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HIGH RISK HPV GENOTYPES: SIX YEAR TRENDS AND DISTRIBUTION IN SOUTH WESTERN UGANDA

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ABSTRACT

Introduction: Persistent infection with High-Risk Human Papillomavirus (hrHPV) causes almost all cervical cancer cases, and the world is steadily moving towards primary HPV testing for cervical cancer screening. We determined the prevalence, distribution, and trends of hrHPV genotypes at Mbarara Regional Referral Hospital (MRRH), south western Uganda.

Methods: In this retrospective study, we reviewed laboratory records at MRRH for a period of six years from January 2020 and July 2025. Data on age, district of residence, HIV status, HPV test result, HPV genotype and date of testing were collected and analyzed using STATA 17.

Results: Out of the 4,852 HPV test results we retrieved, the overall prevalence of hrHPV infection was 28.52% (1379/4,852) with a gradual increase from the year 2020 to 2025. The prevalence of

hrHPV was highest among women aged <19 years (66.71%, 12/18), and it showed a steady decrease with increasing age of participants. More than half (65.12%, 898/1379) of all positive hrHPV tests were non-16/18/45 high-risk HPV genotypes categorized as “other hrHPV”, which also showed a persistent increase in prevalence from 61.04% in 2020 to 63.53% in 2025. “Other hrHPV” genotypes also showed a gradual decline in prevalence with increasing age. Within the HIV positive population, the prevalence of “other hrHPV” genotype group result was consistently high from 64.79% (46/71) in 2020 to 66.55% (189/284) in 2023 to 63.53% (54/85) in 2025. Also, mixed HPV genotype infections showed a steady increase from 11.27% (8/71) in 2020 to 21.18% (18/85) in 2025.

Conclusion: There is an elevated prevalence of hrHPV, especially those HPV genotypes categorized as “Other hrHPV,” and this prevalence is inversely proportional to age. As such, there is a need for implementation of extended hrHPV genotyping and more prospective studies to further describe individual hrHPV genotype prevalence, distribution, and trends.

Key words: HPV, hrHPV, Trends, Cervical cancer

INTRODUCTION

Recent findings indicate that there are over 570,000 new cervical cancer cases worldwide and with over 311,000 deaths mostly in low income countries (1). Cervical cancer incidence and mortality rates are estimated to be 117,316 and 76,745, respectively (2). The estimated cervical cancer incidence rate in the East Africa region stands at 43/100,000 (3). In Uganda, cervical cancer is one of the most common cancers among females (4) with an age-standardized incidence rate of 56.2/100,000 (5). In south western Uganda, the prevalence of cervical intraepithelial lesions is 6% (6) with cervical cancer accounting for 1/10 of all admissions on that gynecology ward of Mbarara Regional Referral Hospital (7).

Persistent infection with hrHPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) as defined by the International Agency for Research on Cancer, has been proven to be a major factor for the development of cervical lesions and eventual progression to cervical cancer (8, 9). Over the years, significant ethno-geographic diversity has been found in Sub-Saharan African countries and regions with regard to HPV genotypes. Several hrHPV genotypes, including HPV-

16, 18, 35, and 52, are found in Africa and have been implicated in the ongoing increase in cervical cancer rates (10).

With the World Health Organisation (WHO) and the Ugandan Ministry of Health advocating for HPV-based interventions for cervical cancer elimination, including primary HPV testing and HPV vaccination (11-13), it is prudent to monitor the local landscape of HPV genotype changes over time. Therefore, understanding the prevalence, trends, and distribution of hrHPV genotypes is crucial for optimizing and adapting targeted interventions, including vaccination, screening, and clinical management. This study aimed to investigate and describe the prevalence, distribution and trends of hrHPV genotypes at a tertiary health facility in South western Uganda.

MATERIALS AND METHODS

Study Site

The study was conducted at Mbarara Regional Referral Hospital (MRRH), a tertiary referral facility located in south western Uganda. MRRH serves a catchment population of approximately 6.3 million people across more than ten districts and also receives patients from neighboring countries, including Rwanda, Burundi, Tanzania, and the Eastern Democratic Republic of the Congo (14). The hospital provides comprehensive cancer care services and functions as the teaching hospital for Mbarara University of Science and Technology Medical School. The study specifically involved the main hospital laboratory, the cervical cancer clinic, and the gynecology ward. HPV testing is performed in the main laboratory, while patients with suspicious cervical lesions are assessed at the cervical cancer clinic and managed at the gynecology ward for clinical staging and treatment. Mbarara Regional Referral Hospital offers specialized services and runs dedicated clinics for various medical conditions, including cervical cancer. Among these is the cervical cancer clinic, which provides screening services to an average of 20 women per day and operates 5 days a week. This clinic is the only cervical cancer clinic in south western Uganda and is staffed by a gynecologic oncologist, medical residents, nurses, and midwives. HPV testing is performed at the main hospital laboratory, which is an accredited diagnostic facility using the Cepheid Xpert HPV assay. This assay is a real time Polymerase Chain Reaction (PCR) platform and detects the E6/E7 region of the viral DNA genome from hrHPV genotypes. This assay tests HPV 16 separately, then detects a combination of HPV 18/45, and then it groups eleven (11)

hrHPV genotypes under the ‘other hrHPV’ category as described previously (15). Confirmatory tests for cervical intraepithelial lesions, including Pap smears and histological examinations, are conducted in the hospital and university pathology department. HPV testing is only performed at the time of screening while follow up of patients after treatment is done with other tests including VIA and pap smear cytology. The gynecology ward has a capacity of approximately 40 patients and is staffed by six gynecologists, a gynecologic oncologist, intern physicians, nurses, and residents. Patients presenting to the cervical cancer clinic with suspicious cervical lesions undergo cervical biopsy, and tissue samples are submitted to the pathology laboratory for histological examination. Following a confirmed diagnosis of cervical cancer, patients are admitted to the gynecology ward for clinical staging. Patients diagnosed with early-stage disease (stage IIA and below) undergo surgical management at MRRH, while those with advanced-stage disease are referred to the Uganda Cancer Institute in Kampala for chemotherapy and radiotherapy.

