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Oncogenic human papillomavirus (HPV) in women from Ghana

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Gynaecological or cervical cancer remains a significant public health problem worldwide, a situation from which Ghana is not exempt. Human papilloma virus (HPV) is an important agent of pre-invasive and invasive cervical cancer. Currently, over 70 genotypes of the virus have been identified, of which twenty or more have actually been isolated from or are reportedly associated with female genital lesions. Anecdotal evidence suggest that clinical cases of cervical cancer in Ghana is on the ascendancy, but our knowledge of HPV genotypes responsible for cervical, vaginal and vulval lesions, as well as precancerous and invasive vaginal, vulval and cervical cancer in Ghanaian women is limited. Women of child-bearing age and of diverse background in the general population who patronized ante-natal services at the largest hospital in Ghana were invited to participate in a cross-sectional study to identify oncogenic HPV genotypes, if any, prevalent in healthy pregnant women. Out of ninety-three (93) samples analyzed, sixty (60) (64.5%) were positive for HPV infection. The HPV genotype profile was complex. Subjects infected with either high risk or low risk genotypes alone or concurrently with 'others', where "other" referred to genotypes that are 'probably carcinogenic' (HPV genotypes 26, 34, 53) or 'probably high risk' (53 and 66) for carcinogenesis were identified. Subjects with oncogenic HPV 16 and 18 infections were also identified in the study group, along with those having single to multiple infections. One had six HPV genotype sequences, while majority (n=26), representing 43.4% had infection with only one HPV genotype.

Key words: Human papilloma virus (HPV), cervical carcinogenesis, Ghana.

INTRODUCTION

Gynaecological or cervical cancer remains an important public health problem worldwide, being the most frequent cancer affecting women in developing countries (Parkin et al., 1999). Cervical cancer was also reported to be the second cause of cancer-related deaths in women (Zur Hausen, 2000, 2002; Valles et al., 2009). This situation persists and Ghana is not exempt. In fact, indications are that the incidence and prevalence of cervical cancer is on the ascendancy (Adanu 2012, Personal Communication). Cervical carcinogenesis is a multi-step process in which

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the aetiological role of the human papillomavirus (HPV) is well established (Walboomers et al., 1999; Bosch and de Sanjose, 2003; Munoz et al., 2003). Virus infection is followed by the development of precursor eruptions referred to as low grade and high grade squamous intraepithelial lesions (LSIL, HSIL) (Lorincz et al., 1992) or cervical intraepithelial neoplasia (CIN). HPV infections occur on the skin and mucous membranes of both sexes through sexual contact. They are highly prevalent, but mostly transient (Schiffman and Kruger-Kjær, 2003). Majority of HPV infections resolve by themselves but about 10% of women progress to carcinomas and cancers (Bosch et al., 2006). Genotyping methods have identified more than 70 different HPV types (Bosch et al., 2006). Of these, at least 20 genotypes have been isolated from or are associated with female genital lesions, while genotypes -16, -18 and -33 have actually been found in cervical carcinoma (Attoh et al., 2010). Globally, infection with HPV type 16 and 18 accounts for more than 70% of invasive cervical carcinomas (Muñoz et al., 2004), with the remaining 30% caused by HPV-31, -33, -35, -39, -45, -51, -52, -56, -58 and -59 (Bouvard et al., 2009).

HPV genotypes -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68 are therefore classified as high-risk (HR) and firmly established as the cause of invasive cervical cancer and its precursor lesions (Bosch and de Sanjose, 2003; Cogliano et al., 2005; Schiffman et al., 2005). These HR genotypes may differ in their progression from LSIL to malignancy (Schlecht et al., 2003). For example, HPVs 35, 39, 51, 56 and 59 supposedly have a lower potential for progression to cancer status. Also, HPV 53 and 66 are “probably high risk” due to their high detection in LSIL, but rarely in cancers. They characteristically have a very low risk for carcinogenesis (Clifford et al., 2005). HPV types -6/11, -40, -42, -43, -44 -54, -61, -70, -72, -81 on the other hand, are low-risk (LR) for carcinogenesis (Valles et al., 2009), while genotypes 26, 34 and 53 are “probably carcinogenic” (Muñoz et al., 2004).

