High-risk HPV in apparently healthy women

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Prevalence of high-risk human papillomavirus genotypes among apparently healthy women with normal and abnormal cervical cytology in Kaduna State, Nigeria

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Abstract:

Background: About 99.7% of cervical dysplasia and cancer cases are caused by persistent genital high-risk human papillomavirus (hrHPV) infection. Most HPV infections are subclinical and self-limiting but may persists in about 5 to 10% of infected women, resulting in pre-cancerous lesions that can progress to invasive cancer years later. This study is aimed at detecting hrHPV among apparently healthy women of reproductive age in Kaduna State, thus providing more information for effective control of HPV and cervical cancer in Nigeria.
Methodology: Cervical smears were taken from 515 randomly selected apparently healthy women across selected secondary and tertiary facilities from 3 Local Government Areas (LGAs) in each Senatorial Zone of Kaduna State, Nigeria. Liquid-based cytology (LBC) technique was used to collect cervical smears and prepare smears for cytology study, while the remaining samples were stored at -80°C for molecular studies. HPV DNA were extracted from the samples and amplified by convectional PCR using specific hrHPV (HPV 16,18,31 and 45) primer sets and a broad spectrum MY09/11 and GP5+/6+ primers for a wider range of HPV genotypes. Data were analysed using the Statistical Package for Social Sciences (SPSS) version 23.0 and relationship between prevalence of hrHPV and socio-demographic factors such as age and marital status were determined using Chi-square or Fisher Exact test with $p<0.05$ considered statistically significant.
Results: The prevalence of total HPV and hrHPV infections in the study population was 11.8% (61/515) and 9.3% (48/515) respectively. A total of 100 HPV genotypes were detected by PCR in the 61 positive smears, with 66 hrHPV types from 48 women, and 34 other HPV types from 13 women. The frequency of hrHPV genotypes detected was HPV 31 (5.8%, n=30), HPV 45 (4.1%, n=21), HPV 16 (1.7%, n=9), and HPV 18 (1.2%, n=6), with other HPV genotypes (6.6%, n=34). The frequency of cervical dysplasia was 6.4% (33/515), which was significantly associated with all HPV genotypes except HPV 16. Single HPV infection was seen in 31 (51.8%) women while multiple infections were seen in 30 (49.2%), with double infection in 21 (34.4%) and triple infections in 9 (14.7%).
Conclusion: The prevalence of hrHPV infection was high among women in Kaduna State, Nigeria. DNA-based screening for hrHPV genotypes and production of new vaccine that will protect against the predominant hrHPV genotypes are thus recommended for the prevention of cervical cancer in Nigeria, Africa and beyond.

Keywords: High-risk human papillomavirus; genotypes; cytology; cervical cancer

Prévalence des génotypes du virus du papillome humain à haut risque chez les femmes apparemment en bonne santé présentant une cytologie cervicale normale et anormale dans l'État de Kaduna, au Nigeria

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Résumé:

Contexte: Environ 99,7% des cas de dysplasie cervicale et de cancer sont causés par une infection génitale persistante au papillomavirus humain à haut risque (hrHPV). La plupart des infections au VPH sont subcliniques

et spontanément résolutives, mais peuvent persister chez environ 5 à 10% des femmes infectées, entraînant des lésions précancéreuses pouvant évoluer vers un cancer invasif des années plus tard. L'étude vise à détecter le hrHPV chez les femmes apparemment en bonne santé et en âge de procréer dans l'État de Kaduna, fournissant ainsi plus d'informations pour un contrôle efficace du VPH et du cancer du col de l'utérus au Nigeria.

Méthodologie: Des frottis cervicaux ont été effectués sur 515 femmes apparemment en bonne santé sélectionnées au hasard dans des établissements secondaires et tertiaires sélectionnés de 3 zones de gouvernement local (LGA) dans chaque zone sénatoriale de l'État de Kaduna, au Nigeria. La technique de cytologie en milieu liquide (LBC) a été utilisée pour collecter des frottis cervicaux et préparer des frottis pour une étude cytologique, tandis que les échantillons restants ont été conservés à -80°C pour des études moléculaires. L'ADN du VPH a été extrait des échantillons et amplifié par PCR convectionnelle en utilisant des ensembles d'amorces spécifiques du hrHPV (HPV 16, 18, 31 et 45) et des amorces MY09/11 et GP5+/6+ à large spectre pour une gamme plus large de génotypes du VPH. Les données ont été analysées à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 23.0 et la relation entre la prévalence du hrHPV et les facteurs sociodémographiques tels que l'âge et l'état civil ont été déterminées à l'aide du test du chi carré ou de Fisher Exact avec $p<0,05$ considéré comme statistiquement significatif.