Study Design and Population

This was a retrospective laboratory-based study analyzing HPV genotyping results collected between January 2020 and July 2025. The study population consisted of women who underwent cervical cancer screening and HPV testing at MRRH during the study period. Only records with complete demographic information and HPV genotype results were included in the analysis.

Sample Size Determination

All eligible laboratory records within the study period were included, yielding a census of data for analysis. No formal sample size calculation was required since the study aimed to analyze all available records that met the inclusion criteria.

Study Selection Criteria

Records were included if they belonged to women aged 18 years and above who underwent HPV testing at MRRH between January 2020 and July 2025 and contained complete demographic and genotype information. Records with missing or incomplete HPV genotype results, duplicate entries, or those outside the defined study period were excluded from the analysis.

Sampling Procedure

A census approach was employed in which all eligible laboratory records within the study period were retrieved and analyzed.

Data Collection

HPV genotyping results and relevant demographic information were systematically extracted from the main laboratory databases, laboratory registers, cervical cancer clinic records, and gynecology ward files using a structured Microsoft Excel data extraction sheet. Extracted variables included patient age, HPV genotype, and year of testing. The data collection process focused on capturing trends and distribution patterns of HPV genotypes across the study period and across the different demographics. We followed the Xpert HPV assay reporting format and categorized HPV genotypes as HPV 16 for those that had a positive result for HPV 16 only; HPV 18/45 for those that had a positive result for HPV 18/45 only; and “other hrHPV” for those that had a positive result for other hrHPV only. For any result that showed positivity with more than one HPV genotype from any of the three other groups, we categorized it as “mixed HPV genotypes”.

Methods of Data Analysis

Data were entered into Microsoft Excel version 17 and exported to STATA version 17 for analysis. Descriptive statistics were used to summarize the prevalence and distribution of high-risk HPV genotypes across the years and patient demographics. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using means and standard deviations for normally distributed data. Trends in genotype prevalence over time were assessed descriptively and visualized using bar graphs, pie charts and line graphs. Statistical significance was capped at $p \leq 0.05$.

RESULTS

Participant demographics

We retrieved HPV test results of 4,852 women whose mean age was 37.3 ± 9.2 years. Most were aged 30–39 years (38.0%, 1,846/4,852) and 40–49 years (33.8%, 1,639/4,852). Participants were predominantly residents of Mbarara district (74.0%, 3,589/4,852), followed by Ntungamo (15.6%, 759/4,852) and Ibanda (4.4%, 213/4,852). More than half (59.6%, 2,890/4,852) of women were HIV-negative, while 40.4% (1,962/4,852) were HIV-positive. There was a gradual increase in the number of HPV tests from 5.1% (247/4,852) in 2020 to 28.6% (1,390/4,852) in 2024. (Table 1)

Table 1: Demographic characteristics of participants from retrieved records across six years.

Variable	Category	Total N=4,852 f (%)
Age (years)		37.3 (+/-9.2)
Age group	18 to 19	18 (0.4%)
	20 to 29	1,010 (20.8%)
	30 to 39	1,846 (38.0%)
	40 to 49	1,639 (33.8%)
	50 to 59	249 (5.1%)
	60 to 90	90 (1.9%)
District of residence	Mbarara	3589 (74.0%)
	Ntungamo	759 (15.6%)
	Ibanda	213 (4.4%)
	Kabale	112 (2.3%)
	Others	179 (3.7%)
HIV status	Negative	2,890 (59.6%)
	Positive	1,962 (40.4%)
Calendar year	2020	247 (5.1%)
	2021	770 (15.9%)
	2022	1,227 (25.3%)
	2023	862 (17.8%)
	2024	1,390 (28.6%)
	2025	356 (7.3%)

Age (years) was presented a continuous variable with mean (standard deviation)

Over all HPV test positivity

Out of 4852 HPV tests results retrieved, 1379 were positive for hrHPV genotypes. This gave an overall prevalence of hrHPV infection of 28.42%. (Figure 1)