In Ghana, only a limited study has been performed to investigate the association and the extent to which HPV genotypes are involved with or responsible for cervical, vaginal and vulval lesions, as well as precancerous and invasive vaginal, vulval and cervical cancer in Ghanaian women (Attoh et al., 2010). In this study, the prevalence of oncogenic HPV genotypes in a population of Ghanaian women of child-bearing age from all walks of life attending ante-natal clinic in the largest tertiary hospital in Ghana was explored. A study to identify oncogenic HPV types among Ghanaian women of child-bearing age could be a justification to embark on a large-scale one involving adolescents in high school around ages of 12 to 15 years and above who may already be or potentially sexually active and therefore susceptible to infection with HPVs including the oncogenic genotypes. The study was therefore undertaken to pilot an investigation into the profile of HPV genotypes in healthy Ghanaian females, with particular interest in the prevalence of oncogenic genotypes.

MATERIALS AND METHODS

Study site, study population and sample collection

The study population was derived from apparently healthy adult pregnant women attending ante-natal clinics in the largest tertiary hospital in Ghana. They were aged between 18 to 41 years at various stages of pregnancy. Ninety three (93), who were negative for HIV infection consented to participate in this cross-sectional study. They were enrolled and specimens of cervical scrapings from epithelial cells were obtained at the Korle Bu Teaching Hospital in 2006. The specimens were frozen in PBS transport medium and transported in cool boxes to the Department of Virology of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana within 24 h.

DNA extraction

The specimens were vortexed, transferred into 15 ml centrifuge tubes and spun at 2500 rpm for 10 min (Kubota, Japan) to pellet the cells. The cells were lysed with 1.0 to 1.5 ml of Lysis Buffer comprising 25 mM Tris, pH 7.5, 0.1 M NaCl, 10 mM EDTA, NP-40 and 0.5% SDS in proportion to the visually estimated pellet size. The cell/buffer suspension was transferred into an appropriately labelled sterile 1.5 ml centrifuge tube and proteinase K (10 mg/ml) added to a final concentration of 2 µl per 100 ml. The cell suspension was incubated 56°C for 3 h, after which 50% chelex-100 was added to the lysate to a final concentration of 5%. The lysate was heated at 95°C for 10 min to inactivate the proteinase K, vortexed and centrifuged at a maximum speed for 3 min in a microcentrifuge. The crude DNA (supernatant) was carefully transferred into a fresh sterile 1.5 ml centrifuge tube and purified or kept at -20°C.

DNA purification by phenol/chloroform extraction

Five hundred micro-litres (500 µl) of crude DNA was transferred into sterile, specimen ID-labeled 1.5 ml microcentrifuge tubes and an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added. The mixture was vortexed vigorously and centrifuged in a microcentrifuge at maximum speed for 3 min. The aqueous layer of the resulting organic and aqueous phases was carefully transferred into a freshly labelled sterile 1.5 ml microcentrifuge tube. Two volumes of reagent grade ethanol was added to the supernatant, thoroughly mixed and centrifuged at maximum speed as previously described to pellet the DNA. The supernatant was carefully aspirated off, the pellet washed with 70% ethanol and dried for 10 min in an oven at ~35 to 40°C. The pellet was re-suspended in 50 µl of sterile distilled water and the purified DNA stored in a -28°C freezer for polymerase chain reaction (PCR).

Polymerase chain reaction (PCR)

PCR for the cellular gene, β_2-microglobulin, was performed to assess the quality of DNA using 5’-CAACTCTATCCACGTTCACC-3’ (PCO4) and 5’-GAAGAGCCAAGGACAGGTAC-3’ (GH20) primers, generating a fragment of ~408 nucleotides. To detect HPV sequences, the amplification mixture contained 1x PCR reaction buffer, 4 mM $MgCl_2$, 0.2 mM of each dNTP, 1.25U Taq polymerase

Table 1. HPV positivity and negativity rate among study patients.

Total No. (%) of subjects tested	No. negative (%) for HPV sequences	No. positive (%) for HPV sequences
93	33	60
(100.0)	(35.5)	(64.5)

and 0.2 µM of each primer. Amplification was performed at 92°C for 4 min, followed by 30 cycles of 30 s at 92°C, 30 s at 53°C and 30 s at 72°C with an extension of 72°C for 15 min.