Résultats: La prévalence des infections totales par le VPH et le hrHPV dans la population étudiée était respectivement de 11,8% (61/515) et 9,3% (48/515). Au total, 100 génotypes HPV ont été détectés par PCR dans les 61 frottis positifs, avec 66 types hrHPV provenant de 48 femmes et 34 autres types HPV provenant de 13 femmes. La fréquence des génotypes hrHPV détectés était HPV 31 (5,8%, n=30), HPV 45 (4,1%, n=21), HPV 16 (1,7%, n=9) et HPV 18 (1,2%, n=6), avec d'autres génotypes de VPH (6,6%, n=34). La fréquence de la dysplasie cervicale était de 6,4% (33/515), ce qui était significativement associé à tous les génotypes de VPH, à l'exception du VPH 16. Une infection unique au VPH a été observée chez 31 (51,8%) femmes, tandis que des infections multiples ont été observées 30 (49,2%), avec double infection chez 21 (34,4%) et triple infection chez 9 (14,7%).

Conclusion: La prévalence de l'infection par le hrHPV était élevée chez les femmes de l'État de Kaduna, au Nigeria. Le dépistage basé sur l'ADN des génotypes hrHPV et la production d'un nouveau vaccin qui protégera contre les génotypes hrHPV prédominants sont donc recommandés pour la prévention du cancer du col de l'utérus au Nigeria, en Afrique et au-delà.

Mots-clés: Papillomavirus humain à haut risque; génotypes; cytologie; cancer du col de l'utérus

Introduction:

It is estimated that viral infections contribute to 15–20% of all human cancers (1). Viruses are obligate parasites which encode proteins that reprogram host cell metabolism and the immune system. Infection by oncogenic viruses can promote different stages of carcinogenesis. Among many types of human papillomaviruses (HPV), around 15 are linked to cancer. Human papillomaviruses are members of the Papovaviridae and consist of almost 8000 bp long circular DNA molecules that are wrapped into a protein shell which is composed of two molecules, L1 and L2 (2).

Cervical dysplasia is caused by the high-risk human papillomavirus (hrHPV) and can develop into cancer at any age. However, follow up and treatment can help to prevent cancer will depend on age of the woman. Human papillomavirus is a common virus that is spread through sexual contact. Persistent infection by certain genotypes of hrHPV plays a crucial role in creating tumors. Human papillomaviruses are a diverse group of viruses that can infect numerous epithelial sites and cause a variety of epithelial lesions, including common warts, verrucous, laryngeal papillomas, and genital condylomata, depending on the HPV types (3).

Human papillomaviruses are found to be present in 99.7% of cervical cancer specimens (4). Most sexually active and unvaccinated men and women get the virus at some point in their life (5). There are over 100 types of HPV which causes of genital warts and may resolve without treatment in immuno-competent individuals but may persist and spread widely in patients with decreased cell-mediated immunity. The different types that infect the female genital tract have been divided into hrHPV, which includes types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68 and low-risk HPV (lrHPV) which are types 6, 11, 40, 42, 54, and 57.

The lrHPVs are associated with benign exophytic genital warts (condylomata acuminata) and are rarely associated with high-grade squamous intraepithelial lesions (HSILs) or invasive squamous cancers. Conversely, HPV 16, the most prevalent virus infecting the cervix, are associated with the entire spectrum of cervical intraepithelial neoplasia (CIN) lesions as well as invasive squamous carcinomas. Recent studies have demonstrated that hrHPV types account for almost 90% of all cervical cancers (6). The study of HPVs has been driven not by these widespread inapparent infections, but by the severity to which some hrHPV-associated diseases can progress. Most significant of these is cervical cancer, which can result from persistent hrHPV infection (3).

Describing the association between multiple HPV infections and cervical disease is important in generating hypotheses regarding its pathogenesis (7). Identifying the individual hrHPV types in each grade of cervical neoplasia is important for the development of HPV vaccines and screening strategies. Provision of appropriate hrHPV vaccines is necessary to protect against many of the HPV strains that

can cause genital warts and cancer. The cervical disease is strongly influenced by cultural and religious practices that govern sexual behavior and transmission of HPV. The sub-Saharan Africa has the highest estimated rates of cervical cancer, and in Guinea, Malawi, and Zambia, the age-standardized incidence rate is over 50 per 100,000 population (8).

In Nigeria, cervical cancer ranks as the second most frequent cancer among women and the second most frequent cancer death among women between the ages of 15 and 44 years (9). The seroprevalence of hrHPV has been reported to be 66.7% and Magaji et al., (10) noted that cancer of the cervix is the most common malignancy among women in Kaduna State, Nigeria. Almost all of cervical cancer deaths could be avoided by early detection and diagnosis of cervical dysplasia. An effective intervention could be made available to women with pre-cancerous lesions caused by persistent HPV after cervical screening. The objective of this study is to determine the prevalence of hrHPV among apparently healthy women of reproductive age in Kaduna State, in order to provide more information for effective control of HPV and cervical cancer in Nigeria.