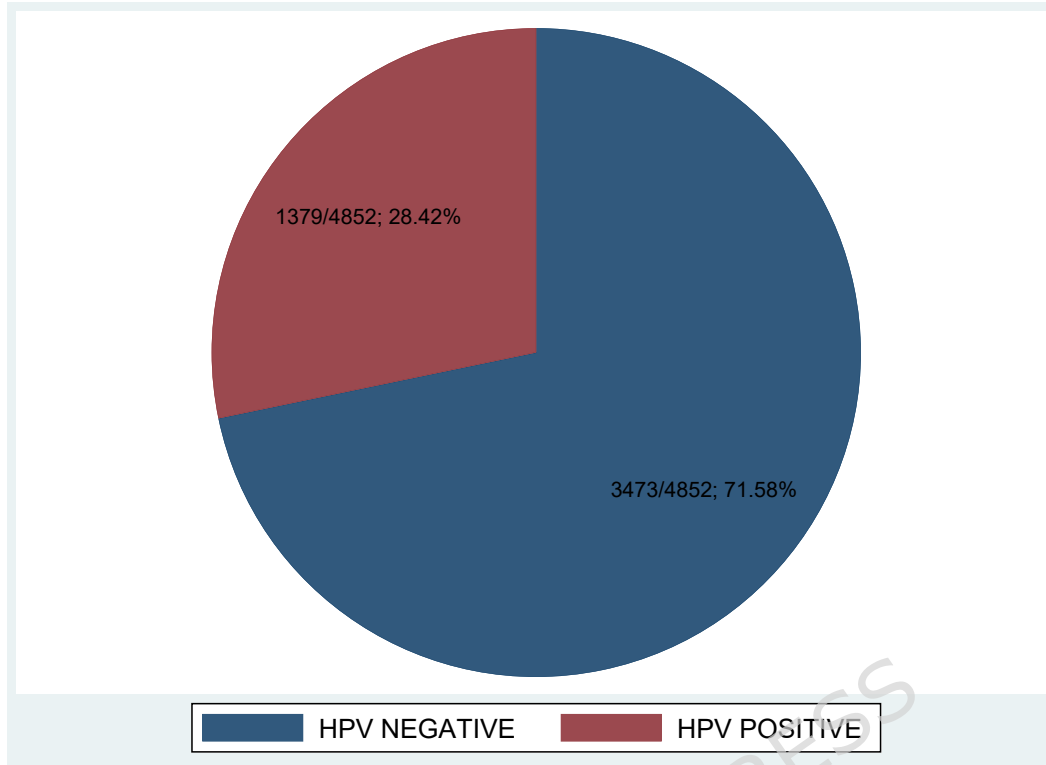


Figure 1: Over all prevalence of HPV infection

Trends of hrHPV prevalence

Across the six-year period (2020–2025), positivity with hrHPV genotypes showed an overall increase. In 2020, the prevalence of hrHPV was 31.2% (77/247), increasing slightly to 32.9% (254/770) in 2021. The prevalence then declined to 27.1% (333/1227) in 2022, followed by another rise to 32.9% (824/862) in 2023. A marked decrease was observed in 2024 (24.9%, 346/1390) and 2025 (23.9%, 85/356) (Figure 2).

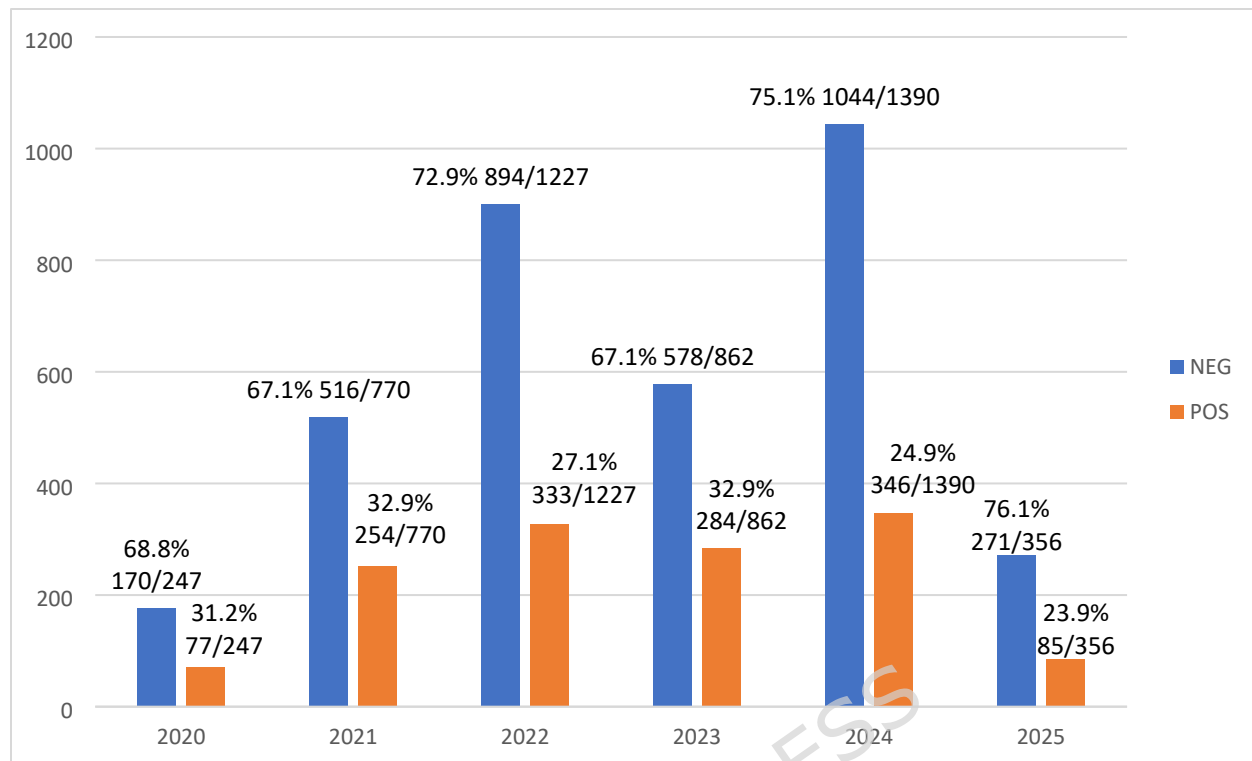


Figure 2: Trends of HPV prevalence by year

Prevalence of hrHPV by age group

Across age groups, the prevalence of hrHPV was inversely proportional to age. Positivity with hrHPV was highest in individuals aged 18 to 19 years with a prevalence of 66.7%, followed by 20-29 years with a prevalence of 37.3%. A much lower prevalence of hrHPV was observed in the age groups of 30 to 39 years (27.7%), 40 to 49 years (25.1%), 50 to 59 years (17.7%), and 60 to 90 years (24.4%). (Table 2)

Table 2: Prevalence of hrHPV across age groups.

