



Prevalence and Distribution of Vaccine-Preventable Genital Human Papillomavirus(HPV) Genotypes in Ghanaian Women Presenting for Screening

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Abstract

Background: Cervical cancer is the most common gynaecologic cancer in Ghana where it is also the second most common cause of all female cancers. A number of vaccines are available to provide both individual and population-level protection against persistent infection with high-risk human papillomaviruses (HR-HPV) and reduce the burden of cervical cancer. Data on the epidemiology of vaccine-preventable papillomaviruses in Ghana is scant.

Methods: A cross-sectional observational study was implemented from May 2011 to November 2014 to understand the epidemiology of genital human papillomavirus (HPV) genotypes and cervical dysplasia in the Greater Kumasi area of Ghana. A nested multiplex polymerase chain reaction (NMPCR) assay incorporating degenerate E6/E7 consensus primers and type-specific primers was used for the detection and typing of eighteen (18) HPV genotypes among women who had never attended cervical screening prior to this study.

Results: The general prevalence of HPV infection in Kumasi was 37.2%. The age-standardized prevalence was 40.9% overall. The frequency of HR-HPV genotypes present in decreasing order were HPV-52, -56, -35, -18, -58, -68, -51, -39, -45, -16, -59, -33 and -31. Low-risk HPVs were also detected in the following order: HPV-42, -43, -66, -6/11 and -44.

Conclusions: The study shows that currently available prophylactic vaccines have the potential to be useful in the primary prevention of HPV infections in the country. This study strengthens the belief that prophylactic HPV vaccination could be a long-term strategy to reduce the burden of HPV infections and potentially reduce the burden of HPV-associated cancers and epithelial cell abnormalities among health-seeking women in Kumasi. Efforts to make vaccines available to young girls should be prioritized.

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Keywords

cervical cancer screening, human papillomaviruses, infectious disease, female reproductive health, human papillomaviruses vaccines, cancer markers

Introduction

In Ghana, cervical cancer is ranked as the second most common cancer in females with an estimated incidence of 27.4 per 100,000 women.^{1,2} Around 2,797 women are diagnosed yearly and approximately 1,699 deaths occur from cervical cancer.^{1,2} The World Health Organization (WHO) predicts that there will be 5,000 emergent cases of cervical cancer in Ghana by year 2025 and 3,361 cervical cancer-related deaths annually.³ In the context of high cervical cancer statistics and low uptake and coverage of cervical screening, the HPV vaccine offers an opportunity to erode the threat of HPV infection and cervical cancer in Ghanaian women. Routine vaccination of girls aged 11–12 years through age 26 years in those who were not adequately vaccinated previously⁴ has been shown to be an effective way of reducing the public health burden of cervical cancer together with empirical data to demonstrate that the genotype coverage of existing vaccines can be effective in the population in focus.⁵

Human papillomaviruses are classified in terms of their ability to persist and cause cervical cancer later in life as high, intermediate and low-risk types. Among high-risk types, HPV-16 and -18 can be detected in almost 70% of all cervical cancer cases, in approximately half of all high-grade cervical lesions and a third of low-grade cervical lesions around the globe.⁶ Other high-risk types designated HPV-31, HPV-33, HPV-35, HPV-45, HPV-52 and HPV-58 account for an additional 20% of cervical cancers worldwide.⁷ Therefore, expanded spectrum vaccines capable of eliciting a neutralizing immune response against common HPV genotypes could potentially prevent the majority of cervical cancers.

Preventive vaccines have been developed and evaluated for efficacy in preventing HPV infection especially when given to young girls before sexual debut. So far, the results have been shown to be safe and efficacious.^{8,9} A number of efficacious HPV vaccines have been approved in many countries as a strategy to reduce the burden of cervical cancers in women. The earliest vaccines could prevent infection with HPV-16 and 18.¹⁰ Currently, the scope of preventable infections has been expanded to cover HPV-31, -33, -45, -52 and -58 VLPs with the introduction of the nonavalent vaccine.^{11,12}

The potential impact of these vaccines in the Ghanaian population has not been decisively answered with supporting data. In general, only a few isolated surveys have been carried out in sub-Saharan Africa to characterize the epidemiology of HPV infection in the population of previously unscreened women. Furthermore, differences in methodology and sensitivity of genotyping assays used in different surveys hinder comparability of HPV studies in Africa.^{13,14} The present study

addresses the prevailing need to determine the epidemiology of HPV types in Ghana to serve as a basis for preventive strategy and future evaluation of primary prevention programmes. Genotyping studies allow for the estimation of the total burden attributable to vaccine preventable HPV types and by extension, the impact of vaccination in the general population.

