HPV infection patterns and viral load distribution: implication on cervical cancer prevention in Western Kenya

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Human papillomavirus (HPV) coinfection remains common globally. However, its clinical significance compared to mono-infection remains controversial. Further, the epidemiology of HPV genotype combination in coinfection is not well studied in Kenya. . Between June and August 2023, a cross-sectional facility-based survey enrolled 434 women aged 16–68 years using purposive sampling strategy. Structured questionnaire was obtained from each woman regarding demographic and sexual behavior characteristics. Cervical specimen was collected from each participant and analyzed using RIATOL assay to determine HPV genotypes and viral load. Overall, HPV 52 was the most frequently detected HPV strain. The mean HPV viral load was elevated among coinfected women than those with mono-infection but there was no evidence to support differences in viral load in the two groups ($P = 0.113$). Mono-infection was common (58.52%). HPV 16 was noted to have a near equal presence both in mono-infection and coinfection (52.17% and 47. 83%), respectively. HPV 33 (alpha 9) and 45 (alpha 7) had the greatest preference for each other compared to all other HPV interactions. HPV 52 is the most prevalent HPV in the population supporting the need for the nonavalent HPV vaccine. Mono-infection with HPV 16 remains common corroborating the relevance of bivalent vaccine in resource limited setting where nonavalent vaccines may be unavailable. The frequent coinfection preference of HPV 33 and 45 (alpha 9 and alpha 7, respectively) pauses the need for further concurrent characterization. HPV vaccination and education on safe sexual behaviors is key in reducing HPV coinfection. *European Journal of Cancer Prevention* XXX: XXXX–XXXX Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc.

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Background

Human papillomaviruses (HPVs) are double-stranded DNA viruses considered core agents of epithelial tissue with high clinical relevance because of its link to cervical intraepithelial neoplasia and cancers (Schiffman *et al*., 1993; zur Hausen, 2002; Ng'andwe *et al*., 2007). There are more than 200 distinct HPV genotypes that have been identified of which 40 occur in the anogenital region (Doorbar *et al*., 2015). HPV genotypes can be classified based on its carcinogenic potential into high-risk HPV (hrHPV, such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) while HPV types 26, 53, 66, 67, 68, 69, 70, 73, and 82 are classified as possible carcinogenic and low-risk HPV (lrHPV, such as HPV 6, 11, 40, 42, 43, and 44), according to their associations with cervical cancer (IARC, 2012). Further, the phylogenetic clade of HPV has been known to be indicative of viral persistence and potential risk of cervical carcinogenesis (Oliveira *et al*., 2008). For instance, HPV 16 which is highly oncogenic belongs to (alpha 9) while HPV 18 belongs to (alpha 7 species). The lrHPV 6 and 11 both belong to (alpha 10 species) (Muñoz *et al*., 2003). It is well known that coinfection with multiple genotypes is common among HPV positive individuals (Chaturvedi *et al*., 2011). Additionally, it has been noted that because anogenital HPV infections are transmitted through sexual activity, people who possess one particular genotype are more likely to carry additional genotypes (Vaccarella *et al*., 2011). It is yet unknown how prevalent a particular combination genotype for coinfection is. Additional, HPV coinfections have gained increasing attention owing to the successful development of prophylactic vaccination. Currently, three types of vaccine are widely licensed and adopted in about 160 countries. They include bivalent vaccine (targeting HPV 16/18), the quadruple vaccine (targeting HPV 16, 18, 6, and 11), and the nonavalent vaccine (targeting 6, 11, 16, 18, 31, 33, 45, 52, and 58).

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In 2019, Kenya joined the list of 115 countries that already began the HPV vaccine program covering type-specific HPV genotypes (Karanja-Chege, 2022). However, the molecular and epidemiologic impact of HPV coinfections is not well known. There have been theoretical arguments on the benefits of removal of certain types of HPV genotypes by vaccination. It is argued that such an activity on the genotypes would result into positive selection pressure on untargeted genotypes thus increasing their prevalence and on the other hand, there is the possibility that the type specific vaccination could as well confer protective immunity against phylogenetically related genotypes (Rousseau *et al*., 2001; Luckett & Feldman, 2016).

While coinfection among HPV types is common among women, their clinical significance remains uncertain. Moreover, the epidemiology of HPV genotype combination is not well studied in Kenya. There are studies that have indicated that coinfection enhances the risk of cervical cancer and presence of multiple HPV types is associated with low response and survival rate of such patients (Chaturvedi *et al*., 2011; Gallegos-Bolaños *et al*., 2017; Wang *et al*., 2018; Luo *et al*., 2023). On the contrary, other authors have found no evidence of synergy or observed viral antagonism during coinfection (Wang *et al*., 2018; Iacobone *et al*., 2019; Luo *et al*., 2023). It is therefore important to determine the epidemiology of mono-infection and coinfections of HPV with the aim of establishing appropriate prevention strategies for example by the design of new vaccines that are tailored to each population. Notably, the present population constitute women who have not been vaccinated against HPV. Further, the hypothesis that HPV genotypes prefer to coinfect with specific genotypes needs further evaluation in other populations.