		Age group						Test	p-value	z
		<19	20 to 29	30 to 39	40 to 49	50 to 59	60 to 90			
		N=18	N=1,010	N=1,846	N=1,639	N=249	N=90			
		n(%)	n(%)	n(%)	n(%)	n(%)	n(%)			
HPV Test Result	Negative	6 (33.3%)	633 (62.7%)	1,334 (72.3%)	1,227 (74.9%)	205 (82.3%)	68 (75.6%)	Cochran–Armitage test for trend	<0.001	−7.64
	Positive	12 (66.7%)	377 (37.3%)	512 (27.7%)	412 (25.1%)	44 (17.7%)	22 (24.4%)			

Prevalence of HPV by genotype groups

Among the 1379 HPV positive tests, more than half (65.12%, 898/1379) were “other hrHPV” genotype group, while 11.49% (158/1379) were HPV16. HPV18/45 group accounted for only

9.62% (136/1379) of all positive HPV test results. Mixed infections accounted for 13.56% (187/1379) of all HPV positive results. (Figure 3)

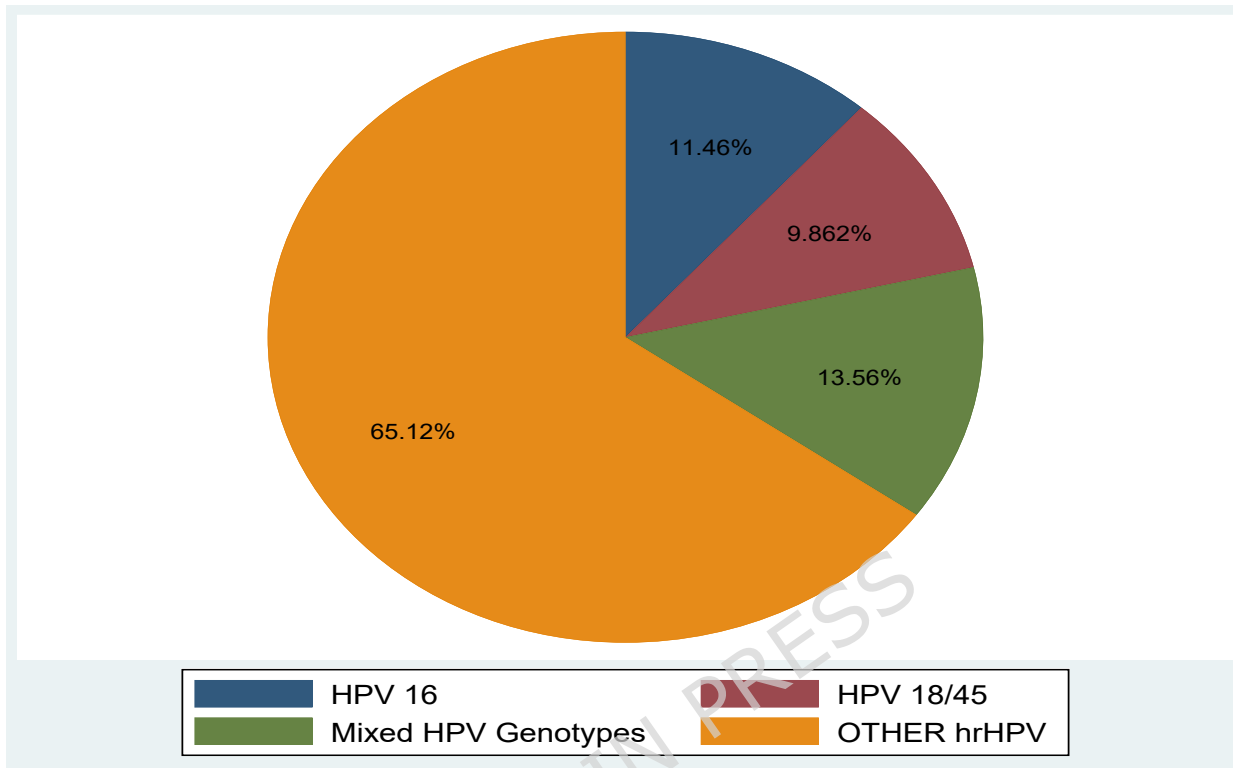


Figure 3: Prevalence of HPV by genotype groups

Trends in proportion of hrHPV genotype groups by year

Across the six-year period from 2020 to 2025, the genotype category of “other hrHPV” showed a consistently high prevalence of 61.04% (47/77) in 2020 all through to 63.53% (54/85) in 2025 compared to other genotype group results. HPV16 test result showed a gradually reducing prevalence from 15.58% (12/77) in 2020 to 8.24% (7/85) in 2025. However, mixed HPV genotypes gradually increased from 11.69% (9/77) in 2020 to 21.18% (18/85) in 2025(Figure 4)

