-positive vulvar cancer (65.2%) and highest in HR-HPV-positive anal (80%) cancer. The fraction attributable to HPV31/33/45/52/58 was higher in HR-HPV-positive cancer in HIV-positive persons (29.5%) than in HIV-negative persons (20.8%) (PR: 1.4, 95% CI: 1.0–2.0), and that of other HR HPV was significantly higher in HR-HPV-positive cancer in men (13.3%, driven by penile cancer) than in women (4.2%) (PR: 3.2, 95% CI: 1.4–7.3). There were no significant differences in type-specific attributable fractions among HPV-positive cancer according to age.

Discussion

This first description of HPV-attributable cancer burden in Rwanda illustrates the preventive potential of a national HPV vaccination program. It also makes a substantial contribution to the sparse data on the HPV-attributable fraction of non-cervical anogenital cancer in sub-Saharan Africa, which varied from about half of penile cancer up to all of anal cancer. Importantly for this region of the world, the impact of HIV infection on the distribution of causal HPV types in HPV-driven cancer was shown to be relatively minimum, confirming the relevance of HPV vaccines irrespective of HIV status: HPV16/18 (the HR types targeted by the current vaccine program) accounted for an estimated 73% of the HPV-attributable cancer burden, and HPV31/33/45/52/58 (which may be cross-protected against by the vaccine), for an additional 22%. A remaining 5% of HPV-attributable cancer was due to other HR-HPV types.

Cervical cancer accounted for the vast majority of the total HPV-related cancer burden in Rwanda, highlighting its priority for prevention efforts. The large majority of cervical cancers were SCC. However, there was also a substantial contribution of ADC (13%), representing a sizeable addition to data previously available on cervical ADC in Africa.[7] Indeed, a relative rarity of ADC (6%) in previous meta-analyses of cervical cancer in sub-Saharan African[4] might partly be due to histopathology assessment issues. In our present study, tumors with any glandular component, including ADSC, were classified as ADC. Although the estimated HPV16/18 attributable fraction was not different between SCC (74%) and ADC (71%), the HR-HPV type distribution between SCC and ADC was strikingly different; whereas HPV16 accounted for the majority of SCC, HPV18 was by far the most common type in ADC.[15] The HR-HPV-negative proportion was also higher in ADC (13%)

than in SCC (3%). Rare histotypes of ADC at the cervical site such as gastric or mesonephric type are known to be unrelated to HPV. Alternatively, a small fraction of ADC may have been misclassified endometrial cancer, unrelated to HPV. Of note, a comprehensive genomic survey has confirmed the existence of a very small HPV-negative fraction of primary cervical cancers that have a gene expression profile resembling endometrial cancer genome.[16]

The most prevalent HR-HPV type in cervical cancer after HPV16 and HPV18 was HPV45 (13%), confirming meta-analytical findings that HPV45 is relatively more important in sub-Saharan Africa[7] than in other world regions.[4] In a previous population-based survey in Rwanda using the same HPV testing protocol,[17] certain other HR-HPV types were more common than HPV18 and 45 in the general female population (e.g., HPV35, 52, 56 and 58), and HPV 58 was the second most common HR-HPV type in HSIL after HPV16. However, these types were relatively rarely found in cancer, highlighting their lower carcinogenicity in comparison to types HPV16, 18 and 45. Of note, of the 13 cervical SCCs that remained HR-HPV negative according to both GP5+/GP6+ and E7-MPG (Table 3), an additional three cases were E7-MPG positive for HPV73, a rare HPV type that is not classified HR,[3] but that has certain similarities to HR HPV in terms of its epidemiological profile.[18,19]

Penile cancer was the second most common HPV-associated cancer in our case series from Rwanda. Although global data remain sparse, penile cancer incidence tends to be considered higher in developing than developed countries,[2] with Rwanda ranking as fifth highest incidence in the world in GLOBOCAN 2018.[1] However, only half (53%) of penile cancer in Rwanda is HPV attributable, consistent with the HPV positivity (48%) estimated in two large global meta-analyses (albeit that did not include any cases from Africa),[20,21] as well as that estimated in a more recent series of 1,010 cases (37% HPV positivity, 19 cases from Africa only)[22] and two small African series (50%[23] and 42%[24]). Thus, there is an important HPV-independent etiology of penile cancer, and the latest WHO classification separates two entities of HPV-positive and HPV-negative penile cancers.[25] The majority of HPV-positive penile cancers were confirmed to be attributable to HPV16.[22]

Combined frequency of vulvar/vaginal cancer was less than penile cancer in our case series, whereas in developed settings it tends to be approximately double.[2,26] The HPV-attributable fraction of vulvar and vaginal cancer, in contrast, was higher than for penile cancer. For vulvar cancer, our estimate of 77% HPV positivity is much higher than that in a global case series (n = 1,709, 29%),[27] as well as that in a global meta-analyses (n = 5,015, 40%),[28] but is consistent with the few African cases included in these analyses (n = 24 only; 71% HPV positivity). This suggests that the HPV-attributable fraction for vulvar cancer in sub-Saharan Africa might be higher in comparison to other world regions.[24,29] This difference could be partly driven by the younger age of vulvar cancer cases in our study compared to those reported from developed countries. Like penile cancer, it is well established that vulvar cancer has two different etiological pathways, HPV related and HPV unrelated, and the contribution of HPV diminishes among older women.[30] Our estimate of 83% HPV positivity for vaginal cancer is also slightly higher than that from a large global case series (n = 408, HPV positivity 74%),[31] in which African cases were not well represented (n = 19, 68% HPV positivity), as well as slightly higher than that in a previous meta-analysis (n = 136, 70%).[30] HPV16 was confirmed to account for the large majority of HPV-positive vulvar and vaginal cancer in Rwanda.[28,31]