This study determined the prevalence of mono-infection and coinfection, possible coinfection patterns and the most frequent HPV genotype interactions among women in Lake Victoria Basin, Kenya. Additionally, the complex interrelationship between type-specific HPV genotype viral load and, HPV infection patterns and age were evaluated.

Methodology

A cross-sectional design was utilized to explore patterns of HPV infection among women attending clinics in selected clinics of Lake Victoria Basin, Kenya. This study was conducted following the STrengthening the Reporting of OBservational studies in Epidemiology reporting guidelines for cross-sectional studies (Cuschieri, 2019). Women in this study were excluded if they were pregnant, had current abnormal bleeding or bloody discharge, hysterectomy, or history of cancer. Purposive sampling was undertaken instead of random sampling. This involved progressively enrolling women who came to the reproductive health clinic for any reason including cervical cancer screening until the numbers were attained. The inclusion criteria for the study included being female, attending reproductive health clinic, and giving consent after being explained the objectives of the study.

Sample size

The sample size was calculated to allow for a prevalence of at least 31.3% for HPV (Omire *et al*., 2020) with a confidence interval of 95% and a power of 80%. The initial calculated sample size was 362. However, women continued to be enrolled on their request until a total of 434 was reached between June and August 2023. This indicated over 100% response rate. While this figure was not anticipated, the authors gave room for reasonable enrollment as it would still improve precision and bring out any outliers.

Data collection

Structured questionnaire

After obtaining written informed consent from each participant, a structured paper questionnaire was privately administered by trained research assistants covering sociodemographic characteristics, and sexual behavior. Details of the questionnaire have been further described (Akinyi *et al*., 2024)

Biological specimen

A sterile vaginal speculum was inserted into the vagina to obtain a cervical swab for HPV testing. The specimens were taken using the multi-collect specimen collection kit (Abbott, Abbott park, Illinous, USA). The collection swab was rotated in the cervical region and then deposited into 1.2 ml of transport buffer that contains guanidine thiocyanate (for DNA stabilization), according to manufacturer instructions. The specimens were then stored at −80 °C in the Western Kenya Cancer Care and Research Center laboratory for preservation till shipment to Antwerp, Belgium for molecular analysis. The RIATOL qPCR HPV assay was used to extract DNA and genotype HPV-DNA at AML, Sonic Healthcare Benelux (Antwerp, Belgium) under ISO15189 accreditation, as previously described (Micalessi *et al*., 2011). Briefly, DNA was extracted in the automated nucleic preparation in chemargic360 (Revvity, Waltham, USA) using the DNA/RNA 360 H96 extraction kit. The extracted DNA was then assigned to master mix solutions and real-time amplification carried out on the Light Cycler 480 (Roche, Basel, Switzerland). The RIATOL qPCR HPV assay is also able to quantify the viral load per genotype using relative quantification to calibration curves. The detailed description of the procedure has been previously discussed (Akinyi *et al*., 2024).

Ethical approval

Ethical approval for the study was obtained from the Institutional Research and Ethics Committee at

Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH ISERC ISERC/JOOTRH/716/23).

Statistics and data-analysis

Data analysis was done using R version 4.3.2. Age was dichotomized into <30 years and ≥30 years. The categorization reflects the WHO 2014 guidelines on cervical cancer screening. The number of HPV infections was also dichotomized into one genotype (mono-infection) and having two or more genotypes (coinfection). The first step involved description of the distribution of HPV types observed among women where the prevalence of genotype was determined. For each HPV type, the Mann–Whitney *U* test was used to compare the HPV viral load of women <30 years and those who are ≥30 years. The total HPV viral load was calculated for each woman. Again, *t*-test was done to compare the mean HPV viral load among the mono-infected and coinfected group. To examine patterns of clustering of HPV types, the prevalence of mono-infection and coinfection was separately determined by calculating proportions from the total number of analyzed patients: HPV positive and HPV negative. The analysis of proportions was done using the χ^2-test. Finally, logistic regression was undertaken to determine factors influencing mono-infection and coinfection. Differences were considered for statistically significance for values of $P < 0.05$.