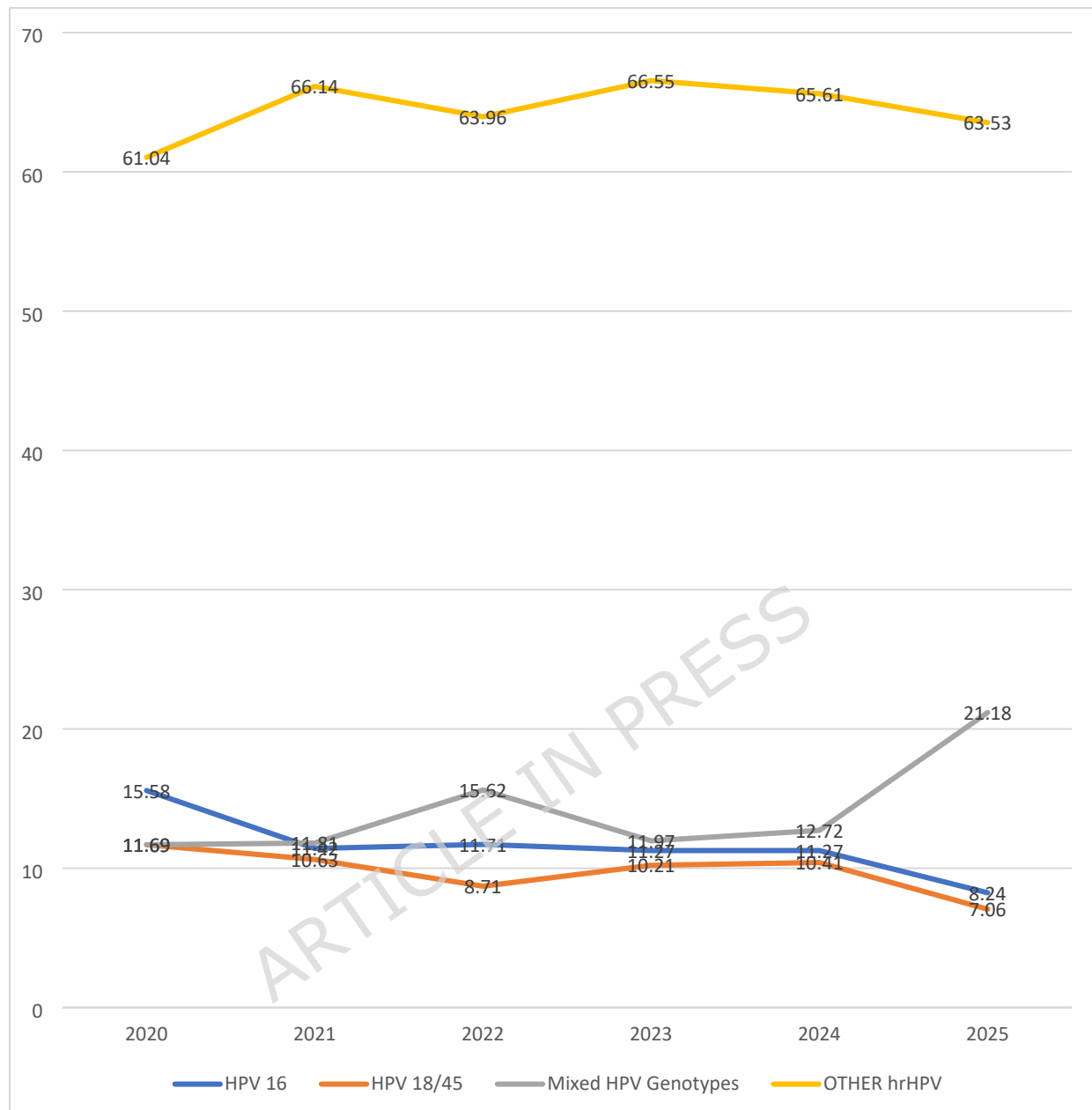


Figure 4: Trends proportion of hrHPV genotype groups across years

Trends and distribution of HPV genotype groups in HIV Negative participants across years

There was an overall increase in the number of HIV negative women being tested for HPV from 176/247, 492/770, 635/1227, 564/862, 572/1390 and 271/356 in 2020, 2021, 2022, 2023, 2024 and 2025 respectively. Within the HIV negative population, HPV16 test result was the most prevalent of all genotypes and showed an increasing trend from 50% (3/6) in 2020 to 100% (2/2) 2021. All HPV infections recorded in 2022 (2/2, 100%) were as a results on mixed HPV genotypes.

However, there were no HPV infections recorded among HIV-negative individuals in 2023-2025. (Figure 5)

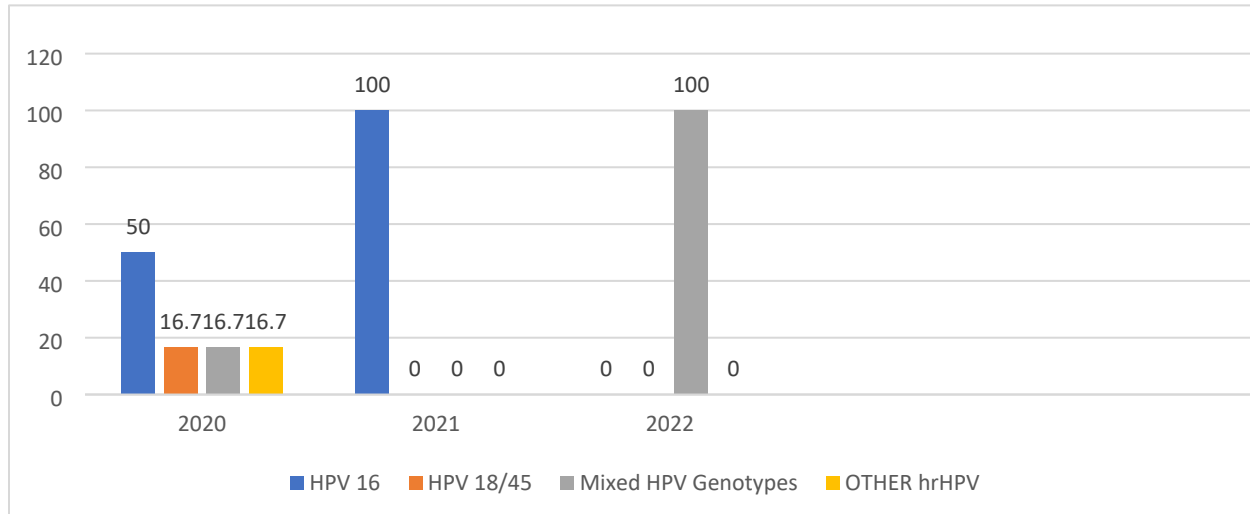


Figure 5: Trends of HPV genotype groups in HIV Negative participants across years

Prevalence of HPV across HIV status

Out of the 4852 results retrieved for this study, 2890 were from HIV negative participants while 1962 were from HIV positive participants. Of all the HIV positive participants, 69.8% (1369/1962) were positive for HPV. Also, of all the HIV negative participants, only 0.3% (10/2890) were HPV positive. Table 3

Table 3: Prevalence of HPV across HIV status

Overall HPV test result	HIV Negative N=2,890	HIV Positive N=1,962	Test	p-value
Negative	2,880 (99.7%)	593 (30.2%)	Chi-square	<0.001
Positive	10 (0.3%)	1,369 (69.8%)		

Distribution of HPV genotype groups across HIV status

We observed that “other hrHPV” genotypes result was the most prevalent (65.5%, 897/1962) among the HIV positive population, followed by mixed HPV genotypes (13.4%, 184/1962). However, in the HIV negative population, HPV 16 genotype result was the most prevalent (50%, 5/10) as shown in table 4.