Plasmid DNA (HPV 16 and 18) were used as positive controls in the amplification, in addition to the DNA extract, previously tested for the β_2-microglobulin gene and sterile distilled water as positive and negative controls, respectively.

Nested multiplex-PCR

A multiplex-PCR using eighteen (18) primer sets in 4 cocktails (Sotlar et al., 2004) was carried out as described subsequently, using the PCR core kit (Sigma, USA). Twenty micromolar (20 µM) primer stocks were used at 0.32 µM final concentrations in a 25 µl reaction volume.

A nested multiplex PCR (NMPCR) assay combining degenerate E6/E7 consensus and type-specific primers was performed to genotype HPV strains (Sotlar et al., 2004).

In the initial step E6/E7 consensus primers: GP-E6-3F/GP-E7-5B/GP-E7-6B was used for the first round amplification to detect generic HPV sequences. GP-E6-3F comprised 5'-GGGWGKKACTGAAATCGGT-3' and two consensus reverse primers GP-E7-5B (5'-CTGAGCTGTCARNTAATTGCTCA-3') and GP-E7-6B (5'-TCCTCTGAGTYGYCT AATTGCTC-3') respectively, where W=A+T, K=G+T, N=A+C+G+T, Y=C+T. In step 2, eighteen (18) type-specific primers for typing of HPV genotypes 6/11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, 39 and 68 were used for the nested PCR reactions. Four different reactions were carried out with 4 primer sets, constituted into 4 cocktails in the following.

Cocktail 1: Primers for HPV 16, 18, 31, 59 and 45,
Cocktail 2: Primers for HPV 33, 6/11, 58, 52 and 56,
Cocktail 3: Primers for HPV 35, 42, 43 and 44,
Cocktail 4: Primers for HPV 68, 39, 51 and 66.

The four second round primer cocktail sets were used for genotyping according to the protocol described by Soltar et al. 2004 (14) with slight modifications.

First and second round PCRs were both performed in a final volume of 25 µl with each PCR mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1% Triton-X100, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 320 nM of each of the primers and 1.25 U of Taq polymerase. The amplifications were done in a thermal cycler (Applied Biosystems 1720, USA) with the following parameters.

For the first round PCR, using 5.0 or 1.0 µl of DNA lysate, an initial 1-round denaturation at 94°C for 4 min was followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 40°C for 2 min and an extension at 72°C for 2 min. A single final extension at 72°C for 10 min was performed before soaking at 4°C. For the second round PCR, 2.0 µl of the first round PCR product were used as the template DNA with each of the four cocktails of type specific primers for genotyping (Sotlar et al., 2004). Cycling conditions were as follows: an initial denaturation at 94°C for 4 min was followed by 35 cycles of, denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and an extension at 72°C for 45 s. This was followed by a single final extension at 72°C of 4 min, before a soaking step at 4°C.

For each round of PCR reaction, HPV-16 or/and HPV-18 genome in plasmid DNA were used as positive control while nuclease free water was used as a negative control. A sample each, found to be HPV negative or positive, was subsequently used as additional negative and positive control, respectively.

Following PCR, 10 µl of the second round products were resolved on a 2% agarose gel stained with 0.001 mg/ml ethidium bromide. The electrophoresis was carried out in 1X Tris Acetate EDTA (TAE) buffer at 100 Volts for 1 h and the gel photographed over a UV trans-illuminator.

RESULTS

Ninety-three samples were analyzed. Thirty three, representing 35.5% of the study population tested negative. Sixty (64.5%) were positive for HPV infection. One (1.7%) patient exhibited sequences to six genotypes simultaneously while 5.0% (n=3) had detectable sequences to 5 genotypes, 8.3% (n=5) with sequences from 4 genotypes, 13.3% (n=8) with sequences from 3 genotypes and 28.3% (n=17) with sequences from 2 HPV genotypes. Majority (n=26), representing 43.4% had sequences of a single HPV genotype (Tables 1 and 2).