Results

Sociodemographics and sexual characteristics

Majority of the women were above the age of 30 (64%). Most women reported to have the first sexual debut between 15 and 19 years (67%). Most women reported having had multiple sex parts (78.1%) while Depo-Provera injection was the most common contraceptive used (71.4%). Results show a strong association between age and HPV infection with women who are 30 years and above being HPV positive ($P = 0.007$) (Table 1).

Table 1 Prevalence categories of age and associated sexual behaviors

	HPV negative Outcome: No = 258		HPV positive Outcome: Yes = 176		
Item	*N*	%	*N*	%	X^2, df, *P*-value
Age category					
<30	93	36	87	49	7.1812, 1,
≥30	165	64	89	51	0.007367
Age of sexual debut					
15–19 years	173	67	113	64	1.6673, 2,
Above 20 years	55	21	35	20	0.4345
Below 15 years	30	12	28	16	
Number of sex partners (more than one)					
Yes	196	76	143	81	1.4117, 1,
No	62	24	33	19	0.2348
Contraceptive type					
Condom	26	10	11	6	6.9822, 6,
Depo	65	25	54	31	0.3225
Implant	61	24	36	20	
Implant condom	0	0	1	1	
ICD	14	5	10	6	
Oral contraceptives	18	7	11	6	
Tubal ligation	3	1	0	0	

HPV, human papillomavirus; ICD, intraterine contraceptive device.

Human papillomavirus genotype distribution

The frequency table (Table 2) shows the distribution of HPV types identified from the sampled population of women. The most prevalent HPV in descending frequency were the HPV 52 (21.02%), HPV 18 (13.64%), HPV 16 (13.07%), and HPV 51/58 (12.50%) (Table 2). HPV 45 had a prevalence of 11.93% (Table 2). Further, a majority of other HPV types ranged from 5 to 12% including HPV 31, HPV 35, HPV 39, HPV 53, HPV 56, HPV 66, and HPV 67 while HP V6 and HP V68 occurred at lower frequencies of 5–6%. The least common were HPV 11, HPV 33, and HPV 59, each accounting for under 5% of infections. Among these, HPV 11 displayed the lowest occurrence. All combined, the top five genotypes HPV 52, HPV 18, HPV 16, HPV 51, and HPV 58 accounted for over 41% of all infections identified. This suggests these were the dominant circulating HPV types, while the remaining HPV strains demonstrated a more even but lower prevalence overall with HPV 11 being the rarest.

Type and pattern of human papillomavirus infection

Mono-infection with HPV was the most frequent among women at 58.52%. Multiple infection occurred with patients having coinfection of two or more HPV genotypes. Four patients had six different HPV genotypes.

Mono-infection was common among patients with HPV 16 (52.17%) being the most frequently occurring genotype in mono-infection (Fig. 1). HPV 66 and HPV 18 were the least occurring mono-infections at 14.29% and 25.00%, respectively. HPV genotype 51 was the hrHPV most frequent in coinfections with other HPV genotypes (86.36%). Further, among coinfected patients, HPV 16 was likely to occur alongside all HPV genotypes except HPV 11 and HPV 33 (Table 3, Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A504*, and Supplementary Table 2, Supplemental digital content 2, *http://links.lww.com/EJCP/A505*).

Analysis was done to check if each genotype was preferably associated with another genotype; within the frequency of each genotype, it was possible to determine the preference for coinfection with another genotype (Table 3, Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A504*, and Supplementary Table 2, Supplemental digital content 2, *http://links.lww.com/EJCP/A505*). Among the hrHPV genotypes, HPV 16 was frequently associated with HPV 52, HPV 18 with HPV 52, HPV 31 with HPV 58, HPV 33 with HPV 45, and HPV 35 with HPV 18. The lrHPV 6 was most frequently

associated with HPV 35, while HPV 11 was frequently associated with HPV 31, 51, and 52. HPV 67, a possibly carcinogenic HPV was mainly associated with HPV 35. Finally, HPV 33 and 45 had the greatest preference for each other compared to all other HPV interactions.

Type specific human papillomavirus viral load distribution by age classification and infection type

Even though HPV 52 was the most prevalent, its viral load among women was low (median 66.9 and 79.9 copies/cell) among <30 and ≥30 years, respectively (Table 4).