Table 4: Distribution of HPV genotype groups across HIV status

HPV genotype group result	HIV Negative n=10	HIV positive n=1962	Test	P value
HPV 16	5 (50.0%)	153 (11.2%)	Fisher's exact	<0.001

HPV 18/45	1 (10.0%)	135 (9.9%)
Mixed HPV Genotypes	3 (30.0%)	184 (13.4%)
Other hrHPV	1 (10.0%)	897 (65.5%)

Trends and distribution of HPV genotype groups in HIV Positive participants across years

There was an equally increasing number of HIV positive women being tested for HPV, with numbers peaking in 2024 (638/1390). Within this group, the “other hrHPV” genotype group remained consistently high from 64.79% (46/71) in 2020 to 66.55% (189/284) in 2023, then slightly reduced to 63.53% (54/85) in 2025. Also, mixed HPV genotype infections showed a steady increase from 11.27% (8/71) in 2020, 11.91% (30/252) in 2021, 15.11% (50/331) in 2022 to 21.18% (18/85) in 2025. (Figure 6)

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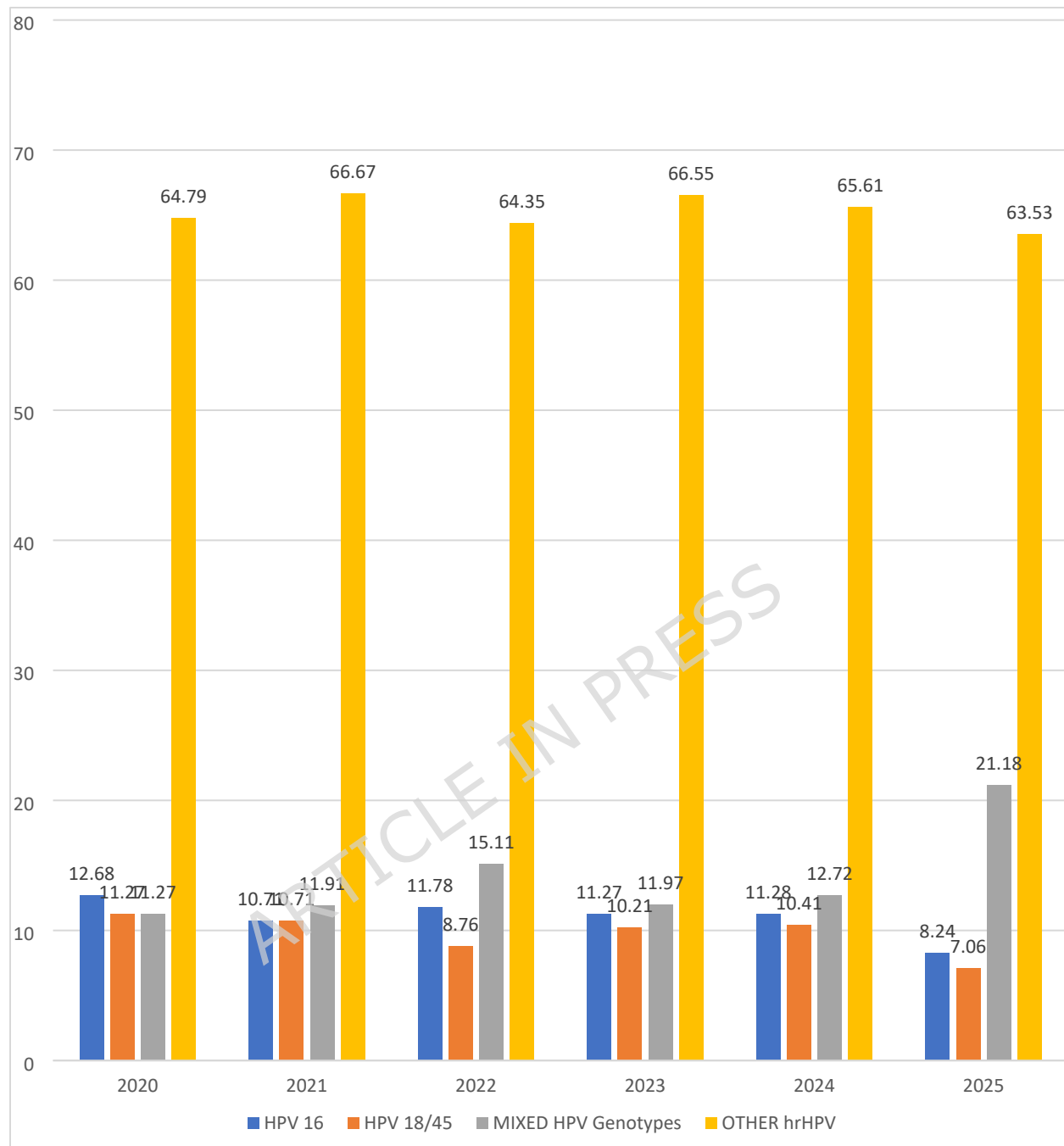


Figure 6: Trends of HPV genotype groups in HIV Positive participants across years

Distribution of HPV genotype groups by age group

We observed a gradually reducing prevalence of “other hrHPV” genotype group with increasing age. For instance, the “other hrHPV” genotype group accounted for 83.33% (10/12), 65.78% (248/377), 66.79% (342/512), 63.35% (261/412), 56.82% (25/44) and 54.55% (12/22) among participants in <19, 20–29, 30–39, 40–49, 50–59, and 60–90-year age groups, respectively. On the

other hand, HPV16 test result showed a gradual increase with increasing age from 0% among participants aged <19 years to 9.81% (37/377), 9.77% (50/512), 13.84% (57/412), 22.73% (10/44) and 18.18% (4/22) among participants aged 20-29, 30-39, 40-49, 50-59 and 60-90 years respectively. Also, mixed HPV genotypes result showed a relatively similar but high proportion across age groups. (Figure 7)