Anal SCC was rarely diagnosed in our case series from Rwanda, even in comparison to other non-cervical anogenital cancers, as seen in an earlier study.[5] This is in contrast to the 2018 global anal cancer statistics, where anal cancer incidence in Rwanda is estimated to be among the highest in the world, and higher than that of vulvar and/or vaginal cancer.[1] This discrepancy is likely due to a substantial proportion of misclassification of the more frequent rectal cancer. Indeed, we excluded *a posteriori* the majority of anal cancers (30 out of 35) because of ADC histology, of which 29 (97%) tested HR-HPV negative. If true, a relatively lower frequency of anal SCC in sub-Saharan Africa may reflect different patterns of homosexual/heterosexual HPV transmission in comparison to high income settings or, again, the relatively young age of our cancer cases compared to reports from Western countries. Of the five anal SCC reported, 100% were attributable to HR HPV, of which the large majority to HPV16, which is consistent with a large global meta-analysis of 2,358 anal cancers (90–100% HPV positivity according to gender and HIV status).[8]

With respect to primary prevention through vaccination, this work suggests that prevention of HPV16/18 infection can be expected to avoid three-quarters of the future burden of HPV-attributable cancer burden in Rwanda, and prevention of HPV31/33/45/52/58 to avoid a large part of the remaining one quarter. These proportions are consistent with worldwide estimates from a meta-analysis.[2] Of note, randomized-controlled trials,[32,33] and post-vaccine surveillance studies[34,35] have shown that HPV16/18-targeting vaccines offer some degree of cross-protection against HPV31/33/45/52/58. Nevertheless, a remaining 5% of HPV-attributable cancer was estimated due to other HR-HPV types, of which HPV35 merits a special note: it was the fifth most frequent type in HPV-attributable cancer in Rwanda (as seen in a wider meta-analysis of African cervical cancer),[7] but is not included in currently licensed vaccines, presumably due to its relative rarity outside the African continent.[36]

HIV is known to increase the risk of the anogenital cancers,[3,6] including in Rwanda.[5] Based upon a crude comparison of HIV prevalence in the present series (17%) with that observed in non-infection related cancers in a recent case-control study in Rwanda as an expected underlying rate (5%),[5] we estimate that approximately 12% of anogenital cancers can be attributable to HIV infection. This excess is driven by cervical cancer, for which HPV is a necessary cause. With respect to other anogenital cancers, the majority of HIV positive cases (20 out of 26) were also HR-HPV

positive, suggesting that HIV was acting also through the HPV-dependent etiological pathway, and cannot explain the HPV-negative fraction. Importantly, however, we were able to demonstrate that the relative distribution of cancer causing types was largely unaffected by HIV status,[7] suggesting similar type-specific relevance of vaccines.

In addition to the large sample size, knowledge of HIV-status and the inclusion of all public Pathology Departments in Rwanda, one of the principal strengths of our study is the double review and quality control of histopathology, allowing exclusion of cases without any evidence of tumor tissue in the specimen for HPV testing, as well as of anal ADC of suspected rectal origin. Furthermore, a rigorous HPV testing approach was used, firstly using an HPV test designed to detect clinically specific HPV infections (GP5+/6+), followed by a second more sensitive test (E7-MPG) to confirm (or not) the absence of HR HPV in the GP5+/6+ HPV-negative cases (see Supporting Information Table S1). We believe that such an approach provides a good estimate of HPV type-specific attributable fractions, while avoiding problems of separating causal from passenger types in a high proportion of multiple infections.[7]

With respect to limitations, an unknown proportion of anogenital cancer cases in Rwanda does not undergo biopsy and receive histological confirmation, and so our data cannot be used to compute incidence rates. However, this is unlikely to affect HPV findings and we nevertheless expect this cancer series to be broadly representative of HPV-related cancer in the country. A slight exception to this is the lack of data on HPV-attributable burden of oropharynx cancer in Africa in our study,[37] but a pilot revealed inadequate specification of head and neck primary cancer site in the diagnosing centers. We also did not use p16 staining in combination with HPV DNA testing, an approach that offers a more conservative attribution of the HPV-related fraction of non-cervical HPV-related cancers.[22,27,31] Neither did we separate non-cervical SCC into warty-basaloid *vs.* non-warty-basaloid types, which has been shown to partly discriminate HPV-positive fractions.[22,27,30,31] Indeed, we did not have sufficient power to study determinants of HPV positivity for any individual cancer sites other than cervix. For example, the HPV-attributable fractions of both vulvar and vaginal,[26,27,31] but not penile,[22,26] cancer have been shown to decrease with age. We could not identify any significant age effect but the mean age of our cancer cases was relatively young compared to developed settings. One quarter of vaginal cancers was of ADC histology, but HPV positivity (5 out of 6) was similar to vaginal SCC (14 out of 17).

In conclusion, we were able to produce a picture of the HR-HPV type-specific attributable burden of cancer in Rwanda, according to cancer site, age, gender and HIV status. These data provide an important baseline for predicting today, and demonstrating (by repeat series) in the future, HPV vaccine impact on cancer in Rwanda. They offer support for continued investment in the Rwandan national HPV vaccine program, as well for programs in other sub-Saharan African settings.

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