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Genotypic characterisation of human papillomavirus infections among persons living with HIV infection; a case–control study in Kumasi, Ghana

Denis Dekugmen Yar[1], Samson Pandam Salifu[1,2], Samuel Nkansah Darko[1], Augustina Angelina Annan[1], Akosua Adumea Gyimah[3], Kwame Ohene Buabeng[4] and Ellis Owusu-Dabo[1,5]

1 *Kumasi Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
2 *Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
3 *Ghana Health Service, Kumasi South Hospital, Ashanti Region, Ghana*
4 *Department of Clinical and Social Pharmacy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
5 *School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

Abstract

OBJECTIVES The objective of this study is to describe the burden of human papillomavirus (HPV) infection among women living with HIV and non-infected women in Ghana.

METHODS A case–control study was conducted involving 107 women living with HIV aged between 18 and 59 years (cases) and 100 non-HIV-infected apparently healthy women (controls) who were recruited from the Kumasi South Hospital, from July to December, 2014. Cervicovaginal swabs were taken from study participants to characterise 28 high- and low-risk HPV genotypes using a multiplex real-time PCR.

RESULTS The overall mean age for the participants was 40.10 ± 9.76 years. The prevalence of high-risk (hr)-HPV genotypes was significantly higher among the cases than the controls (77.4% *vs.* 41.6%, $P < 0.0001$). Overall, HPV 58 and 54 were the most predominant high-risk (18.8%) and low-risk (15.0%) genotypes detected. The two most common hr-HPV genotype isolates were 58 (18.8%) and 35 (15.9%) with 58 being the most prevalent among age group 35–44 years compared with hr-HPV 16, 18, 35 and 45, found predominantly among 18–34 age group.

CONCLUSIONS Significant variations exist in HPV genotypes among HIV-infected and uninfected women.

keywords human papillomavirus (HPV), Genotypes, HIV, Ghana

Introduction

Human papillomavirus (HPV) is a major risk factor for cervical cancer worldwide [1]. Genotypes of this virus designated as high-risk include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This is based on their association with cervical intra-epithelial neoplasia (CIN) with terminal outcomes among patients with cervical cancer. Other genotypes, including 26, 53, 69, 73 and 82, have been suggested as probable high-risk group [2, 3]. Globally, several studies reported HPV 16 and 18 as the most predominant high-risk genotypes [2, 4, 5]. Although sub-Saharan Africa has the highest burden of HPV infection [6], vaccines against this infection have been developed based on studies conducted mainly among Caucasian populations with about 91–95% efficacy [3]. A recent study [7] conducted among patients diagnosed with invasive cervical cancer in sub-Saharan Africa also reported HPV genotypes 16, 18, 35, 45, 33 and 52 in descending order of prevalence. A study [8] conducted in Ghana among patients diagnosed with cervical cancer, however, reported HPV genotype 18 as being the most prevalent, followed by 16 and genotypes 35, 39, 45, 52, 56 and 66 in that order.

HIV-infected women have higher rates of persistent HPV infection, severe cervical CIN lesions and aggressive cervical cancer, than the general population [9–11]. In a prospective observational study in Europe, HIV-positive African women had 43% prevalence of hr-HPV with high HPV positivity among those younger than 30 years [12].

The odds of HPV infection in HIV-infected women are double those in non-HIV-infected women in South and East Africa with 16, 35 and 45 as the most prevalent genotypes [13–15]. In Cameroun, the predominant

hr-HPV genotypes among women living with HIV (WLWHIV) were 45 and 58; these women were also more susceptible to co-infections with other hr-HPV genotypes than healthy participants [16]. Among WLWHIV in South Africa and Tanzania, the most predominant hr-HPV genotypes are 16 and 52, suggesting regional differences [15]. Other studies from the Eastern and Southern Africa focused on HPV genotype distribution among WLWHIV have reported hr-genotypes 16, 35, 45 and 58 using reverse hybridisation [5, 17–19].