Methods

This study used a multi-centre cross-sectional design to estimate the prevalence of genital HPV genotypes and their distribution among an unscreened population of women in Kumasi. Women found within the precincts of 3 major health centres in Kumasi; namely, Kumasi South Regional Hospital, Tafo Government Hospital and Suntreso Government Hospital, were purposively invited to be screened for infection with 1 of 18 different HPV types. By means of recorded messages broadcast on radio stations and public address systems located within markets, all women received education about cervical cancer prevention through routine examination at the health facilities and post-screening counselling from a midwife. Women who had never had a Pap smear, were eligible for inclusion. Pregnant women were excluded to fulfil local institutional review requirements. Women with a medical history of hysterectomy or a Pap smear test were also excluded from the study.¹⁵

DNA Sample Collection

All women provided cervical swabs during clinical examination by midwives trained in genital swab preparation. Exfoliated cervical cells sampled with cotton-tipped applicators were recovered in a commercial preservative buffer (DNA Gard, Biometrica, San Diego, USA) and transported to the Pathology Department of Korle-bu Teaching Hospital for HPV-DNA detection.

HPV DNA Purification, Amplification and Detection

Human papillomavirus-DNA isolation was performed with a commercially available kit (Qiagen Ltd, Maryland, USA) in line with enclosed instructions.¹⁶ Briefly, cervical cells were lysed by incubation at 56°C with 20 µl QIAGEN Proteinase K in a proprietary buffer AL (300 µl). Next, the exposed DNA was primed by addition of 400 µl ethanol (96–100%) and bound to a special matrix in 2 mL spin columns. The sample was then successively washed with buffer AW1 and AW2 by centrifuging

at 8000 r/min for 1 min and 14,000 r/min for 3 min, respectively. Finally bound, purified DNA was eluted into 50 µl Buffer AE and stored at -70°C in duplicate until further processing.

Human papillomavirus genotype identification was carried out by nested multiplex PCR (NMPCR) as previously described.^{16,17} A single consensus forward primer (GP-E6-3F) and 2 consensus back primers (GP-E7-5B and GP-E7-6B) were used for the general primer PCR. Subsequently, a nested multiplex PCR using 18 type-specific primers capable of detecting common genital HPVs was performed to screen for presence of HPV infection. Details of PCR reaction conditions and electrophoresis on 2% agarose gel were as disclosed previously ([Supplementary File 1: Laboratory Protocols](#)).^{16,17}

The study was endorsed by the Ethical Research Committee at the Kwame Nkrumah University of Science and Technology with reference CHRPE 42/11. Letters of approval and support were also obtained from the Ghana Health Service (GHS). All participants were sufficiently educated about the study rationale and design, and about potential risks incurred by providing biological samples for research before providing documented consent for samples and biological data to be analysed and published anonymously.

Data Analysis

Exploratory data analysis was carried out in SPSS Statistics software version 26 (IBM Corp., New York, USA) to obtain descriptive statistics. Categorical variables were examined for association by Chi-square test whilst differences between quantitative variables examined by Student's t-test. Chi-square test for trend was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software Inc, San Diego, California, USA). Continuous variables were tested for homogeneity of variances and normality before analysis. For categorical outcomes, proportions are reported (with 95% confidence interval in parenthesis). For continuous variables, data is given as mean (with 95% confidence interval in parenthesis). The reporting of this study conforms to the STROBE guidelines for cross-sectional studies.¹⁸

Results

This study investigated the association of selected demographic and behavioural characteristics with the detection of 18 low-risk and high-risk genital human papillomaviruses (HPV) in women recruited in Kumasi for cervical screening. Cervical samples were originally obtained from 595 women from age 18 to 93 years; out of which 500 eligible women with an HPV DNA result were selected for the present study. Data on sociodemographic characteristics are available in [Table 1](#). The mean age of the participants was 42.3 years (SD = 11.6 years) and the median age was 42 years. Age and all other continuous variables studied were found to follow a Gaussian distribution according to D'Agostino-Pearson normality test.

The majority of the population had completed middle school/junior high school (40.4%) or university education (17.4%). However, 10.2% of the women in the study had never received formal education. More than half of women disclosed their status as legally married (56.2%). Only 6.8% of women had never been pregnant. The average number of lifetime pregnancies was 4.5 (SD = 2.8). Almost half of the women (49.2%) reported 5 pregnancies or more. The mean number of all abortions reported per woman was 1.74 (SD = 2.8) and the overall abortion prevalence rate was 72.8%.