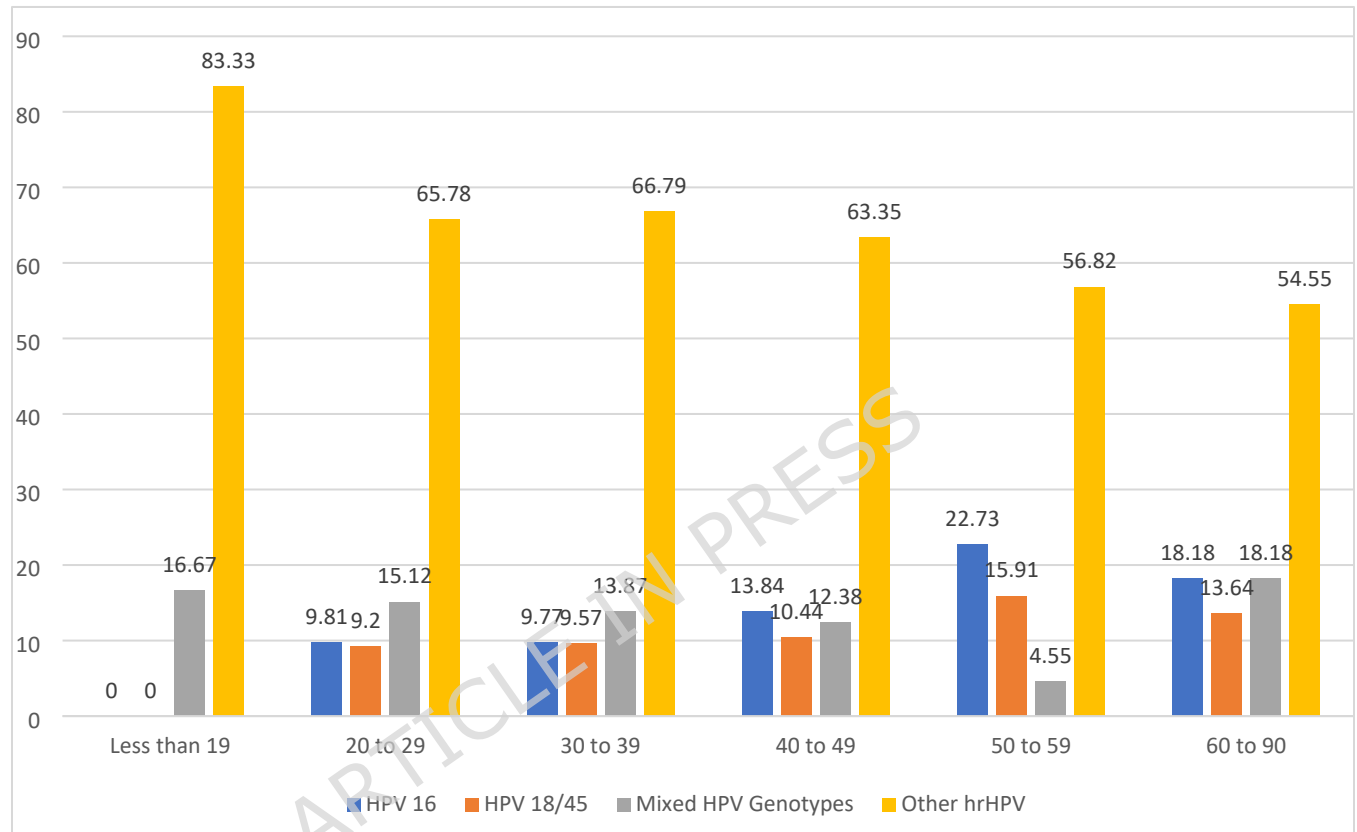


Figure 7: Distribution of HPV genotype groups by age group

DISCUSSION

In this retrospective review of records, we aimed to describe the prevalence, distribution and six year trends of hrHPV genotypes at Mbarara Regional Referral Hospital, south western Uganda. We observed an overall prevalence of hrHPV of 28.42%. We present an increased prevalence of hrHPV genotypes among the young population. The prevalence also shows a steadily decreasing trend with increasing age. Studies from other parts of the world equally reported increased prevalence of hrHPV genotypes (16-19), especially among women aged below 25 years of age (20), which most likely represents the average age of sexual debut (21, 22). In Africa, there have been reports showing increased prevalence of hrHPV in women who are younger than 20 years (23). In South Africa and Tanzania, studies have reported higher prevalence estimates (66.7% and

32.5% respectively) among women aged 16 to 22 years (24, 25). This observation could be explained by the fact that young girls tend to have higher rates of new sexual relationships, increased biological susceptibility of their cervical epithelium and engage in sexual intercourse at an early age, all of which expose them to hrHPV infections (8, 21, 26-29) and persistence (24, 30, 31). The observation of hrHPV prevalence being inversely proportional to age has also been described previously (18, 32-34) and is most likely as a result of a reduction or total absence of risk factors like number of sexual partners as well as general sexual activity which are observed as women advance into menopause (35, 36). In the long run, these reduce chances of HPV infection. Close to half of the HPV results included in this study were from HIV positive women. This reflects the current WHO's recommendation of HPV testing as the primary cervical cancer screening method (13). As a result, the Ugandan ministry of health is implementing primary cervical cancer screening with HPV tests, especially for HIV positive women (12). Also, the AIDS Control Program in the Uganda Ministry of Health (11) has expanded cervical cancer prevention services for women living with HIV. This has not only increased the number of HIV clinics offering cervical cancer screening but it has also raised the number of such women taking up cervical cancer screening in Uganda (37). However, we observe that there are years when HPV tests were unexpectedly few, especially in 2020 and 2025. This could be explained by COVID-19 movement restrictions in 2020 as well as partial data obtained for the year 2025.