Materials and method:

Study setting:
This study was conducted in 3 selected health facilities from each senatorial district in 9 randomly selected Local Government Areas (LGAs) of Kaduna State, Nigeria. The health facilities included the Ahmadu Bello University Teaching Hospital Zaria in Kaduna North Senatorial district, Barau Dikko Teaching Hospital (BDTH) in Kaduna Central Senatorial district and the General hospitals located in the selected LGAs in the State.

Study design:
The study is a hospital based cross-sectional comparative study which was conducted among apparently healthy women of reproductive age (15-65 years), irrespective of ethnicity, educational status and place of residence who were attending the Reproductive Health and Family Planning clinics in the selected facilities.

Sample size and sampling method:
The minimum sample size was determined using the Fisher formula (11), $n=Z^2p(1-p)/d^2$, where 'Z' is the standard normal variate (=1.96), 'p' is the local prevalence of 48.1% from a previous study (12), and 'd' is the degree of precision (=0.05). After adjusting for 10% attrition, the sample size of 422 was obtained, although a total of 515 consenting women were eventually enrolled for the study. Participants were consecutively recruited at the clinics of each selected facility until the sample size was obtained. Exclusion criteria were women older than 65 years and those with previous operative or therapeutic history of related to gynaecologic diseases.

Ethical consideration:
Ethical approvals for the study were obtained from the ethics committees of Ahmadu Bello University Teaching Hospital Zaria (ABUTH), Barau Dikko Teaching Hospital (BDTH) and Kaduna State Ministry of Health. Informed consent was obtained from each participant and the procedure for obtaining samples was explained to each participant before samples for investigations were collected.

Data collection:
A predesigned structured questionnaire was interviewer-administered to collect socio-demographic information such as age, education, occupation, type of family, geographic location, annual income, and possible risk factors from each participant.

Sample collection and transportation:
Liquid-based cytology (LBC) technique was used to collect cervical smears by the attending physician assisted by a reproductive health nurse following standard procedures. This was carried out by using a cytology brush to take the smears. The brush was rotated over the whole surface of the cervix, making sure that the squamo-columnar junction was well and truly scrapped. The smeared brush was detached into a special vial containing the preservative liquid and labeled appropriately.

The specimens were transported to the Pathology laboratory of the Ahmadu Bello University Teaching Hospital Zaria where an aliquot of the smear was vortexed to obtain homogeneous mixture and strained to remove other elements such as mucor. The mixture was then centrifuged into layers based on density gradient. With the aid of a special polycarbonate filter, a thin layer of cells was placed on a clean grease free glass slide and deposit was processed using standard procedures. The remaining sample was stored at -80ºC for molecular studies at the Institute of Human Virology Regional Laboratory, Jos, Nigeria.

Cytology technique:
A total of 5ml of vortexed cervical sample was centrifuged at 3000 revolution per minute (rpm) for 2 mins. The supernatant was decanted and 2ml of clearing fluid was added and vortexed again after which it was centrifuged at 3000 rpm for 2 mins, and the supernatant discarded. Two drops of polymer base were added to the sediment and thoroughly mixed to make the LBC smear on a clean grease-free glass slide.

The slides were washed with distilled water, stained in a solution of Haris haemat-

oxylin for 5 mins and washed in distilled water. Scott tap-water was used to blue the stained slide before washing in 95% ethanol following which Orange-G-6 was applied to stain the smear for 90 seconds and washed with 95% ethanol again. The smear was stained with Eosin Azure 50 for another 90 seconds and washed again in 95% ethanol. The preparation was finally dehydrated in absolute alcohol, cleared with xylene and mounted on Distrene Polystrene Xylene (DPX) for microscopic examination by a cytologist using the Bethesda system of reporting cytology slides.

DNA extraction:

Extraction of the DNA was performed using Quick-DNA™ viral kit (Zymo Research) following the manufacturer's instructions. Briefly, in a 1.5 ml Eppendorf tube, 800 μl of viral DNA buffer containing beta-mercaptoethanol to a final dilution of 0.5% (v/v), was added to 200 μl of each sample followed by brief vortexing and incubation for 10 mins at room temperature. Subsequently, the mixture was transferred to Zymo-Spin™ immuno chromatographic (IC) column in a collection tube and centrifuged at 10, 000 x g for 1 min.

The samples were washed with 300 μl DNA wash buffer containing 100% ethanol and the columns dried by centrifugation at 10,000 x g for 1 min. The flow through with collection tubes were discarded. The Zymo-Spin™ IC columns were transferred into new 1.5 ml Eppendorf microcentrifuge tubes and the DNA was eluted by addition of 10 μl DNA elution buffer directly onto the column matrix, incubated at room temperature for 1min and centrifuged at 10, 000 x g for 1 min to elute the DNA. The processed samples were then stored at -20°C until analysis.