The study documented the ages at cessation of menses, first pregnancy and sexual experience. The average reported age at menopause was 48.4 years ranging from 34 to 60 years (SD = 5.6). The average reported age of first sexual experience (coitarche) was 14.8 years ranging from 12 to 31 years (SD = 9.3). In all, 50.9% of respondents had their first sexual encounter before 18 years ([Table 2](#)). Furthermore, approximately 2 in 3 women (58.3%) reported becoming pregnant for the first time by age 20 years. However, the average reported age for first conception was 21.3 years ranging from 12 to 36 years (SD = 4.5).

Approximately 79.1% of respondents had more than 1 sexual partner in their entire lifetime. Overall, there was a reported average of 3 lifetime sexual partners per woman ranging from 1 to 15 lifetime partners (median = 3.0). Additionally, 18.3% of married women suspected that their husbands had had extramarital sexual relationships; 45.7% intimated that their husbands had been married before and 56.5% of married women were found in polygamous relationships.

Cervical swabs were analysed for 18 HPV types. [Table 3](#) shows the Prevalence of human papillomavirus (HPV) types detected in cervical specimens from these women. Also shown is the prevalence of high-risk HPV (types -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68) and low-risk HPV (types -6/11, -42, -43, -44 and -66) in the specified population.

The prevalence of HPV oncogenic DNA of any type among the study population was 37.2% (95% CI: 33.4–41.6). The prevalence of LR HPV was 14.2% (95% CI: 11.3–17.6) (38.2% of HPV positive cases) while that of HR HPV was 31.4% (95% CI: 27.4–35.7) (84.4% of all 18 HPV genotypes screened were detected). An accounting of the distribution of HPV types covered by the 3 vaccines: Cervarix, Gardasil and Gardasil 9 in the population of unscreened women is presented in [Table 3](#). A bivalent vaccine that prevents infection from HPV-16 and -18 may potentially prevent 16.7% (95% CI: 11.3–22.0%) of HPV infections among unscreened women; a quadrivalent vaccine that prevents infection from HPV-6/11 in addition to -16 and -18 may potentially prevent 21.5% (95% CI: 15.6–27.4%) of circulating HPV infections and a multivalent vaccine that prevents infection from HPV-6/11, -16, -18, -31, -33, -45, -52, and -58 may potentially prevent 68.8% (95% CI: 62.2–75.5) of circulating HPV infections. Among 157 women with high-risk HPV infections, the prevalence of vaccine preventable

Table 1. Population Demographics of Women Screened in Kumasi, Ghana.

Characteristic	No. (%*)
Age (N=493)	
<25	20 (4.1)
25–34	120 (24.3)
35–44	154 (31.2)
45–54	128 (26.0)
55–64	50 (10.1)
≥65	21 (4.3)
Education (N=462)	
never attended	51 (11.0)
primary	60 (13.0)
middle/JHS	202 (43.7)
SHS	41 (8.9)
technical/vocational	21 (4.5)
university	87 (18.8)
Marital status (N=476)	
single	64 (13.4)
divorced/widowed	81 (17.0)
married	281 (59.0)
cohabiting	50 (10.5)
Ethnicity (N=455)	
Akan	383 (83.4)
Mole-Dagbani	42 (9.2)
Ewe	17 (3.7)
Ga	13 (2.8)
Parity	
Gravidae (N=461)	
0	34 (7.4)
1 to 2	86 (18.7)
3 to 4	135 (29.3)
≥5	206 (44.7)
Para (N=461)	
0	65 (14.1)
1 to 2	150 (32.5)
3 to 4	156 (33.8)
≥5	90 (19.5)
Abortion (N=459)	
0	125 (27.2)
1	118 (25.7)
≥2	216 (47.1)
Age at first pregnancy (years) (N=391)	
≤17	66 (16.9)
18–21	162 (41.4)
22–25	94 (24.0)
>25	69 (17.6)

Abbreviation: JHS, junior high school; SHS, senior high school.
*Percentage fraction of respondents.

HPV genotypes was as follows: Cervarix (19.7% of HR HPV infections, n = 31), Gardasil (19.7% of HR HPV infections, n = 31), and Gardasil 9 (75.8% of HR HPV infections, n = 119).

Table 2. Sexual History of Women Screened in Kumasi, Ghana.