Table 2 Frequency of HPV genotype distribution

HPV genotypes	Clade	Frequency	%	Cumulative frequency	%
HPV 52	Alpha 9	37	12.0	37	12.0
HPV 18	Alpha 7	24	7.8	61	19.8
HPV 16	Alpha 9	23	7.5	84	27.3
HPV 51	Alpha 5	22	7.1	106	34.4
HPV 58	Alpha 9	22	7.1	128	41.6
HPV 45	Alpha 7	21	6.8	149	48.4
HPV 35	Alpha 9	19	6.2	168	54.5
HPV 53	Alpha 6	19	6.2	187	60.7
HPV 67	Alpha 9	18	5.8	205	66.6
HPV 31	Alpha 9	16	5.2	221	71.8
HPV 56	Alpha 6	16	5.2	237	76.9
HPV 39	Alpha 7	16	5.2	253	82.1
HPV 66	Alpha 6	14	4.5	267	86.7
HPV 59	Alpha 7	11	3.6	278	90.3
HPV 68	Alpha 7	11	3.6	289	93.8
HPV 6	Alpha 10	9	2.9	298	96.8
HPV 33	Alpha 9	8	2.6	306	99.4
HPV 11	Alpha 10	2	0.6	308	100.0

HPV, human papillomavirus.

HPV 33 among women <30 years had the highest viral load (median 11 532 copies/cell). Overall, women whose age <30 years had an elevated HPV viral load (Fig. 1). The overall HPV viral load was 1.7 times higher among women <30 years compared to those ≥30 years (median 384 copies/cell vs. 78 copies/cell; $P = 0.017$). Among women <30 years, HPV 6, 11, 31, 33, 39, 45, 51, 56, 58, 66, and 67 were elevated. HPV 16 was elevated among women ≥30 years.

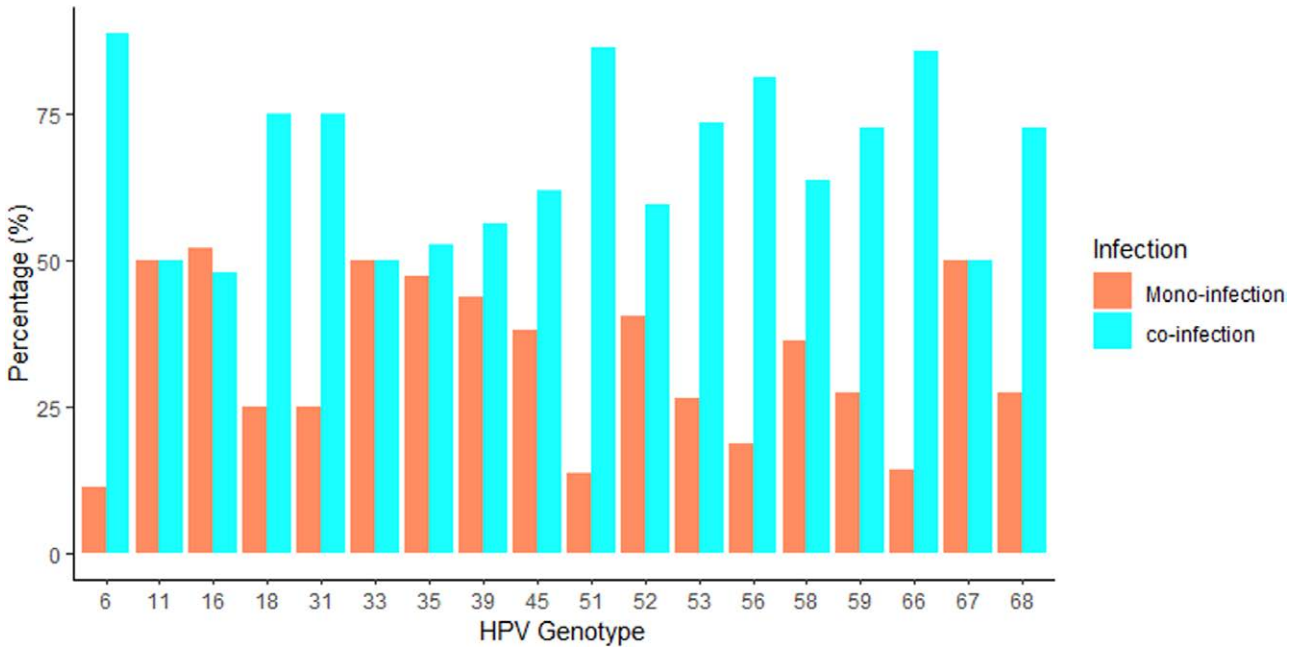
The average (mean) viral load among women in the mono-infection group was 11 782.5 copies/cell while in the coinfection group it was substantially higher at 78 276 copies/cell (Table 5). However, despite the elevated viral load in the coinfection group, there was no statistical evidence to conclude a difference in viral load distribution between the mono-infection and coinfection group ($P = 0.113$) (Figs. 2 and 3).

There are factors that influence the infection pattern of HPV in women. The logistic regression analysis on Table 5 shows the associations between factors and HPV infection patterns. In this study, women who are ≥30 years are less likely to have coinfection compared to women who are <30 years. As such, the odds of having coinfection are 0.33 times lower for women who are ≥30 years.