What is known about the prevalence of HPV genotypes with the highest burden among WLWHIV in Africa is largely from Eastern and southern Africa. Data on HPV genotype distribution in West Africa are limited, with few studies mainly focusing on non-HIV-infected women [7, 20, 21]. Moreover, there is no comparative study on the prevalence of HPV genotypes distribution among women with and without HIV in Ghana. This study therefore sought to determine the distribution of hr-HPV genotypes among HIV- and non-HIV-infected Ghanaian women.

Methods

Study design and setting

We conducted a case–control study at Kumasi South Hospital, the Regional Hospital in Kumasi metropolitan area, Ghana. This health facility serves as referral hospital for other health facilities within and outside Kumasi. The average monthly Outpatient Department (OPD) attendance for 2013 was about 10 603 of which about 58% of the patients were women aged 18–59 years. This Hospital runs a specialised HIV/AIDS clinic together with other chronic illnesses and antiretroviral therapy (ART) services designated as Chronic Care Unit (CCU) with more than 4200 clients. From July 2014 to December 2014, we collected cervicovaginal swabs from 107 WLWHIV aged 18–59 years with intact uterus attending the CCU as cases and 100 non-HIV-infected women as controls from the OPD. Data on CD4 counts and viral load were not collected for all 107 cases who were on ART. We obtained ethical approval from the Scientific and Ethical Review Committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST) and the Komfo Anokye Teaching Hospital, Kumasi. We further obtained signed informed consent from each participant at the time of recruitment.

Data collection

A questionnaire was administered via a personal digital assist device (PDA) to record each participant's biodata. High cervicovaginal swabs were taken from each participant for RT-PCR to determine the presence of HPV genotypes. We estimated a sample size of 100 in a 1:1 ratio for cases and controls to test 30% positive for high-risk HPV genotype using multiplex RT-PCR with a power of 80% at 95% confidence interval.

Laboratory tests

DNA extraction. High cervicovaginal swabs were taken using the Copan eNat™ Collection and Transport System (Copan, Italy), an L-shaped applicator swab with nylon fibre tip in a preservation media. Samples were immediately placed on ice packs and transported to the laboratory at Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR). The Copan eNat tubes were vortexed for 30s and DNA extraction was done using Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Fisher, USA) with modifications. Briefly, 400 µl of samples was added to 400 µl of lysis solution and 20 µl of proteinase K solution. DNA was eluted in 100 µl of elution buffer.

Multiplex RT-PCR detection of HPV genotypes: HPV DNA was determined using an Anyplex II HPV 28 Detection kit (Seegene, South Korea). The kit contains two TOCE Oligo Mixes (TOMs), 4× HPV 28 TOM A and 4× HPV 28 TOM B, for amplification and detection of 14 genotypes of HPV each. The A set contains primers targeting DNA from 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), while the primers of the B set target allows amplification and detection of five probable high-risk types (26, 53, 69, 73, 82) and nine low-risk (lr) HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70).

For each sample, 5 µl of extracted DNA was added to a mixture of 5 µl of 4× Anyplex PCR Master Mix, 5 µl of RNase-free water and 5 µl of 4× HPV 28 TOM A for the A set. A similar mixture but with TOM B was made for the B set. The two sets were run side by side for a set of samples on 8-strip PCR tubes. For each set, three positive controls and one negative control were used. In the positive controls, 5 µl of the HPV 28 PC1 (consisting of HPV genotypes: 26, 33, 42, 43, 44, 51, 56, 66 and an internal control), HPV 28 PC2 (for HPV genotypes: 35, 39, 40, 45, 54, 59, 61, 68, 69 and 82) and HPV 28 PC3 (for HPV genotypes: 6, 11, 16, 18, 31, 53, 58, 70 and 73), substituted the sample DNA, and for the negative controls 5 µl of RNase-free water.

RT-PCR was performed using the CFX96™ Real-time PCR System (Bio-Rad). The protocol for RT-PCR was programmed as described by kit manufacturer and analysed with Seegene viewer (Seegene).