Characteristic	No. (%*)
Age at Coitarche (N=375)	
≤15	39 (10.4)
16–18	152 (40.5)
19–21	121 (32.3)
≥22	63 (16.8)
Lifetime sex partners (N=388)	
single	81 (20.9)
multiple	307 (79.1)
Main partner ever married (N=265)	
No	139 (54.3)
Yes	117 (45.7)
Husband with unmarried (extramarital) partners (N=257)	
no	162 (63.0)
yes	47 (18.3)
not sure	48 (18.7)
Wife of polygamous relationship (N=269)	
no	117 (43.5)
yes	152 (56.5)
Main partner's age (N=265)	
≤34	33 (12.5)
35–44	83 (31.3)
45–54	87 (32.8)
55–64	47 (17.7)
≥65	15 (5.7)
VIA (N=500)	
normal	465 (93.0)
abnormal	35 (7.0)

Abbreviation: VIA, visual inspection with acetic acid.
*Percentage fraction of respondents

Tables 4 and 5 show the relative frequencies of HPV types detected in the study. The most common HR types were, HPV-52 (11.6%; 58 out of 500 women), HPV-56 (7%; 35 out of 500 women), HPV-35 (5%; 25 out of 500 women), HPV-18 (4.8%; 24 out of 500 women), HPV-66 (3.8%; 19 out of 500 women) and HPV-58 (3.2%; 16 out of 500 women). HPV-42, a low-risk type, was also common (7.8%; 39 out of 500 women). The frequency of HPV-16 (the most common genotype associated with cervical cancer worldwide) detection was 1.4% (7 out of 500 women).

The estimated age-related prevalence rates ranged from 32% among women aged 55–64 years to 47.6% among women from the age 65 and above category. There were no statistically significant differences in HPV detection according to marital status or across the various strata for number of conceived pregnancies, parity, number of abortions, age at first pregnancy and age at coitarche. At the 5% level of significance, HPV DNA detection was significantly associated with lifetime sex partners ($\chi^2 = 3.92$, $df = 1$, $P = .048$), extramarital activity of woman's partner ($\chi^2 = 7.34$, $df = 2$, $P = .026$), but not with woman's age at coitarche, polygamy, marital history of woman's partner and main partner's age (Table 6).

Table 3 : Prevalence of Various Categories of Human Papillomavirus Infections (HPV).

Parameter	n	% ^a (95% CI)	% ^b
HPV negative	314	62.8 (58.6–67.0)	
HPV positive	186	37.2 (32.9–41.4)	
HPV-16, -18	31	6.2 (4.1–8.3)	16.7 (11.3–22.0)
HPV-6/11, -16, -18	40	8.0 (5.6–10.4)	21.5 (15.6–27.4)
HPV-6/11, -16, -18, -31, -33, -45, -52, -58	128	25.6 (21.8–29.4)	68.8 (62.2–75.5)
Low-risk HPV only ^c	29	5.8 (3.8–7.9)	15.6 (10.3–20.8)
High-risk HPV only ^d	115	23.0 (19.3–26.7)	61.8 (54.9–68.8)
Both high and low-risk	42	8.4 (5.9–10.8)	22.6 (16.6–28.6)
Multiple HPV infections	85	17.0 (13.7–20.3)	45.7 (38.5–52.9)

Abbreviation: HPV, Human Papillomavirus.

^aPercentages calculated using total number of cases (N= 500) as common denominator.

^bPercentages calculated using number of positive cases (N= 186) as common denominator.

^cWomen infected with only LR HPV. Low-risk HPV types include 6/11, 42, 43, 44 and 66.

^dWomen infected with only HR HPV. High-risk HPV types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

Table 4. Prevalence of High-Risk Human Papillomavirus (HPV) Genotypes.

HPV Type	n*	% ^a (95% CI)	% ^b (95% CI)	% ^c (95% CI)
HPV-52	58	11.6 (8.8–14.4)	31.2 (24.5–37.8)	36.9 (29.4–44.5)
HPV-56	35	7.0 (4.8–9.2)	18.8 (13.2–24.4)	22.3 (15.8–28.8)
HPV-35	25	5.0 (3.1–6.9)	13.4 (8.5–18.3)	15.9 (10.2–21.7)
HPV-18	24	4.8 (2.9–6.7)	12.9 (8.0–17.7)	15.3 (9.7–20.9)
HPV-58	16	3.2 (1.7–4.7)	8.6 (4.6–12.6)	10.2 (5.5–14.9)
HPV-68	16	3.2 (1.7–4.7)	8.6 (4.6–12.6)	10.2 (5.5–14.9)
HPV-51	12	2.4 (1.1–3.7)	6.5 (2.9–9.2)	7.6 (3.5–11.8)
HPV-39	11	2.2 (.9–3.5)	5.9 (2.5–9.3)	7.0 (3.0–11.0)
HPV-45	9	1.8 (.6–2.9)	4.8 (1.8–7.9)	5.7 (2.1–9.4)
HPV-16	7	1.4 (.3–2.4)	3.8 (1.0–6.5)	4.5 (1.2–7.7)
HPV-59	3	.6 (.0–1.3)	1.6 (.0–3.4)	1.9 (.0–4.1)
HPV-33	3	.6 (.0–1.3)	1.6 (.0–3.4)	1.9 (.0–4.1)
HPV-31	2	.4 (.0–.9)	1.1 (.0–2.6)	1.3 (.0–3.0)

Abbreviation: HPV, Human Papillomavirus.