Those infected with either high risk or low risk genotypes alone or concurrently with others were determined (Tables 2 and 3). In this study, "other" was reported as genotypes that were 'probably carcinogenic' (HPV genotypes 26, 34, 53) or 'probably high risk' (53 and 66). Twenty six (26)/60 (43.4%) patients had a single infection. Of these, 15/26 (57.7%) had an infection from high risk (HR) genotypes, while 9/26 (34.6%) were infected with low-risk (LR) HPV genotypes. Two (7.7%) had a single infection from HPV genotypes described as "other" in Tables 2, 3 and 4 (Genotypes 26, 34, 53 and 66). Thirty four (34/60) (56.7%) had simultaneous HPV infections, involving both LR and HR- genotype combinations or between LR/HR/ other' genotypes. Of the patients with dual infections (n=17), 4 had both viruses being HR HPV genotypes. In one patient, both genotypes were LR, while eight had a combination of HR and LR. Two patients each had infections from genotypes classified as either HR/other or LR/other. Eight patients (13.3%) were infected with 3 HPV genotypes as follows: two had LR genotypes; four had HR/LR genotype while two each had either HR/other or LR/other genotypes.

Five patients had infections from 4 HPV genotypes simultaneously which were HR/LR/other in nature. Three patients had infections from 5 HPV genotypes simultaneously, of which 2 were HR/LR and 1 was HR/LR/other in nature. Finally, one patient had six infections

Table 2. Proportion of positive patients infected with either single or multiple HPV genotypes.

Type of infection	Number of infecting genotypes	No of patients (%)	Category of HPV infections observed and number of subjects affected						
			HR only	LR only	Other	HR/LR	HR/other	LR/other	HR/LR/ other
Single	1	26 (43.4)	15	9	2	-	-	-	-
	2	17 (28.3)	4	1	-	8	2	2	-
	3	8 (13.3)	-	2	-	4	-	1	1
Multiple	4	5 (8.3)	-	-	-	-	-	-	5
	5	3 (5.0)	-	-	-	2	-	-	1
	6	1 (1.7)	-	-	-	1	-	-	-
-	-	60 (100)	-	-	-	-	-	-	-

LR: Low risk, HR: high risk, Other: genotypes 'probably carcinogenic' (HPV genotypes 26, 34, 53) or 'probably high risk' (53 and 66).

Table 3. Profile of categorized HPV genotypes observed in 26 single infected patients.

S/N	Sample ID of patient	Genotype identified	HR only	LR only	Other
1	002	43	-	1	-
2	006	42	-	1	-
3	007	**52**	1	-	-
4	013	**58**	1	-	-
5	015	**68**	1	-	-
6	017	**68**	1	-	-
7	018	**18**	1	-	-
8	027	**66**	-	-	1
9	029	**68**	1	-	-
10	041	42	-	1	-
11	045	42	-	1	-
12	048	56	1	-	-
13	056	16	1	-	-
14	070	35	1	-	-
15	071	43	-	1	-
16	074	66	-	-	1
17	076	43	-	1	-
18	081	43	-	1	-
19	083	42	-	1	-
20	093	42	-	1	-
21	105	58	1	-	-
22	106	33	1	-	-
23	109	35	1	-	-
24	120	56	1	-	-
25	124	68	1	-	-
26	129	35	1	-	-

LR: Low risk, HR: high risk, Genotypes in bold print = HR. Other: Genotypes 'probably carcinogenic' (HPV genotypes 26, 34, 53) or 'probably high risk' (53 and 66).

simultaneously which were HR/LR. The exact profile of these infections is detailed in Tables 2, 3 and 4.

Table 3 shows the 26 patients with a single infection, their ID number and the infecting genotype, characterized as HR, LR or other. Patients with ID numbers 027 and 074 were infected with genotype 66 characterized as 'other'. Patients with ID numbers 018 and 056 were infected with HR genotypes 18 and 16, respectively.

Table 4. Profile of categorized HPV genotypes observed in 34 multiple infected patients.