Data management

Data were collected using PDAs and sent via Internet for cloud storage on a central computer and aggregated into Microsoft Excel. Subsequently, data were analysed with Stata version 12 (Stata Corp, USA). Descriptive statistics was used to summarise the distribution of various variables into tables and graphs. Genotype prevalence was presented as weighted prevalence; genotype presence was divided by group size. Differences between discrete and continuous variables were analysed using chi-square and Mann–Whitney test, respectively, with 95% confidence level and 5% margin of error.

Results

Overall mean age for the study participants was 40.10 ± 9.76; and for the case and control groups, 39.39 ± 8.08 and 40.86 ± 11.27, respectively (Table 1). Overall HPV positivity for both cases and controls was 72.0% (86.9% and 56.0% for cases and controls, respectively). The percentage weighted overall prevalence for high-risk HPV genotypes was 59.9% for both cases and controls (76.6% and 42.0% for cases and controls, respectively). The weighted overall mean prevalence for low-risk HPV genotypes for both cases and controls was 41.1% (49.5% and 32.0% for cases and controls, respectively) at 95% CI. The odds for both high- and low-risk HPV genotype infections in the case group were approximately four and two times higher, respectively, than for controls. Conversely, the majority of women without HPV were in the control group (44.0%) rather than the case group (13.1%).

Excluding the probable hr-HPV 82, all the other hr-HPV genotypes had higher percentage weighted averages in the cases than in the controls (Table 2). hr-HPV genotype 58 had the highest overall prevalence of 18.8% (26.4% for cases and 10.9% for controls; $P = 0.0075$). This was followed by hr-HPV genotype 35 (15.9%), 21.5% and 10.0% for cases and controls, respectively. The overall prevalence for hr-HPV genotypes 16 and 18 was 7.7% and 8.7% [hr-HPV 16 (13.1% for cases *vs.* 2.0% for controls, $P = 0.0032$); hr-HPV 18 (16.0% cases *vs.* 1.0% for controls, $P < 0.0001$)], respectively. The prevalence of hr-HPV genotype 68 among cases and controls was 21.5% and 3.0% ($P < 0.0001$), respectively.

As shown in Table 3, lr-HPV genotypes 6 and 44 were significantly more common in the case group than the control group [lr-HPV 6 (8.4% and 0.0%), $P = 0.0034$; lr-HPV 44 (15.9% *vs.* 5.0%), $P = 0.0128$], respectively. Among the low-risk genotypes, lr-HPV 54 had the highest overall percentage weighted prevalence (15.0%): 19.6% and 10.0% for cases and controls, respectively.

Figure 1 shows the percentage age group distribution with most predominant hr-HPV genotypes: 16, 18, 35, 45 and 58. The hr-HPV 16 was the most prevalent (20%) among the 18–24 age group and least (4%) in the 45–54 age category. The age group 25–34 had the highest frequency (16%) for the hr-HPV 18, followed by the 55–64 age group (11%). hr-HPV genotype 35 was the most prevalent (25%) among the age group 25–34 and the least (10%) prevalent in the age group 18–24. hr-HPV genotype 45 was distributed only among three age groups: 18–24 (10%), 25–34 (2%) and 35–44 (1%). hr-HPV genotype 58 was mostly distributed among the age group 35–44 (22%) with the least in the age group 18–24 (10%).

We found prevalences of 12.1% for hr-HPV 16 and 13.1% for hr-HPV 18 co-infected with other high-risk genotypes among cases (Table 4). A prevalence of 20.6% for hr-HPV 31 co-infections with other high-risk genotypes was detected among cases; this was 1.0% in controls. The three most frequent hr-HPV genotypes co-infected with other hr-HPV genotypes among cases were 58 (21.5%), 68 (20.6%) and 35 (19.6%). Co-infections of hr-HPV with lr-HPV genotypes were more common among cases than controls. A prevalence of 17.8% hr-HPV 31 co-infections with lr-HPV genotypes was detected in the case group, and hr-HPV 58 (9.0%) in controls.