*Because of multiple infections, a woman may be counted more than once.

^aPercentages calculated using total number of cases (N = 500) as common denominator.

^bPercentages calculated using number of positive cases (N = 186) as common denominator.

^cPercentages calculated using number of HR cases (N = 157) as common denominator.

Age-specific HPV prevalence estimates for all women and according to normal and abnormal cytology result are shown in [Supplementary Table S2](#): HPV Distribution According to Demographic Categories. In general, HPV prevalence was highest in women younger than 25 years and prevalence decreased in the 35–44 year group. A second but smaller peak in HPV infections occurred in the 45–54 year group followed by another decline ([Supplementary Figure S3A and S3B](#): Age Trends in HPV Prevalence). However, for women with abnormal cytology report, HPV infection was remarkably low in women younger than 25 years. However, the rate of infections peaked among middle-aged women (35–44 year group) and remained significantly elevated thereafter ([Supplementary Figure S3C](#): Age Trends in HPV Prevalence).

Discussion

Prevalence estimates provide a measure of the percentage of persons in a population who have new, persistent or recurring HPV infection at a particular point in time. The variability in HPV detection rates is an indication that very robust and highly sensitive methods are desirable for establishing authoritative, reproducible estimates. Using a highly-sensitive nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers, the prevalence of HPV infection found in this study was 37.2%. This figure is consistent with existing reports of the elevated prevalence of HPV in women in Sub-Saharan Africa.^{19–21} The WHO estimates that at least 21.5%

of women from Western Africa carry HPV infection at a given time³. Yar and colleagues conducted a case-control study involving 107 women living with HIV aged between 18 and 59 years (cases) and 100 non-HIV-infected

apparently healthy women as controls. Overall HPV positivity for cases and controls was 86.9% and 56.0% respectively. The excess HPV positivity among control women may be explained by the expanded genotype portfolio used. In that study, cervicovaginal swabs were taken from study participants to characterize 28 high- and low-risk HPV genotypes while the present study captured 18 HPV including all types of major interest.

Human papillomavirus statistics on sub-Saharan Africa have generally shown relatively high prevalence with some variation. But oftentimes, the findings of these studies are difficult to compare directly owing to the differences in sampling and testing methods. As it is, both random population surveys and opportunistic surveys from clinical settings have been reported to use both immunologic and nucleic acid protocols operating at varying sensitivities.¹³ Again, estimates may vary considerably, owing to modalities for the selection of study subjects (randomised population sampling or opportunistic series from clinical settings) and to the tests used to detect HPV DNA.²² Using the second generation hybrid capture (HC) assay II, prevalence estimates range from 17% (for HR HPV types) in rural Uganda²³ to 25% among

Table 5. Prevalence of Low-Risk Human Papillomavirus (HPV) Genotypes.

HPV Type	n*	% ^a (95% CI)	% ^b (95% CI)	% ^c (95% CI)
HPV-42	39	7.8 (5.5–10.2)	21.0 (15.1–26.8)	54.9 (43.4–66.5)
HPV-43	30	6.0 (3.9–8.1)	16.1 (10.8–21.4)	42.3 (30.8–53.7)
HPV-66	19	3.8 (2.1–5.5)	10.2 (5.9–14.6)	26.8 (16.5–37.1)
HPV-6/11	9	1.8 (.6–2.9)	4.8 (1.8–7.9)	12.7 (4.9–20.4)
HPV-44	3	.6 (.0–1.3)	1.6 (.0–3.4)	4.2 (.0–8.9)

Abbreviation: HPV, Human Papillomavirus.

*Because of multiple infections, a woman may be counted more than once.

^aPercentages calculated using total number of cases (N = 500) as common denominator.

^bPercentages calculated using number of positive cases (N = 186) as common denominator.

^cPercentages calculated using number of LR cases (N = 71) as common denominator.

Table 6. Sexual History and Human Papillomavirus (HPV) Prevalence Among women.