Polymerase chain reaction set up:

Each sample was amplified by PCR as adapted from the work of Shikova et al., (13) using the following primer sets; consensus primers MY09/MY11 and GP5+/GP6+, and the type-specific primers for HPV16, 18, 31 and 45 as shown in Table 1. PCR assays were carried out in a final volume of 50 μl containing 25 μl One Taq Quick-Load 2X Master Mix (New England Biolabs, Inc.), 2 μl of each primer, 13 μl of water and 10 μl of genomic DNA sample. Amplifications were performed with the following cycling outline; initial denaturation at 94°C for 5 mins, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 40°C for 2 mins, and extension at 72°C for 1 min, followed by a final extension of 10 mins at 72°C.

Gel electrophoresis of PCR amplicons:

The PCR products were run on a 2% agarose gel stained with ethidium bromide. The amplicons were loaded into wells created in the agarose gel with a gel comb. The first well was loaded with 100 bp size ladder (Promega) which served as the standard DNA marker. Electrophoresis was carried out at 90V for 30 mins and the gel was visualized under UV light in Bio-Rad Gel Doc™ Universal Hood II Imaging System Lab.

Statistical analysis:

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 23.0, and presented in frequency distribution tables, and bar and pie charts. The relationship between prevalence of hrHPV and socio-demographic factors such as age and marital status were determined using Chi-square or Fisher Exact test with $p<0.05$ considered statistically significant.

Table 1: Sequence of primers used in the study

Primer name	Primer sequence	No of bp
MY09	CGT CCA CAA GAG GGA TAC TGA TC	23
MY11	GCA CCA GGG ATC ATA ACT AAT GG	23
GP5	TTTGTTACTGTGGTAGATACTAC	23
GP6	GAAAAATAAACTGTAAATCATATTC	25
16L1F	TGC TAG TGC TTA TGC AGC AA	20
16L1R	ATT TAC TGC AAC ATT GGT AC	20
16E6/F	TTG CTT TTC GGG ATT TAT GC	20
16E6/R	AGA TCA GTT GTC TCT GGT TGC A	22
18F	AAG GAT GCT GCA CCG GCT GA	20
18R	CAC GCA CAC GCT TGG CAG GT	20
31F	ATG GTG ATG TAC ACA ACA CC	20
31R	GTA GTT GCA GGA CAA CTG AC	20
45F	ACC AGA TTT GTG CAC AGA AT	20
45R	TTT TTT CCA GTG TCT CTC CA	20

bp=base pair

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Results:

Cervical smear samples of 515 apparently healthy women were examined for the presence of HPV and hrHPV DNA (16, 18, 31, and 45). Human papillomavirus DNA was detected in smears of 61 women, of which 48 were positive for hrHPV (and 13 were positive for other HPVs), giving HPV and hrHPV prevalence rates of 11.8% and 9.3% respectively among the study participants.

Figures 1, 2 and 3 are of the gel electrophoresis pictures of representative DNA amplicons from the samples. A total of 100 HPV DNA types were detected by PCR in the 61 positive smears, with 66 hrHPV DNA types from 48 women, and 34 other HPV DNA types from 13 women. The frequency of hrHPV genotypes detected was HPV 16 (n=9), HPV 18 (n=6), HPV 31 (n=30) and HPV 45 (n=21), with other HPV genotypes (n=34) (Fig 4).
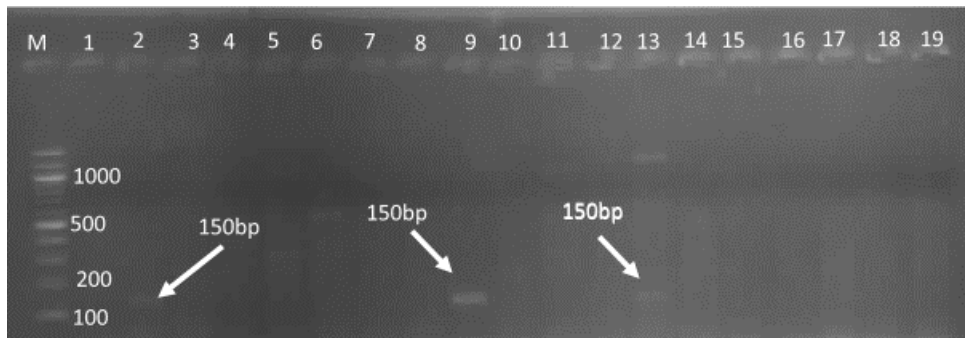