Discussion

The present study reported a high prevalence of HPV 52 (21.9%) with women harboring one HPV genotype

Fig. 1



Bar graph of HPV genotypes distribution pattern. The prevalence of each HPV genotype identified in mono-infection and coinfection in the tested women. Data displays percentage mono-infection and coinfection for each genotype. HPV, human papillomavirus.

(mono-infection) being most common compared to multiple infection (coinfection). HPV 52 has been reported as one of the most prevalent HPV in Kenya and other parts of Sub-Saharan Africa (Luchters *et al.*, 2010; Omire *et al.*, 2020; Sweet *et al.*, 2020; Seyoum *et al.*, 2022). The HPV genotype variation is greatly influenced by the vast ethnogeographical differences and key vulnerable populations. A past systematic review with meta-analysis conducted to study the genotype distribution of HPV in sub-Saharan African women revealed high prevalence of HPV 52 and 16 in Eastern and Southern Africa (Seyoum *et al.*, 2022). On the other hand, HPV 35 and 16 was found to be more prevalent in Western African countries.

Table 3 HPV type and its preferred coinfecting genotypes

HPV type	Preferred coinfecting HPV types
HPV 6	16, 18, 31, 35*, 39, 51, 53, 58, 59, 66, 67, 68
HPV 11	31*, 51*, 52*
HPV 16	6, 18, 31, 35, 39, 45, 51, 52*, 53, 56, 58, 59, 66, 67, 68
HPV 18	6, 16, 33, 35, 51, 52*, 53, 56, 58, 59, 66, 67, 68
HPV 31	6, 11, 16, 33, 35, 39, 45, 51, 52, 53, 56, 58*, 59, 68
HPV 33	18, 31, 35, 45*, 53, 58
HPV 35	6, 16, 18*, 31, 33, 39, 45, 53, 58, 66, 67, 68
HPV 39	6, 16, 31, 33, 35, 45*, 51, 53, 56, 59, 66, 67, 68
HPV 45	16, 31, 33*, 35, 39, 45 51, 52, 53, 56, 58, 59, 66, 67, 68
HPV 51	6, 11, 16, 18, 31, 39, 45, 52, 53, 56, 58, 59, 66*, 67, 68
HPV 52	11, 16*, 18*, 31, 45, 51, 53, 56, 58*, 59, 66, 68
HPV 53	6, 16, 18, 31, 33, 35, 39, 45, 51, 52*, 56, 58, 59, 66, 68
HPV 56	16, 18, 31, 39, 45, 51*, 52*, 53,59, 66, 68
HPV 58	6, 16, 18, 31, 33, 35, 45, 51, 52*, 53, 59
HPV 59	6, 16*, 18, 31, 39, 45, 51, 52, 53, 56, 58, 66, 67, 68
HPV 66	6, 16, 18, 35, 39, 45, 51*, 52, 53, 56, 59, 67
HPV 67	6, 16, 18, 35*, 39, 45, 51, 59, 66, 68
HPV 68	6, 16, 18, 31, 35, 39, 45 51, 52, 53*, 56, 59, 67

Asterisk (*) indicates the most frequent HPV coinfection among the genotypes (see Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A504* and Supplementary Table 2, Supplemental Digital Content 2, *http://links.lww.com/EJCP/A505*).
HPV, human papillomavirus.

The common occurrence of mono-infection in the present study illustrates the mixed pattern of HPV infection among various subpopulations. Clinically, mono-infection is often encountered in vast majority of cases compared to multiple infection (Liao *et al.*, 2020). However, these findings were not consistent with previous investigations done among female sex workers in Western Kenya (Menon *et al.*, 2017). In this study, the prevalence of coinfection was higher compared to mono-infection. The reported HPV pattern could be attributed to the sub-population studied. Female sex workers are known to engage in risky social behavior such as having multiple sex partners increasing the risk of an HPV infection when compared to other women probably accounting for the findings (Luchters *et al.*, 2010). Importantly, this study has been done among women seeking reproductive health services in facilities and without any selection bias of a subpopulation with particular sociobehavioral characteristics or behavior. In the present study, HPV 16 had a near equal presence both in mono-infection and coinfection. HPV 16 is a genotype of clinical significance owing to its high carcinogenic potential. The tendency of HPV 16 to coinfect with other HPV has been well documented (Piana *et al.*, 2011; Gallegos-Bolaños *et al.*, 2017; Jesus *et al.*, 2018; Wu *et al.*, 2019). For instances, the study in North Sardinia, Italy focusing on patterns of behavior of HPV 16 genotype revealed it had the highest capacity to coinfect with all tested genotypes suggesting a probable synergistic interaction in the carcinogenesis (Piana *et al.*, 2011). There are possible reasons for why HPV 16 coinfects more than other types such as its transmission factors, immune response, and viral properties. It is suggested that HPV 16 has viral properties that make it more likely to coinfect than others (Oyervides-Muñoz *et*