Discussion

This study found that HIV-infected women are at nearly twice the risk of being infected with both high- and

Table 1 Mean ages and HPV testing of cases and controls

	Controls ($n = 100$)	Cases ($n = 107$)	Total ($N = 207$)	OR (95% CI)
Age (mean ± SD)	40.86 ± 11.27	39.39 ± 8.08	40.10 ± 9.76	
HPV-positive	56 (56.0)	93 (86.9)	149 (72.0)	5.2 (2.6–10.4)
Hr-HPV-positive	42 (42.0)	82 (76.6)	124 (59.9)	3.7 (2.0–6.9)
Lr-HPV-positive	32 (32.0)	53 (49.5)	85 (41.1%)	2.1 (1.2–3.7)
HPV-negative	44 (44.0)	14 (13.1)	58 (28.0%)	0.2 (0.1–0.4)

Hr-HPV, high-risk human papillomavirus; lr-HPV, low-risk human papillomavirus.

Table 2 Percentage weighted prevalence of Hr-HPV genotypes

Hr-HPV genotype	Controls (n = 100) (%)	Cases (n = 107) (%)	P-value	Total (N = 207) (%)
Hr-HPV 16	2 (2.0)	14 (13.1)	0.0032	16 (7.7)
Hr-HPV 18	1 (1.0)	17 (16.0)	<0.0001	18 (8.7)
Hr-HPV 26*	1 (1.0)	8 (7.5)	0.0360	9 (4.4)
Hr-HPV 31	7 (7.0)	22 (20.6)	0.0051	29 (14.0)
Hr-HPV 33	0	9 (8.4)	0.0034	9 (4.4)
Hr-HPV 35	10 (10.0)	23 (21.5)	ns	33 (15.9)
Hr-HPV 39	4 (4.0)	3 (2.8)	ns	7 (3.4)
Hr-HPV 45	0	3 (2.8)	ns	3 (1.5)
Hr-HPV 51	2 (2.0)	3 (2.8)	ns	5 (2.4)
Hr-HPV 52	5 (5.0)	15 (14.0)	0.0342	20 (9.7)
Hr-HPV 53*	5 (5.0)	18 (16.8)	0.0076	23 (11.1)
Hr-HPV 56	0	9 (8.4)	0.0034	9 (4.4)
Hr-HPV 58	11 (10.9)†	28 (26.4)†	0.0075	39 (18.8)†
Hr-HPV 59	1 (1.0)	8 (7.5)	0.0360	9 (4.4)
Hr-HPV 66	1 (1.0)	8 (7.5)	0.0355	9 (4.4)
Hr-HPV 68	3 (3.0)	23 (21.5)	<0.0001	26 (12.6)
Hr-HPV 69*	0	0		0
Hr-HPV 73*	0	15 (14.0)	<0.0001	15 (7.3)
Hr-HPV 82*	10 (10.0)	6 (5.6)	ns	16 (7.7)

Hr-HPV, high-risk human papillomavirus; ns, not significant.
*Probable high-risk.
†Highest.

Table 3 Percentage weighted prevalence of Lr-HPV genotypes

Lr-HPV genotype	Controls (n = 100) (%)	Cases (n = 107) (%)	P-value	Total (N = 207) (%)
Lr-HPV 6	0	9 (8.4)	0.0034	9 (4.3)
Lr-HPV 11	0	1 (0.9)	ns	1 (0.5)
Lr-HPV 40	1 (1.0)	3 (2.8)	ns	4 (1.9)
Lr-HPV 42	5 (5.0)	11 (10.3)	ns	16 (7.7)
Lr-HPV 43	5 (5.0)	9 (8.4)	ns	14 (6.8)
Lr-HPV 44	5 (5.0)	17 (15.9)	0.0128	22 (10.6)
Lr-HPV 54	10 (10.0)*	21 (19.6)*	ns	31 (15.0)*
Lr-HPV 61	2 (2.0)	4 (3.7)	ns	6 (2.9)
Lr-HPV 70	10 (10.0)	12 (11.2)	ns	22 (10.6)