Characteristic	N	Prevalence of HPV,			
		Any HPV n(% [#])	N	Low-risk HPV n(% [#])	High-risk HPV n(% [#])
Age at Coitarche (N=375)					
≤15	39	12(30.8)	12	5(41.7)	11(91.7)
16–18	152	63(41.4)	63	20(31.7)	56(88.9)
19–21	121	45(37.2)	45	16(35.6)	37 (82.2)
≥22	63	17(27.0)	17	6(35.3)	14(82.4)
(χ^2 , df = 3, P-value)		4.64; .200		.51; .917	1.49; .685
Lifetime sex partners (N=388)					
Single	81	23(28.4)	23	10(43.5)	17(73.9)
multiple	307	124(40.4)	124	42(33.9)	106(85.5)
(χ^2 , df = 1, P-value)		3.92; .048		.78; .376	1.91; .168
Main partner ever married (N=265)					
No	139	42(30.2)	42	18(42.9)	31(73.8)
Yes	117	45(38.5)	45	12(26.7)	43(95.6)
(χ^2 , df = 1, P-value)		1.93; 0.165		2.52; .112	8.08; .004
Husband with unmarried (extramarital) partners (N=257)					
No	162	50(30.9)	50	19(38.0)	42(84.0)
Yes	47	16(34.0)	16	7(43.8)	12(75.0)
Not sure	48	25(52.1)	25	7(28.0)	23(92.0)
(χ^2 , df = 2, P-value)		7.34; .026		1.19; .551	2.20; .333
Wife of polygamous relationship (N=269)					
No	117	42(35.9)	42	15(35.7)	37(88.1)
Yes	152	52(34.2)	52	19(36.5)	42(80.8)
(χ^2 , df = 1, P-value)		.08; .774		.01; .934	.93; .335

[#] Row percentages are computed to show within-group HPV prevalence estimates. Significant values are highlighted in bold font

HIV-negative women in Harare, Zimbabwe.²⁴ Polymerase chain reaction-based assays showed HPV prevalence of 40% in rural Mozambique,¹⁹ 31% in Harare, Zimbabwe,²⁵ 18% in Dakar and Pikene, Senegal²¹ and 44% in Nairobi, Kenya.²⁶

The proportion of HR-HPV genotypes in this study (84% of HPV genotypes detected) was relatively high but consistent with studies involving women attending routine gynaecological screening. Said *et al.*²⁷ investigated the prevalence and distribution of HPV genotypes in women with squamous abnormalities and normal cervixes participating in a community-based microbicide study. Among women with normal cervixes, 73% of the HPV positive specimens were HR-HPV types even though the women were mainly volunteers from the local community. In a cross-sectional study by Brandful *et al.* aimed to estimate HPV prevalence in pregnant women (18–41 years) in Ghana, overall HPV prevalence was 65% and of this number 72% were infected with high-risk HPV genotypes.²⁸ In a similar study among women drawn mostly from Accra and also from Kumasi, the prevalence of HPV was 43.5%, with high-risk HPV cases accounting for 35% of all HPV positive.²⁹ In addition, the prevalence of the bivalent vaccine types was 8.2%, quadrivalent vaccine types was 9.1% and nonavalent vaccine-types was 28.4% among all women. Prevalence estimates can vary depending on the demographic and sexual behaviour characteristics of the group under study. Therefore, differences in study population characteristics may account for the differences in result. However, in spite of the nuances of relative genotype distributions across populations, Oksana *et al.*,²⁹ estimated that the 3 available vaccines would prevent between 24.3% and 78.4% of high-risk HPV infections compared to 19.7% and 75.8% for the present study which is most significant for public health policy regarding the introduction of HPV vaccines to prevent high-risk infections that may progress to cancer especially in high-risk sub-populations. Another interesting dimension of HPV studies is the frequency of detection of HPV-16 and -18, the 2 most common high-risk oncogenic genotypes and the 2 covered by the vaccines Cervarix and Gardasil. In this study, there was a similar combined rate of detection of HPV-16 and -18 oncogenes (6.2%), to previous studies in Ghana (6.6–8.2%).^{28,29} This is a regular feature of HPV studies in the general population of previously unscreened cervixes and lies in sharp contrast to the scenario seen in global HPV studies involving histologically confirmed cancer tissue.^{30–32} HPV-16 and HPV-18 remain under-represented in women with normal cytology by comparison with their importance in severe cervical lesions. Yar *et al.*³³ reported a low overall prevalence of HR-HPV-16 and -18 similar to this study's observation.