Table 4 Viral load of HPV types [$n \geq 0$ copy/cell; median (IQR)]

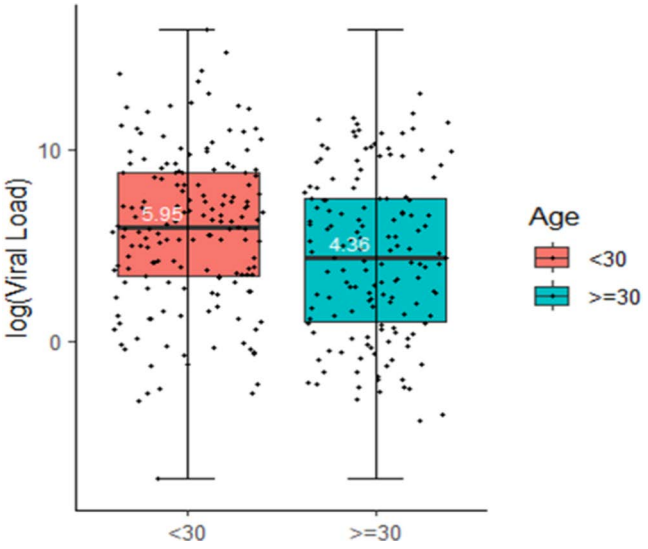
HPV type	Age < 30 *N* = 164 *n*; median (Q1–Q3)	Age ≥ 30 *N* = 146 *n*; median (Q1–Q3)	*P*-value
HPV 6	7; 3393.3 (714–8376.2)	2; 196 (98.1–293.9)	0.500
HPV 11	1; 6712.9 (6712.9–6712.9)	1; 1.5 (1.5–1.5)	1.000
HPV 16	13; 731.8 (15.7–1456)	10; 1084.1 (11.7–26 009.4)	0.923
HPV 18	12; 3.3 (0.2–2234.2)	12; 0.6 (0.2–9.9)	0.432
HPV 31	7; 7415.5 (1694.1–35 000.6)	9; 802.2 (202.6–11 667.9)	0.114
HPV 33	4; 11 532 (459.6–64 218.4)	4; 9.3 (1.8–193.9)	0.114
HPV 35	9; 338.8 (44.5–1469.5)	10; 824.4 (107.7–3830.5)	0.549
HPV 39	10; 263.9 (162.1–1075.8)	6; 9.5 (1.7–38.8)	0.142
HPV 45	11; 193.5 (22.5–311.3)	10; 50.1 (26.6–307)	0.780
HPV 51	11; 686.2 (246–108 348.3)	11; 11.5 (0.8–9991.8)	0.545
HPV 52	23; 66.9 (11.7–1426.9)	14; 79.9 (1.5–600.9)	0.724
HPV 53	8; 7.4 (3.5–120.3)	11; 55.4 (1.8–8540.4)	0.152
HPV 56	10; 13 545.9 (1173.2–58 944)	6; 783.1 (7.2–6063)	0.145
HPV 58	10; 1051.8 (579.1–4117.4)	12; 399.6 (83.5–12 675.5)	0.464
HPV 59	4; 328.5 (65.9–782.9)	7; 546.8 (57.7–41 243.2)	0.191
HPV 66	9; 526 (59.4–2062.7)	7; 27.6 (11.2–879.4)	0.174
HPV 67	7; 1142.9 (91.4–4847.6)	11; 65.2 (3.4–1219.7)	0.408
HPV 68	8; 30.9 (2.7–171.7)	3; 78 (68.3–113.2)	1.000
Overall	**164; 384.1 (29.4–6305.2)**	**146; 77.9 (2.8–1691.3)**	**0.017**

Note: *P*-value – mood's median test also known as the median test; nonzero viral load were used in the analysis.
HPV, human papillomavirus; IQR, interquartile range.