Fig 1: Gel electrophoresis picture of HPV DNA amplicons (Lanes 2, 9, and 13) with consensus primer GP5+/GP6+ (150 bp)

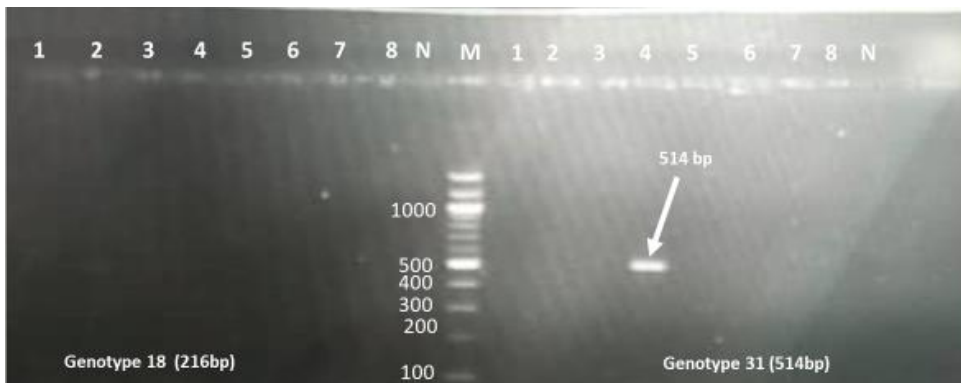Fig 2: Gel electrophoresis picture of PCR amplicon of HPV 16 in lane 2 (152 bp)

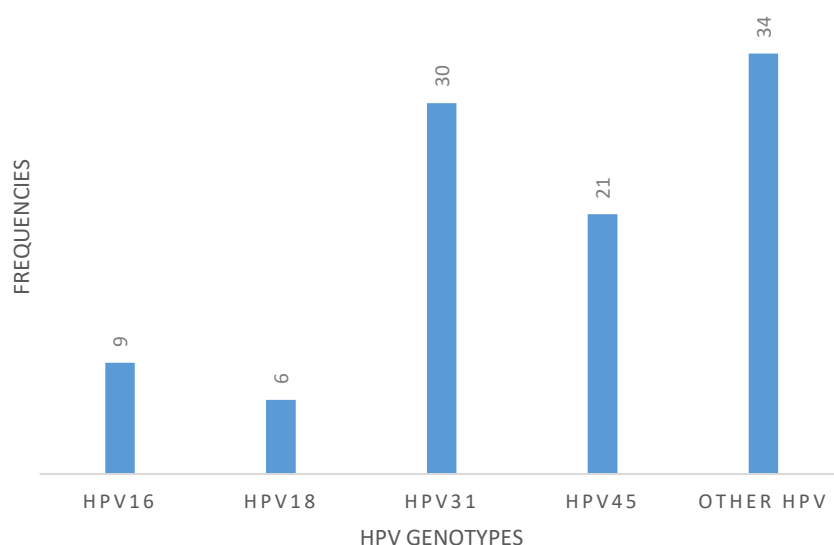Fig 3: Gel electrophoresis of PCR amplicon of HPV 31 in lane 4 (514 bp)

Fig 4: Distribution of HPV genotypes among women of reproductive age in Kaduna State, Nigeria

Table 2: Distribution of human papillomavirus genotypes and cervical dysplasia in association with socio-demographic characteristics of apparently healthy women in Kaduna State, Nigeria

Socio-demographic Variable	Number of women examined	No of women with HPV infection (%)	X^2	*p* value	No of women with cervical dysplasia (%)	X^2	*p* value
Age group (years)							
16-25	31	2 (6.5)	9.09	0.0609	0	19.069	0.0008*
26-35	119	16 (13.4)			7 (5.9)		
36-45	173	29 (16.8)			5 (2.9)		
46-55	116	8 (6.9)			17 (14.7)		
56-65	76	6 (7.9)			4 (5.3)		
Total	515	61 (11.8)			33 (6.4)		
Educational status							
None	66	4 (6.1)	2.585	0.694	5 (7.6)	0.3919	0.9831
Primary	74	10 (13.5)			4 (5.4)		
Secondary	144	19 (13.2)			10 (6.9)		
Tertiary	199	24 (12.1)			12 (6.0)		
Qur'anic	32	4 (12.5)			2 (6.3)		
Total	515	61 (11.8)			33 (6.4)		
Marital status							
Single	34	3 (8.8)	1.173	0.7596	3 (8.8)	3.914	0.2710
Married	422	53 (12.6)			23 (5.5)		
Divorced	33	3 (9.1)			4 (12.1)		
Widowed	26	2 (7.7)			3 (11.5)		
Total	515	61 (11.8)			33 (6.4)		
Ethnicity							
Hausa	224	25 (11.2)	5.942	0.1145	12 (5.4)	1.575	0.6651
Yoruba	38	1 (2.6)			4 (10.5)		
Igbo	65	12 (18.5)			4 (6.2)		
Others	188	23 (12.2)			13 (6.9)		
Total	515	61 (11.8)			33 (6.4)		