Lr-HPV, low-risk human papillomavirus; ns, not significant.
*Highest.

low-risk HPV genotypes as non-HIV-infected women. The most prevalent hr-HPV genotypes among WLWHIV and non-infected women were 58 and 35, predominantly among the age groups of 35–44 and 25–34, respectively. These genotypes (hr-HPV 58 and 35) are mostly concomitant with other hr-HPV genotypes. Single and co-infections were observed among a broad spectrum of HPV genotypes. These co-infections also occurred between the hr-HPV and the lr-HPV genotypes and in diverse combinations. In this study, we found the most frequent co-infections with hr-HPV 58, 68 and 35. Co-infections were more associated with HIV cases than the control group. Co-infections on the widely reported hr-HPV 16 and 18 were also associated with WLWHIV. Our results further confirmed that HIV-positive women were more prone to co-infections of HPV genotypes than HIV-negative women [7, 8].

In a Ghanaian study, Brandful *et al.* [22] reported co-infections of HPV genotypes among healthy pregnant women with hr- and lr-HPV genotypes occurring alone,

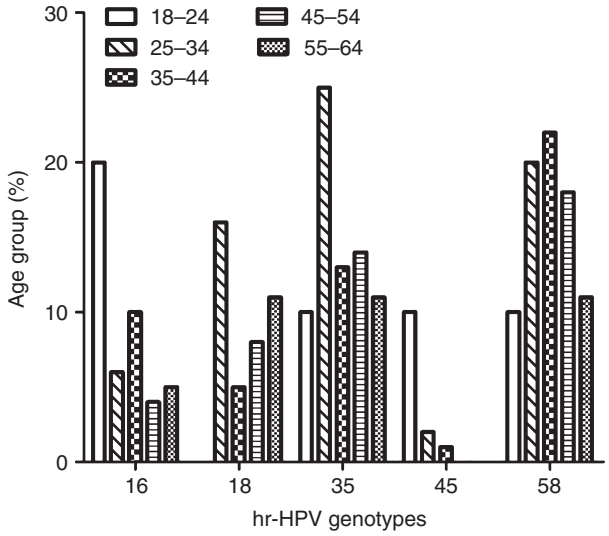

Figure 1 Age distribution *vs.* high-risk human papillomavirus genotypes.

or concurrently with others, fairly similar to our findings. Attoh *et al.* [8] also reported several hr-HPVs co-infections among Ghanaian women diagnosed with cervical cancer. However, Attoh and colleagues observed that co-infections among HPV genotypes occurred predominantly with the hr-HPV genotypes 16 and 18. This contrasts with our study, in which co-infections were more associated with the hr-HPV genotypes 58, 68 and 35. Denny *et al.* [7] reported multiple HPV genotype co-infections in HIV-positive and HIV-negative women in sub-Saharan Africa fairly similar to our findings.

The overall HPV positivity in our study was 72.0%, higher than the 64.5% reported by Brandful *et al.* [22] among healthy pregnant women and relatively lower than the 98% reported by Attoh *et al.* [8] among cervical cancer patients in Ghana. The marked variations in prevalence observed in these studies in Ghana could be due to the differences in the study population. Among WLWHIV, we found 86.9% HPV positivity, similar to prevalence reported in a study [7] among HIV patients in sub-Saharan Africa. It is important to note that prevalence of HPV infection among WLWHIV in our study was nearly twice that reported in a meta-analysis of data from four continents, among HIV-positive women without cytological abnormalities, and fairly similar to those with associated cervical abnormalities [7]. The participants in this study had no previous history of known cervical abnormalities and yet had very high HPV positivity comparable to women with known cervical abnormalities [4, 7]. This finding in our study confirms previous studies that HIV-infected women have higher rates of persistent HPV infection [9–11]. However, the prevalence of HPV positivity among HIV-negative women in our study was much lower than that reported in sub-Saharan Africa [7].