There were notable fluctuations in HPV genotype prevalence especially the decline in HPV 16 and 18 not only across the years but also among women in younger age groups. This could be as a result of increasing advocacy and eventual uptake of HPV vaccination in Uganda. Uganda introduced HPV vaccination in 2015 through pilot demonstration projects using the quadrivalent vaccine (Gardasil) and subsequently integrated HPV vaccination into the national immunization program for girls aged 9–14 years(38). The Gardasil vaccine offers protection against four HPV genotypes including 6, 11, 16, and 18, and with the current improvements in HPV vaccination uptake (73.8%)(39) and completion rates (43.3%)(40), this might have influenced both the fluctuations and the reducing prevalence of specific HPV genotypes (especially HPV16 and 18) in younger women and also across the years.

We also observed no HPV infections recorded among HIV-negative individuals in 2023-2025 despite the increasing attendance. This may reflect the lower susceptibility to HPV infection

among HIV-negative persons compared with those living with HIV, potentially due to preserved immune function and more effective viral clearance. However, the absence of detected HPV infections should be interpreted cautiously, as it may also be influenced by several factors such as a generally low underlying HPV prevalence in this subgroup.

We also report a consistently increased prevalence of hrHPV genotypes categorized as “other hrHPV” according to the Cepheid Xpert HPV assay. This category excludes HPV 16, HPV 18 and HPV 45, though they too are relatively increased. This corroborates well with our earlier observation though in an HIV population (15). We also observe that HPV 16, 18 and 45 follow “Other hrHPV” in prevalence and are almost present in all genotype combinations, and their presence cannot be ignored and they have as well been implicated as such from previous studies (16, 41-44) which signifies their long known footprint in cervical carcinogenesis.

A major strength of this study is the large number of HPV results included in the analysis. Therefore, the provided prevalence, distribution and trends of hrHPV genotypes could reflect a real life picture and are easily extrapolatable to other populations. However, this study’s major limitation is the inability to provide the prevalence, distribution and trends for individual hrHPV genotypes not categories. Also, the age category of <19 had very few observations, and this could have impacted the exact prevalence and genotype distribution in this category. In the current presentation, it is impossible to ascertain the contribution of each of the individual genotypes. Also, this being a retrospective review of records, we do not present data on several participant demographics and clinical characteristics. Therefore, we cannot make any inferences on hrHPV prevalence, distribution and trends in regards to possible participant demographics and clinical characteristics. We take note that this study was purely descriptive, did not aim at testing any hypothesis. We only demonstrated existence of any patterns of associations and we did not use statistical methods that show trends for example in calendar years, age groups etc.

Conclusion

There is an elevated prevalence of hrHPV, especially those HPV genotypes categorized as “Other hrHPV,” and this prevalence is inversely proportional to age. Considering the steadily increasing number of vaccinated women and the rapidly changing screening recommendations moving towards primary hrHPV testing, we note that the “other hrHPV” genotype category needs more specific focus. Therefore, this highlights the need to implement and integrate extended hrHPV

genotyping into routine cervical cancer screening programs to better address patient needs. The same logic extends to other medical centers within Uganda and beyond. The current observation of diversity of HPV genotypes may also warrant a closer look at other interventions including HPV vaccination with a focus on relevant hrHPV genotypes. We recommend prospective studies to further characterize the prevalence, distribution, and trends of individual hrHPV genotypes.

Abbreviations

HPV Human Papilloma Virus

hrHPV High- Risk Human Papilloma Virus

HIV Human Immunodeficiency Virus

WHO World Health Organisation

MRRH Mbarara Regional Referral Hospital

STATA Statistical Software for Data Science

DECLARATIONS

Acknowledgement

We thank the management and staff of Mbarara Regional Referral Hospital cervical cancer clinic and the diagnostic laboratory for their assistance in all procedures during data collection during this study.

Availability of data and materials

All data from which this paper was prepared is available from the corresponding author, only upon reasonable request.

Ethical considerations

This study was reviewed and approved by The Mbarara University of Science and Technology Research Ethics Committee (MUST-2025-334). However, the need for informed consent was waived due to the nature of the study. Never the less, we sought and obtained administrative clearance from the director Mbarara Regional Referral Hospital in order to access the medical records. Also, all methods involved in this study were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Competing interests

All authors declare no conflicting interests.

Author contribution

Data curation, Frank Ssedyabane, Hope Mudondo and Elly B Atuhumuza; **Formal analysis**, Frank Ssedyabane, Hope Mudondo and Elly B Atuhumuza; **Funding acquisition**, Frank Ssedyabane, Joseph Ngonzi, Cesar M Castro, Hakho Lee, Thomas C Randall, Deusdedit Tusubira and Geoffrey Mwesigwa; **Methodology**, Frank Ssedyabane, Joseph Ngonzi, Cesar M Castro, Hakho Lee, Thomas C Randall, Deusdedit Tusubira, Joy Muhumuza, Alexcer Namuli and Geoffrey Mwesigwa; **Project administration**, Frank Ssedyabane, Joseph Ngonzi, Cesar M Castro, Hakho Lee, Thomas C Randall, Deusdedit Tusubira, Joy Muhumuza, and Alexcer Namuli; **Resources**, Frank Ssedyabane, Joseph Ngonzi, Cesar M Castro, Hakho Lee, Thomas C Randall, Deusdedit Tusubira, Joy Muhumuza, Alexcer Namuli and Geoffrey Mwesigwa; **Supervision**, Frank Ssedyabane, Joseph Ngonzi, Cesar M Castro, Hakho Lee, Thomas C Randall, Deusdedit Tusubira, Joy Muhumuza, and Alexcer Namuli; **Writing original draft**, Frank Ssedyabane; **Review & editing**, Frank Ssedyabane, Joseph Ngonzi, Cesar M Castro, Hakho Lee, Thomas C Randall, Deusdedit Tusubira, Joy Muhumuza, Alexcer Namuli, Elly B Atuhumuza and Geoffrey Mwesigwa. All authors are accountable for all aspects of this manuscript.

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