*Statistically significant at *p*<0.05

The socio-demographic characteristics of the participants as presented in Table 2 shows that HPV infection was highest among women in the age group 36-45 years with 16.7% (29/173), and lowest among women in age group 16-25 years (6.4%, 2/31), although there was no significant difference in HPV prevalence among the different age group of participants (x^2=9.09, *p*=0.0609). However, the prevalence of cervical dysplasia was highest among women in the age group 46-55 years (14.7%, 17/116), which was statistically significant (x^2=19.069, *p*=0.0008).

Women with primary and secondary education have higher frequency of HPV infection with 13.5% and 13.2% respectively (x^2= 2.585, *p*=0.694) while cervical dysplasia was highest in women with no education (7.6%)

and women with secondary level education (6.9%) (x^2=0.392, p=0.9831), although the prevalence differences in HPV infection and cervical dysplasia were not statistically significant with respect to level of education.

The prevalence of HPV infection was highest among married women (12.6%, p=0.7596) while cervical dysplasia was highest among divorced (12.1%) and widowed women (11.5%), but the difference was also not statistically significant (p=0.2710). Women of the Igbo tribe had the highest frequency of HPV infection (18.5%, p=0.1145) while women of Yoruba tribe had the highest frequency

of cervical dysplasia (10.5%, p=0.6651) but the difference was not statistically significant.

In all, hrHPV 31 was the most prevalent high-risk HPV genotype occurring in 30 (49.2%) of the 61 women with HPV infection. The distribution of single and multiple HPV infections in Table 3 showed that 31 (51.8%) women had single and 30 (49.2%) women had multiple infections [21 (34.4%) double and 9 (14.7%) triple HPV infections] (Fig 5). Single HPV infection was highest among women with HPV 45 (n=6) and lowest among those with HPV 18 (n=3).

Table 3: Distribution of single and multiple HPV genotypes among apparently healthy women in Kaduna State, Nigeria

| Type of infection/HPV genotype	No of participants (%)
Single infection	
HPV 16	4 (6.5)
HPV 18	3 (4.9)
HPV 31	5 (8.2)
HPV 45	6 (9.8)
Other HPV	13 (21.3)
	31 (50.8)
Double infection	
HPV 16, Other HPV	1 (1.6)
HPV 18, Other HPV	1 (1.6)
HPV 31, Other HPV	11 (19.6)
HPV 16, HPV 45	1 (1.6)
HPV 31, HPV 45	7 (11.5)
	21 (34.4)
Triple infection	
HPV 16, HPV 31, HPV 45	1 (1.6)
HPV 16, HPV 45, Other HPV	2 (3.3)
HPV 18, HPV 31, Other HPV	2 (3.3)
HPV 31, HPV 45, Other HPV	4 (6.5)
	9 (14.8)
Total	**61 (100.0)**
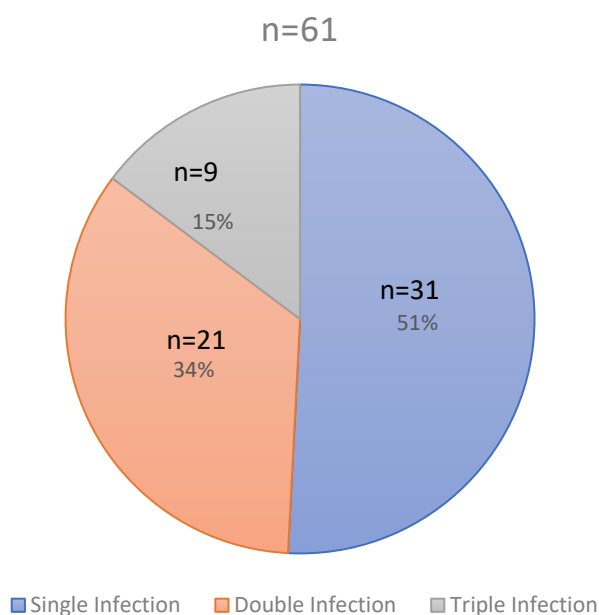
Fig 5: Distribution of single and multiple HPV infections among women in Kaduna State, Nigeria

Table 4 displays the distribution of HPV genotypes in the 33 women with cervical dysplasia, which when compared with women without cervical dysplasia, showed significant association of cervical dysplasia with all the HPV genotypes detected except HPV 16. The frequency of cervical dysplasia was highest among women with other HPV infections with 30.3% (10/33, OR=8.297, 95% CI=3.552-19.383, *p*<0.0001) followed by women with HPV 31 infections with 21.2% (7/33, OR= 5.373, 95% CI=2.111-13.672, *p*=0.0016), HPV 45 with 12.1% (4/33, OR=3.773, 95% CI=1.192-11.942, *p*=0.0385) and HPV 18 with 9.1% (3/33, OR=15.967, 95% CI=3.089-82.531, *p*=0.0042). The frequency of cervical dysplasia was lowest in women with HPV 16 infections (6.1%, 2/33), and when compared with the frequency of HPV 16 infections in women without cervical dysplasia (1.5%, 7/482), was not statistically significant (OR=4.378, 95% CI=0.8722-21.975, *p*=0.1081).