It is interesting to note that the WLWHIV in our study were all on ART. The higher level of prevalence among the cases may be an indication that ART either has no/marginal effect on persistent HPV infection among WLWHIV [9–11]. It is more likely that immunosuppression, which acts in synergy with sexually transmitted infections in hindering HPV clearance, may have resulted in persistent infection among the HIV-positive individuals compared with the control group [23, 24].

This finding agrees well with the assertion that HIV infection compromises the host immune system, making the individuals susceptible to co-infections of HPV genotypes that persist during the initial infections over time [7, 9, 11]. It has been previously reported that reactivation of latent HPV infections in a previously non-HIV-infected individual due to immunosuppression could possibly catalyse co-infections [7]. We further found a higher level of co-infections among high-risk genotypes than high-risk with low-risk genotype co-infections among cases. The reason(s) for the higher prevalence of hr-HPV genotypes co-infections compared with the lr-HPV genotypes among the cases is unclear. However, it is plausible that immune suppression favours persistent infection of hr-HPV genotypes compared with low-risk-genotypes.

We reported low-risk HPV 54 and 70 more frequently in both HIV-positive and HIV-negative women contrary to HPV 11, 53 and 61 reported in a meta-analysis [4], suggesting variations in low-risk HPV genotypes in different geographical settings and populations.

This study detected a low overall prevalence of hr-HPV 16 (7.7%) and 18 (8.7%), compared with that reported in Europe, America and some African populations with varied stages of CIN [14, 25]. These rates were also lower than others reported from sub-Saharan Africa, which ranged between 18% and 58% for hr-HPV genotypes 16 and 18, respectively [5, 19, 21], which HPV vaccines target. The top five hr-HPV genotypes reported among the control group in this study were 58, 35, 31, 52 and 39 in descending order. This suggests regional differences of these last two high-risk HPV genotypes (16, 18) reported to be highly associated with cervical cancer. Other studies [8, 22] in Ghana, however, reported these genotypes as the most predominant ones among their study populations. This further suggests that there exist even differences in HPV genotypic distributions within the same regional populations and study subjects. Our study was conducted in the middle-forest belt, rather than the coastal belt as the other studies in Ghana.

Table 4 Frequency of high-risk genotypes in single infections and co-infections with low-risk and other high-risk types

	Case (N = 107)			Control (N = 100)		
hr-HPV genotype	Single (n [%])	Co-infection with lr-HPVs (n [%])	Co-infection with other hr-HPVs (n [%])	Single (n [%])	Co-infection with lr-HPVs (n [%])	Co-infection with other hr-HPVs (n [%])
16	1 (0.9)	8 (7.4)	13 (12.1)	1 (1.0)	0	1 (1.0)
18	3 (2.8)	9 (8.4)	14 (13.1)	0	0	1 (1.0)
26*	0	2 (1.9)	8 (7.5)	0	0	1 (1.0)
31	0	19 (17.8)	22 (20.6)	6 (6.0)	3 (3.0)	1 (1.0)
33	1 (0.9)	5 (4.6)	8 (7.5)	0	0	0
35	2 (1.9)	14 (13.1)	21 (19.6)	2 (2.0)	8 (8.0)	8 (8.0)
39	0	2 (1.9)	3 (2.8)	2 (2.0)	2 (2.0)	2 (2.0)
45	0	2 (1.9)	3 (2.8)	0	0	0
51	0	2 (1.9)	3 (2.8)	1 (1.0)	0	1 (1.0)
52	0	11 (10.3)	15 (14.0)	1 (1.0)	4 (4.0)	4 (4.0)
53*	2 (1.9)	14 (13.1)	16 (15.0)	1 (1.0)	1 (1.0)	4 (4.0)
56	1 (0.9)	5 (4.6)	8 (7.5)	0	0	0
58	5 (4.7)	14 (13.1)	23 (21.5)	2 (2.0)	7 (7.0)	9 (9.0)
59	1 (0.9)	6 (5.6)	7 (6.5)	0	0	1 (1.0)
66	1 (0.9)	8 (7.4)	7 (6.5)	0	0	1 (1.0)
68	1 (0.9)	14 (13.1)	22 (20.6)	1 (1.0)	2 (2.0)	2 (2.0)
69*	0	0	0	0	0	0
73*	0	10 (9.3)	0	0	0	0
82*	0	4 (3.7)	6 (5.6)	7 (7.0)	2 (2.0)	3 (3.0)