S/N	No. of genotypes identified	Sample ID of patient	Genotypes identified	HR only	LR only	Other	HR/ LR	HR/ Other	LR/ Other	HR/ LR/ Other
1		005	39, 68	1	-	-	-	-	-	-
2		008	42, 43	-	1	-	-	-	-	-
3		009	**39, 68**	1	-	-	-	-	-	-
4		010	6/11, 43	-	1	-	-	-	-	-
5		012	42, **68**	-	-	-	1	-	-	-
6		014	**58, 68**	1	-	-	-	-	-	-
7		019	6/11, 43	-	1	-	-	-	-	-
8		021	**58**, 66	-	-	-	-	1	-	-
9		024	**52**, 66	-	-	-	-	1	-	-
10	2	046	42, **56**	-	-	-	1	-	-	-
11		049	16, 42	1	-	-	1	-	-	-
12		064	42, **58**	-	-	-	1	-	-	-
13		069	44, **68**	1	-	-	-	-	-	-
14		087	42, **52**	-	-	-	1	-	-	-
15		092	42, 66	-	-	-	-	-	1	-
16		101	44, **56**	-	-	-	1	-	-	-
17		112	**35, 39**	1	-	-	-	-	-	-
18		122	43, **45**	-	-	-	1	-	-	-
19		128	44, 66	-	-	-	-	-	1	-
1		016	**18**, 42, 43	-	-	-	1	-	-	-
2		060	42, **56**, 66	-	-	-	-	-	-	1
3		085	42, 43, 66	-	-	-	-	-	1	-
4	3	091	42, **45, 56**	-	-	-	1	-	-	-
5		110	6/11, **35, 39**	-	-	-	1	-	-	-
6		114	**35**, 44, **58**	-	-	-	1	-	-	-
7		125	6/11, **35**, **39**	-	-	-	1	-	-	-
8		137	44, **51**, **56**	-	-	-	1	-	-	-
1		095	**35,** 42, **58,** 66	-	-	-	-	-	-	1
2	4	135	**39,** 43, **45, 59**	-	-	-	1	-	-	-
3		136	43, **45, 51, 68**	-	-	-	1	-	-	-
4		066	6/11, 43**,45,56**	-	-	-	1	-	-	-
1		038	42, 43**, 58,** 66**, 68**	-	-	-	-	-	-	1
2	**5**	094	**35,** 42, **45, 52, 68**	-	-	-	1	-	-	-
3		111	6/11, **35,** 43, **45, 58**	-	-	-	1	-	-	

LR: Low risk, HR: high risk, Genotypes in bold print = HR. Other: Genotypes 'probably carcinogenic' (HPV genotypes 26, 34, 53) or 'probably high risk' (53 and 66).

Table 4 details the infecting HPV genotype profiles for patients with multiple infections. These infecting genotypes were also classified as HR only, LR only, 'other', HR/LR, HR/other, LR/other and HR/LR/other. Thus, patient with ID number 021, for instance, had dual infection with genotypes 58 and 66, categorized as HR/other.

In Table 5, the frequency distribution and prevalence of HPV genotypes in our population of 60 was shown. In all, 16 out of ~70 genotypes were observed. LR genotypes 42 and 43 were the most frequently occurring, affecting 19 and 14 of the patients representing 31.7 and 23.3% prevalence, respectively. The HR genotypes 16 and 18 each had a frequency of 2 or 3.3% prevalence, that is, genotype 16 sequences, for example, were seen in one patient (ID 056) with a single infection and in another (ID 049) with a dual infection. Genotype 18 sequences were observed in patient (ID 018) with a single infection and also in patient (ID 016) with a triple infection.

Genotype 66 ("other") was observed in 10 patients (16.7%), 2 (3.3%) with a single infection and 8 (13.3%) with multiple infections. Globally, even though HR sequences were more than LR, individual frequencies of HR were lower than LR genotypes 42 and 43.

Table 5. HPV frequencies and prevalence observed in 60 genopositive patients.

S/N	Category of HPV	HPV genotypes	Number of infecting HPV genotypes						HPV Frequency	HPV Prevalence (%)
			Single infection	Multiple infections						
			1	2	3	4	5	6		
1	HR	6/11	1	2	2	1	1	-	6	10.0
2		16	1	1	-	-	-	-	2	3.3
3		18	1	-	1	-	-	-	2	3.3
4		**33**	1	-	-	-	-	-	1	1.7
5		**35**	3	1	-	3	1	1	9	15.0
6		**39**	-	1	-	-	-	-	1	1.7
7	LR	42	5	7	4	1	2	-	19	31.7
8		43	3	2	4	2	2	1	14	23.3
9		44	-	3	2	-	-	-	5	8.3
10	HR	45	-	1	1	3	2	-	7	11.7
11		51	-	-	1	1	-	-	2	3.4
12		**52**	1	2	-	-	1	-	4	6.7
13		**56**	2	2	3	-	1	-	8	13.3
14		**58**	2	3	1	1	1	1	9	15.0
15	Other	**66**	2	3	2	1	1	-	9	15.0
16	HR	**68**	4	5	-	1	2	-	12	20.0