Table 5 shows the distribution of hr-HPV genotypes among women with cervical epithelial cell abnormalities (CEA) which indicates that HPV 16 and HPV 18 were implicated in 2 cases of low-grade squamous intra epithelial lesion (LSIL). High-grade squamous intra-epithelial lesion (HSIL) was detected in 3 women infected with HPV 31 and 4 women with unidentified (other) HPV genotypes. Multiple HPV genotypes were also detected among 4 women who had HSIL and LSIL.

Table 4: Frequency distribution of HPV genotypes and association with cervical dysplasia among the women participants

HPV types	No of HPV genotypes detected in all women (%) (n=515)	No of HPV detected in cervical dysplasia-positive women (%) (n=33)	No of HPV detected in cervical dysplasia-negative women (%) (n=482)	χ^2	OR (95% CI)	*p* value
HPV16	9 (1.7)	2 (6.1)	7 (1.5)	1.608	4.378 (0.8722-21.975)	0.1081
HPV18	6 (1.2)	3 (9.1)	3 (0.6)	12.584	15.967 (3.089-82.531)	0.0042*
HPV31	30 (5.8)	7 (21.2)	23 (4.8)	12.368	5.373 (2.111-13.672)	0.0016*
HPV45	21 (4.1)	4 (12.1)	17 (3.5)	3.842	3.773 (1.192-11.942)	0.0385*
Other HPV	34 (6.6)	10 (30.3)	24 (4.9)	28.146	8.297 (3.552-19.383)	<0.0001*
Total	100 (19.4)	26 (78.8)	74 (19.4)	75.427	20.479 (8.573-48.918)	<0.0001*

OR=Odd Ratio; CI=Confidence Interval; *= Statistically Significant at *p*<0.05

Table 5: Distribution of HPV genotypes in samples of women with cervical epithelial cell abnormalities in Kaduna State, Nigeria

No of HPV genotypes	No of HPV detected in sample with cervical dysplasia			No of HPV detected in sample with cervical non-dysplasia lesions		
	LSIL	HSIL	ASCUS	Anthropic changes	Inflammation	Cervicitis
HPV16 (n=9)	2	0	0	1	1	0
HPV18 (n=6)	2	1	0	0	0	0
HPV31 (n=30)	3	3	0	0	1	1
HPV45 (n=21)	4	1	0	0	1	1
Other HPV (n=34)	4	4	2	2	1	1
Total (n=100)	**15**	**9**	**2**	**3**	**4**	**3**
Multiple HPV (n=30)	4	4	1	1	2	1
Single HPV (n=31)	4	0	1	2	1	0
Total (n=61)	**8**	**4**	**2**	**3**	**3**	**1**

LSIL=Low-grade Squamous Intra-epithelial Lesion; HSIL= High-grade Squamous Intra-epithelial Lesion; ASCUS=Atypical Squamous Cells of Undermined Significance

Discussion:

Despite the high burden of cervical cancer morbidity and mortality in Nigeria, there is no reliable national prevalence of HPV genotypes in women. This study determined the prevalence of some hrHPV among the study population in Kaduna State, Nigeria and found that many women who were apparently healthy were actually infected with hrHPV. The prevalence of cervical HPV in this study is 11.8 % while the prevalence of hrHPV is 9.3%.

The HPV prevalence in our study is low compared to rates reported by similar studies across Nigeria. In Kano, northwest Nigeria, a high HPV prevalence of 76.0% was reported by Auwal et al., (14). Manga et al., (12) reported cervical HPV prevalence among women of 48.1% in a study carried out in Gombe, northeast Nigeria. In southwest Nigeria, cervical HPV was detected in 26.3% of sexually active women above 15 years in Ibadan and 14.7% among 1282 women in Irun (15). In Okene, northcentral Nigeria, a prevalence of 21.6% among 231 women was reported, and in Abuja, the Federal Capital of Nigeria, the prevalence was 37% among 275 women studied (16). The variation in the HPV prevalence in our study from those of others could be due to the differences in methodology and nature of samples used. Most studies determined HPV seroprevalence using ELISA but our study used molecular detection of HPV DNA on cervical samples using liquid-based technique. There is high probability of false positive detection of HPV antigens or antibodies in serological studies without detection of viral DNA in the cervical scrapings as done in our study.