Table 5 Logistic regression on factors influencing the odds of HPV infection pattern (having coinfection vs. mono-infection)

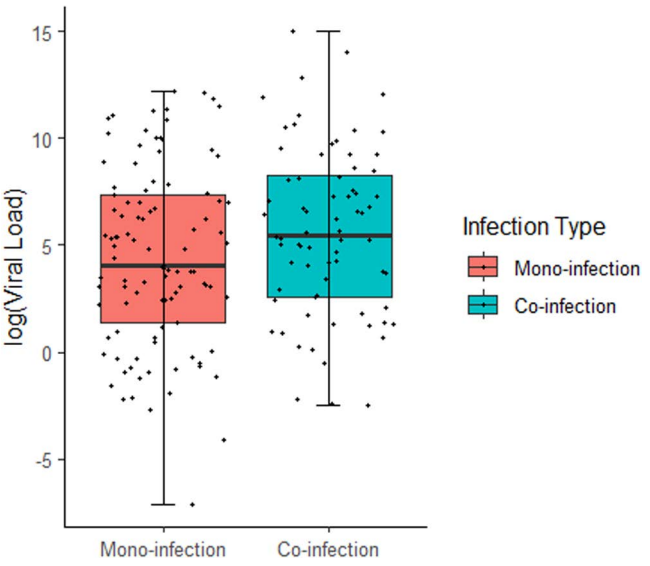
	OR	2.5% CI	97.5% CI	*P*-value
(Intercept)	1.39	0.19	9.06	0.732
age_group: ≥30	0.33	0.14	0.75	0.009
first_sex_age: Above 20 years	1.98	0.66	5.98	0.216
first_sex_age: Below 15 years	2.36	0.79	7.17	0.125
More than_one_sex_patner: Yes	0.45	0.14	1.38	0.164

Fig. 2



Boxplot showing the distribution of the HPV viral load grouped by the age ($P = 0.017$). The plot is plotted on the log-scale and the number on the plot refers to the median values. HPV, human papillomavirus.

Fig. 3



Boxplot showing the distribution of the HPV viral load by infection type ($P = 0.113$). The plot is plotted on the log-scale and the number on the plot refers to median values. HPV, human papillomavirus.

al., 2020). However, this argument is inconclusive and needs further research. Moreover, coinfection does not always mean dominance (Wu *et al*., 2019).

The average HPV viral load of each genotype present in coinfection group is elevated compared to mono-infection. However, there is no statistical evidence to account for the difference in the viral load measures in each genotype between the two groups. A higher viral load among genotypes in the coinfected groups could indicate increased viral replication of HPV in coinfections (Trottier *et al*., 2006). This could be attributed synergistic effects of HPV genotypes on each other and probable favorable vaginal environment.

In research conducted among Mexican women, multiple HPV infections was associated with higher viral load and potential persistent infection (Oyervides-Muñoz *et al*., 2020). However, there has been a controversial argument regarding the role of single infection and coinfection in cervical cancer prognosis (Y. Zhou *et al*., 2023). Particularly, it has been argued that coinfection will not enhance the incidence of cervical cancer and that the severity of cervical lesions does not depend on the number of HPV genotypes present but rather to the virulence of the HPV genotype (Li *et al*., 2021). This study compared the HPV viral loads of women in mono-infection and coinfection but did not relate them to cervical pathological grades. It is recommended to perform further research with a large sample size that focuses on HPV infection pattern and cervical lesions to provide a clearer picture on the role of mono-infection and coinfection in this population.

Further, HPV viral load was established to be elevated among women who are less than 30 years old. Moreover, coinfection is less likely among women who are 30 years and above compared to those who are below 30 years. The findings of this study contradicts the results of a study done among women who needed colposcopy evaluation at Weifang Medical University in China. In this study, increasing age correlated with a higher viral load (Lu *et al*., 2021). Similarly, another study that was done among Mexican women to determine the association between hrHPV DNA load and cervical lesion established that oldest women had the highest viral load (Hernández-Hernández *et al*., 2003). However, the observation of the current study has been explained by the fact that the effect of age on viral load is likely associated to the balance between acquisition of HPV infection and HPV clearance. Further, there is a study that was done among the Chinese population to establish the age-specified and genotype distribution of HPV multiple infection. Interestingly, the proportion of HPV coinfection among HPV positive individuals increased with age of people older than 30 years unlike in the present study (Y.-X. Zhou *et al*., 2024). Primarily, women who are below the age of 30 years are considered young and are likely

to harbor HPV due to the sexual debut before an adequate immune response could be established (Syrjänen *et al.*, 2004). It is therefore probable that the rate of new acquisition of HPV infection exceeds the clearance rate leading to a high viral load among the younger women. However, the low viral load but persistent HPV in older women in this study suggest the need for further evaluation, cytology, and clinical follow-ups as well. While HPV infections have been known to be transient especially among young women, HPV infection among older women may be persistent with decline in spontaneous clearance because of physiological and immunological changes. As a result the persistent infection are often associated with progression to precancerous and cancerous lesion (Lu *et al.*, 2021). This study therefore suggest that the older group of women are the ones highly susceptible to develop cervical cancer hence need for effective intervention. In addition, due to the time it takes to induce cellular changes, long persistence leads to more cellular changes and hence dysplasia and malignancy.