Genotypes in bold print = HR

DISCUSSION

Single and multiple infections were observed from a broad spectrum of HPV genotypes in a cross-sectional study involving 60 subjects. These were attendees of antenatal clinics in the largest hospital in Ghana. Multiple infections occurred between HR and LR, as well as in combination with other genotypes. From our data, 3 to 7% of this cohort had infection with HPV 16 and 18, the two extremely high risk oncogenic genotypes. This prevalence suggested a low burden of disease from these genotypes. This was also observed in another study in Ghana (Attoh et al., 2010) and a second from Burkina-Faso, a country with which Ghana shares a common border (Didelot-Rousseau et al., 2006). In Ghana, this situation contradicts reports that HPV 16 is the most common type associated with invasive cervical carcinoma (Muñoz et al., 2004; Wheeler et al., 2009; Attoh et al., 2010).

With the reported increase in the prevalence of cervical cancer in Ghana and the observation of a broad spectrum of HPV genotypes, however, other HR (and probably non-HR) genotypes may be assuming more importance and relevance in cervical carcinogenesis than previously thought.

In our apparently healthy population, it was observed for instance, that LR genotypes 42 and 43 occurred more frequently than the HR genotypes. In combination with other genotypes, the virulence of the resulting phenotype could not be readily ascertained.

Infections with HPV genotypes are fundamentally independent of each other (Erickson et al., 2013). There is hardly any evidence of interaction between different carcinogenic HPV types, while minimal interaction occurred at the viral-viral level regarding persistence, clearance or acquisition. However, the Ludwig-McGill Study Group investigating the natural history of HPV and cervical neoplasia reported that infections with multiple HPV types seemed to act synergistically to promote cervical carcinogenesis (Trottier et al., 2006). The profile of infecting HPV genotypes in our study population left unclear what their role could be in cervical carcinogenesis and the extent to which the subjects were at risk for cancer. This underscores the need to conduct a large scale longitudinal study to investigate multiple infections with HPV and their contribution to any potential cervical carcinogenesis in Ghanaian women. In 2013, Ghana embarked on a pilot vaccination programme to immunize school children ahead of a policy decision on routinely including HPV screening and vaccination in the Expanded Programme on Immunization of the Ghana Health Service (GHS, 2013). The decision to undertake routine prophylactic vaccination in such populations or otherwise can be justified from a follow-up to this study. Adolescents in high school around ages of 12-15 years could be screened on a large scale to determine the

prevalence of HPV genotypes, especially the known oncogenic ones.

A comprehensive HPV typing is reportedly better at disease prognosis. As such it is preferable to follow a protocol (as used in this study) which identifies most genotypes than one that merely detects only the main oncogenic HPV types (Castle et al., 2005; Schiffman and Castle, 2005). A preventative vaccine from recombinant L1 capsid protein virus-like particles (Villa et al., 2005, Didelot-Rousseau., 2006) has shown good immunogenicity, especially targeting the most prevalent high-risk HPVs (HR-HPV) worldwide, namely genotypes 16 and 18 (Koutsky et al., 2002; Ault et al., 2004) but also capable of immunizing persons infected with other genotypes.

In conclusion, our HPV genotype profile of 60 adult healthy pregnant women from the general population of Ghana varied. 3 to 7% were infected with HPV genotypes 16 and 18. The frequency of some LR HPVs was higher than HR genotypes. Several subjects had sequences of multiple HPVs of all categories. Against the observation of increasing cervical cancer cases in Ghana, other non HPV genotypes may be assuming a greater prominence in carcinogenesis than previously thought. This necessitates further studies, longitudinal in nature, to investigate the possible role of such other genotypes in mixed infections generated from a population-based group in Ghana, profiling HPV genotypes as a marker for the potential development of cervical cancer.

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Conflict of interest

The authors declare “no conflict of interest” with regard to the publication of this manuscript.

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