Hr-HPV, high-risk human papillomavirus; lr-HPV, low-risk human papillomavirus.
*Probable high-risk.

Moreover, previous sexual exposures in the different study populations may explain the marked variation in HPV genotype distributions reported.

We also reported the top five predominant hr-HPV genotypes 58, 35, 68, 31 and 18, among WLWHIV in Ghana, contrary to studies reported elsewhere [4, 7, 8, 10, 11]. The high prevalence of HPV 58 in this study population is inconsistent with previous studies reported in Africa. A multicentred predominantly HIV-negative population reported HPV 45 [7] as the most prevalent genotype, while HPV 52 and 45 were frequently reported in sub-Saharan Africa among HIV-positive participants [20, 25]. These findings indicate differences in prevalence and distribution of hr-HPV genotypes other than genotypes 16 and 18 among HIV-infected and non-HIV-infected female in sub-Saharan African populations. This therefore calls for urgent need for a population-based study taking into consideration differences in age groups, regional and or ethnic groups as well as populations of patients diagnosed with either HIV or cervical cancer. This, we hope, will provide up to date profile of HPV genotypic distribution in any given area and in different subjects. This will allow for precise and targeted effective vaccine development for specific geographical settings and populations.

It is worthy of note that HPV 16 was more prevalent in the 18–24 years age group, similar to a study conducted by Dols *et al.* [15], while HPV 18 was more associated with the 25–34 years age group. This could suggest that HPV genotypic distribution is age dependent, which could be beneficial for vaccination programmes that target the hr-HPV 16 and 18 and the age group. There are currently two licensed HPV vaccines: a bivalent vaccine (Cervarix®; GlaxoSmithKline, Belgium) which targets hr-HPV 16 and 18, and a quadrivalent (GardasilTM; Merck and Co., Inc, USA) against lr-HPV 6 and 11 in addition to hr-HPV 16 and 18. Ghana, like other nations, is considering vaccinating young girls aged 9–11 years with these two licensed vaccines. However, the marked differences of hr-HPV genotypes in WLWHIV reported in our study maybe grounds for taking into account the possible inclusion of the aforementioned population in planned vaccination programmes. Data generated from this study were from a relatively adult population and therefore caution must be taken for extrapolation into policy.

There were some limitations. We mainly focused on the genotypic prevalence and distribution of HPV genotypes among HIV-infected and non-HIV-infected women. Hence, cytological and histological data associated with

single and multiple HPV genotype infections were not considered. Other screening tests to determine the presence of cervical precancerous lesions or cervical cancer were not examined. The limitation in not conducting examinations on other sexually transmitted infections in synchrony with HPV evaluation inadvertently omitted; therefore, results must be interpreted with caution. Moreover, the level of CD4 counts of HIV-positive women was not considered in this study, which could shed more light on the influence of host immunity on HPV infections. Further, the determination of HPV genotypes, which are initiators of multiple infections, was beyond the scope of this study.

In conclusion, we found significant variations in HPV genotypes among WLWHIV and non-HIV individuals with very high prevalence of high-risk-HPV genotypes and co-infections among the cases. Current efforts at vaccine development should aim at including the HPV malignant strains targeted perhaps at different age-dependent populations.

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Corresponding Author **Ellis Owusu-Dabo**, Kumasi Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology (KNUST), UPO, PMB Kumasi, Ghana. E-mail: owusudabo@kccr.de