The interaction among multiple HPVs has potential oncogenic risks that still remain unclear. The data revealed the most common HPV coinfection pairing was HPV 33/45. HPV 33 is member of the alpha 9 group while HPV 45 belongs to the alpha 7 group. This corroborates the controversial pathway of phylogenetic clustering of HPV. A study was conducted among patients seeking pathology services in Brazil to establish the pattern of genotype distribution in multiple HPV infection. Results showed coinfections with oncogenic types from different clades were significant for high-risk clades of alpha 7 and alpha 9 revealing that HPVs of the same phylogenetic clade may not always cluster together (Oliveira *et al.*, 2008). Further, findings from a study done among female sex workers in Western Kenya did not support phylogenetic clustering (Menon *et al.*, 2017). In the study, the most frequently observed pairings were HPV 18/31 (alpha 7 and alpha 9) among HIV negatives and HPV 31/52 (alpha 9) among HIV positives. Further, in a large multicenter study done across China to look at multi-infection and coinfection patterns of HPV among women HPV 16 and 31 (alpha 9) were the most frequent pairing (Liao *et al.*, 2020). The significant differences in clustering of HPV have been attributed to distinct variables such as age, number of sexual partners, transmission routes, and biological properties of each genotype (Oliveira *et al.*, 2008). The present study used cross-sectional design hence it was not possible to evaluate whether the presence of previous mono-infection was a risk factor for acquisition of subsequent HPV types, hence coinfection. There is a need to further study phylogenetic clustering as a means of predicting the likelihood of concurrent HPV infection. Risky sexual behaviors has often been noted as potential risk factors in HPV infection and cervical cancer (Huang *et al.*, 2020). The potential biological mechanism can be explained by the fact that having intercourse with more than one partner increases exposure to HPV and persistence. The findings of this study are in tandem to that of a systematic review and meta-analysis undertaken to determine whether multiple sex partner was a risk factor for cervical cancer (Liu *et al.*, 2015). In this review, it was established that having multiple sex partner was an independent risk factor for HPV infection. The finding reveals the need for safe sex education among women.

One major strength of this study was high sensitivity of HPV DNA diagnostic used that covers more than the common 14 h-HPV (Akinyi *et al.*, 2024). The HPV assay used detected up to 18 HPVs including the possibly carcinogenic types. Our sample size was fairly large. However, this study did not obtain cervical smear for cytological purposes hence it is not possible to correlate pattern of HPV infection and cytological abnormality.

Clinical implications and future perspectives

The high prevalence of HPV 52 underscores the need for a vaccine that targets the genotype in the Western Kenya setting. Currently, the bivalent and quadrivalent HPV vaccine have been rolled out in Kenya. The nonavalent vaccine that includes HPV 52 may not be available and accessible in resource-limited settings like Western Kenya pausing a need for its crucial introduction to the population.

Further, cervical cancer screening awareness should be enhanced in the general Kenyan population of women especially among older women. HPV testing is recommended as the primary screening for women above the age of 30 years. However, this has not been possible owing to resource limitation and therefore visual inspection with acetic acid/visual inspection with Lugol's iodine (VIA/VILI) has been used as the primary screening method. VIA has also been noted to have limitations of problematic sensitivity especially for older women with endocervical lesion. Comprehensive HPV testing such as the one in this study shows that viral load is necessary for both the younger and older women. Moreover, while the study found no significant difference in viral load between mono-infection and coinfection, further research is critical to explore the potential of viral load to act as a biomarker for disease progression.

The study further highlights the complexity of HPV infection due to coinfection patterns. The frequent coinfection pairing of HPV 33 and 45 which belong to alpha 9 and alpha 7 pauses the need for further characterization and possible role and influence on vaccine efficacy and phylogenetic clustering.

In perspective, this study reveals epidemiological gaps and therefore suggest the need for future longitudinal studies to track the natural history of HPV infection including the viral clearance, persistence, and progression to cervical cancer among women of different age groups.

Conclusion

This is the first study in the post COVID season to do extended HPV genotyping, pattern of infection, and HPV viral load measures in selected facilities of Lake Victoria Basin of Kenya. HPV 52 remains the most prevalent HPV. The common simultaneous occurrence of HPV 16 in mono-infection and coinfection explains persisting burden of cervical carcinogenesis in Kenya. Further, its presence in multiple infections requires that the micro epidemiology of concurrent be explained. Finally, the low HPV viral load reported among women ≥30 years suggests the need for regular cervical cancer screening, strictly following with cytology and histology when necessary.

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Conflicts of interest

There are no conflicts of interest.

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