The higher prevalence of HPV-16 and -18 in cancerous women compared with women who remain unscreened for cervical abnormalities reflect the tendency of HPV-16 and -18 to be more persistent in infection and induce more aggressive cell-level changes that predispose infected women to cervical dysplasia. It does not necessarily mean that existing bivalent/

quadrivalent HPV vaccines may be ineffective in preventing a great fraction of HPV infections that would eventually result in cancer. HPV-16 and/or HPV-18 contribute to more than 50% of the infections detected in high-grade squamous intraepithelial lesions, 70% of infections in invasive cervical cancer, and 81.5% of infections detected in adenocarcinomas.^{34,35} In a recent study by Awua and colleagues, HPV-specific DNA was detected in 89.8% of 230 paraffin embedded tissue from confirmed cervical cancer cases diagnosed at the Korle-Bu Teaching Hospital during the period January 2004 to December 2006.¹⁷ The 4 commonly detected (overall prevalence) HPVs were HPV-18 (47.4%), HPV-59 (42.2%), HPV-45 (37.4%) and HPV-16 (10.0%). The same 4 HPV genotypes were the most common infecting genotypes in both multiple and single infections but in different sequence. In an earlier study of 50 similarly processed samples collected between January and December 2003 at the same hospital and using the same HPV detection methods, an HPV positivity of 98.0% was reported.³⁶ The most common detected genotypes were HPV-18 (84%), HPV-16 (24%), HPV-45 (6%), and HPV-39 (4%).

A cross-sectional, multi-centre, epidemiological study conducted in women from Ghana, Nigeria and South Africa, who had cervical lesions clinically suggestive of ICC has been published.³² In that study, HPV positivity was 90.4% and the most common detected genotypes were HPV-16 (50.7%), HPV-18 (19.2%), HPV-45 (10.1%), HPV-35 (9.7%), HPV-33 (5.0%) and HPV-52 (4.5%). Additionally, a prevalence of HPV DNA (89.4%), was reported by a study of cervical tumour samples in neighbouring Cote d'Ivoire.³⁷ The most common HPV types were HPV-16 (45%), HPV-18 (21%), HPV-45 (10%), HPV-35 (8%), and HPV-31 (3%). Similarly, a study in Benin reported HPV-59 (24.6%), HPV-35 (22.5%), HPV-16 (17.6%) and HPV-18 (14.8%) as the common HPV genotypes detected.³⁸ Also, in a study in Burkina Faso, HPV-52 (14.7%), HPV-35 (9.4%), HPV-58 (9.4%) and HPV-51 (8.6%) were the common genotypes.³⁹ Furthermore, several studies from other regions in and outside Africa confirm the assertion that although HPV-16 and HPV-18 are not the most common HPVs in the general population of women, they are unequivocally the most common genotypes in cervical cancers globally.

A peculiar age pattern of HPV prevalence was observed with an initial peak of HPV infection (mainly HR HPV types) among women younger than 25 years and a consistently high prevalence among middle-aged women despite a decrease in the 35–44 year group. A second peak in HPV infections occurred in the 45–54 year group followed by another decline in older women ([Supplementary File S3: Age Trends in HPV Prevalence](#)). The age trend of HPV found in Kumasi suggests that middle-aged women retain a significantly elevated prevalence of HPV. Domfeh *et al.*,⁴⁰ studied a cross-section of women selected from the gynaecology outpatient clinic of the Korle-Bu Teaching Hospital, Accra, Ghana; and also found a high crude prevalence of HPV in older age groups. This age-related pattern of HPV infection has been similarly reported in

populations with a generally high risk for cervical cancer, namely, in Ibadan, Nigeria²⁰ and Chennai, Southern India.⁴¹ The highest HR HPV prevalence was found in women aged below 45 years and this finding is concurrent with a higher number of recent sexual partners linked with this age group. Elsewhere, a similar observation was made in women younger than 30 years.^{42,43}

A high prevalence of HPV in middle and old age in this study could be attributed to 1 or more non-mutually exclusive mechanisms, such as reactivation of latent infections acquired earlier in life,⁴⁴ acquisition of new infections due to sexual contacts with new partners later in life,⁴⁵ or to histologic changes associated with menopause. A waning of vaginal secretions after menopause reducing vaginal lubrication may increase the likelihood of micro-tears in the vaginal mucosa and allow HPV the opportunity to infect basal cells. Similarly, reduced secretions may result in low levels of protective viral neutralising antibodies in the vaginal environment again favouring HPV infection. In older women, inflammatory processes and reduced humoral immune surveillance may result in reactivation of latent infections and delayed clearance or persistence of infection respectively, both of which may result in accumulation of infections and thus a high prevalence.⁴⁶

This study represents the largest effort to determine the genotype distribution of HPV among women in Kumasi to date embracing data from 500 women attending cervical screening at the 3 major centres in the metropolis over a 2-year period. The frequency of high-risk HPV genotypes present in decreasing order were HPV-52, -56, -35, -18, -58, -68, -51, -39, -45, -16, -59, -33 and -31. HPV-18 was found to be more prevalent than HPV-16. Low-risk HPV were also detected in the following order: HPV-42, -43, -66, -6/11 and -44.

A few studies have reported HPV genotype distribution among the general population of Ghanaian women. Yar *et al.*,³³ recently published findings from a pilot case-control study involving HIV women. The most common high-risk HPVs detected were -58, -35, -31, -68, -53, -52, -18 and -16. The authors concluded that significant variations exist in HPV genotypes among HIV-infected and uninfected women. The high HIV prevalence could explain the observed HPV genotype distribution, since HIV-infected women are reported to acquire a broader spectrum of HPV genotypes compared to HIV-naïve women.^{39,47,48}

An interesting finding of our study was the higher prevalence of HPV-18 compared to -16. This finding has been documented in many other available local studies both in normal cervixes^{28,33} and cancerous tissue.^{17,36} In the study by Attoh *et al.*, HPV-18 was found to be more prevalent than HPV-16 among Ghanaian women with cervical cancer, mostly adenocarcinomas, followed by HPV -45, -39, -35, -52 and -56.³⁶ There is emerging concern about the possibility of certain HPV types being more common in Sub-Saharan African women than elsewhere. HPV-35, for instance, was slightly more common than HPV-16 in Mozambique both in women with normal cytology and in those with HSIL or worse.¹⁹ HPV-52 was found slightly more

frequently than HPV-16 or HPV-35 in Kenya²⁶ and in colposcopically normal women in Zimbabwe.²⁵ In Senegal, HPV 16 and 58 were the most common types overall and in women with cervical lesions.²¹ However, a low prevalence of HPV-16, HPV-53 and HPV-18 has been reported in the USA⁴⁹ and Greece⁵⁰ similar to that of the present study.

Limitations

Cross-sectional studies provide a snapshot of infections within the population under study at a particular time unlike cohort studies that follow subjects across time and can provide data on the natural history of infections. This means that there is a potential for bias through an over-representation of infections by HPV types that are more resistant to immune clearance compared to those that are more easily cleared by the immune system. Thus, the natural history of the different types of HPV infections could introduce bias that the study was not designed to overcome. The study also did not investigate the immune status of participants. Any interpretation of our findings, therefore, must take these limitations into consideration.

Conclusions

Using a highly sensitive and specific nested-multiplex PCR assay, we have successfully estimated the prevalence and distribution of genital HPV genotypes in a population of women attending cervical care clinic in the Kumasi Metropolis, Ghana. To this end, the study provides evidence that HPV infection in the general population may be high (37.2%) and deserving of attention because of the possibility of arousing epithelial cell abnormalities that portend invasive cancer. The most common HR types are at present vaccine-preventable with the exception of HPV-56 (7%; 35 out of 500 women) and HPV-35 (5%; 25 out of 500 women). Without taking the cross-protective potential of available vaccines into account, the prevalence of vaccine-preventable HPV genotypes among unscreened women attending cervical care in Kumasi was as follows: Cervarix (19.7% of HR HPV infections), Gardasil (19.7% of HR HPV infections) and Gardasil 9 (75.8% of HR HPV infections).

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Authors' Contributions

RHA and ETD optimized study protocols, carried out the PCR analysis and participated in the writing of the manuscript. ETD was

responsible for data collection and statistical analysis. ETD carried out the genomic DNA extraction. FAY, EOD, EKW and RHA conceived the study, provided supervision, designed the study and reviewed and refined the manuscript prior to submission. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics Approval

Ethical clearance for commencement of the study was obtained from the Committee on Human Research, Publication and Ethics (CHRPE), Kwame Nkrumah University of Science and Technology, School of Medical Sciences (KNUST-SMS), & Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana (ref. CHRPE 42/11). Study participants were adequately informed of the purpose, nature, procedures, risks and hazards of the study.

Consent for Publication

Participant recruitment followed a standard informed consent procedure requiring explanation of procedures in a comprehensible format, providing study leaflets with contacts of principal investigator and review board and culminating in documented signatures. Participation was strictly voluntary with full freedom to withdraw from the study without compromising right to medical care. All participants were fully in support of disseminating anonymized study findings.

Availability of Data and Materials

The data that support the findings of this study has been provided in this manuscript and its supplementary files (Supplementary file S4: Data file).

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Supplemental Material

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Appendix

Abbreviations

CHRPE	Committee for Human Research Publication and Ethics	KNUST	Kwame Nkrumah University of Science and Technology
DNA	Deoxyribonucleic acid	LR HPV	Low-risk human papillomavirus
HIV	Human immunodeficiency virus	NMPCR	Nested multiplex polymerase chain reaction
HR HPV	High-risk human papillomavirus	PCR	polymerase chain reaction
JHS	Junior high school	SD	Standard deviation
KATH	Komfo Anokye Teaching Hospital	SHS	Senior high school
		USA	United States of America
		VIA	Visual inspection with acid
		VLP	Virus-like particle
		WHO	World Health Organization