Age is a significant socio-demographic factor because the chances of a woman developing cervical dysplasia increases with increasing age. According to Mosuro et al., (17), the mean age for developing dysplasia and carcinoma-in-situ ranged from 34.7 to 38.6 years, and 39.6 to 43.5 years, respectively. In our study, the age group 46-55 years had the highest frequency of cervical dysplasia (14.7%, 17/116) that was statistically significant ($p=$ 0.0008), which agrees with the reports of Oguntayo and Samaila (18) who observed a peak age specific prevalence rate of cervical intraepithelial neoplasia (CIN) in their study. The prevalence of HPV infection however was highest among the age group 36-45 years in our study (16.2%, 28/173), although this was not statistically significant ($p=$0.0609). This finding differs from those of Kolawole et al., (19) and Akarolo-Anthony et al., (15) in similar HPV studies in Lokoja and Abuja, Nigeria respectively, where they reported high HPV infection rates among younger women less than 30 years of age, with decrease in HPV infection rate with age. This is probably bec-ause younger women including teenagers are now more sexually active with higher number of partners compared to the older women.

Reports from different parts of Nigeria indicate that there is paucity of data on the prevalence of HPV infections. Several authors have raised the possibility of certain HPV types being more common in sub-Saharan African women than elsewhere. Our study showed that HPV 31 and HPV 45 were more commonly detected compared to HPV 16 and HPV 18 in Kaduna State. This agrees with the finding of Nejo et al., (20) who reported that HPV 31 followed by HPV 35, were the most predominant high-risk circulating HPV in Ibadan, south west Nigeria. HPV 35, for instance, was slightly more common than HPV 16 in Mozambique both in women with normal cytology and in those with high-grade squamous intra-epithelial lesion (HSIL) or worse (21,22). Our finding is however contradicted by that of Ezebialu et al., (16) who reported that HPV 16 and HPV 18 rates were higher than other hrHPV genotypes in their study among women in Awka, southeast Nigeria. Manga et al., (14) also reported that HPV 16 and 18 were the most prevalent HPV genotypes in northern Nigeria. Geographical and socio-cultural diversities in the different regions of Nigeria may be account for variations in hrHPV prevalence rates in these studies.

Emeribe et al., (23) noted that data on HPV genotypes, geographical distribution and risk factors among women of child bearing age are important to determine the best vaccines needful for the protection against cervical cancer. In our study, 49.2% (30/61) of the women infected with HPV had more than one HPV genotypes. This observation has been a common occurrence in most HPV genotype studies across Nigeria. A systematic review of 16 studies reported that HPV 31 poses a similar or higher risk for CIN3+ disease compared to HPV 18, above the 4% American Society for Colposcopy and Cervical Pathology (ASCCP) immediate risk of CIN3+ threshold for referral to colposcopy (24).

The prevalence of multiple HPV infections in this population is high as 30% (9/30) of women who had multiple hrHPV infection were reportedly positive for cervical dysplasia. This finding agrees with that of Schmitt et al., (7) who reported that multiple HPV were found in 75.9% of HPV positive samples. The distribution of hrHPV genotypes among women with cervical dysplasia in this study showed that hrHPV 31 and hrHPV45 may play significant roles in the development of cervical cancer in Kaduna State, Nigeria. A similar study in Maiduguri, northeast Nigeria, reported that both single and multiple high-risk HPV infections were observed among slides prepared from women with cervical cancer (25). Further

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observations were reported in studies from two African countries, Malawi and Ghana, with multiple HPV infections that accounted for 54.0% and 52.2% respectively (26,27).

Conclusion:

The prevalence of hrHPV genotypes of 9.3% in this study is high among women in Kaduna State, Nigeria. Our study showed that HPV 31 and HPV 45 were predominant geno-types detected both as single and multiple infections. This justifies the need to review the currently available HPV vaccines with the view to developing new types that will be potent against other predominant oncogenic hrHPV types such as HPV 31 and 45 in Nigeria and Africa.

DNA-based screening for hrHPV geno-types and vaccination of young girls are reco-mmended for early detection of hrHPV and preventive management of cervical dysplasia, which can result in significant reduction in cervical cancer in Nigeria and beyond. Our study contributes to the understanding of HPV epidemiology and allows for hrHPV genotype screening programs to better assess the can-cer-developing risks associated with individual hrHPV infections.

Contributions of authors:

ADS was involved is study conceptua-lization, project administration, investigations, methodology, resources and writing of the original draft; MA was involved in supervision, validation of data, review and editing of the manuscript; EE was involved in supervision, methodology, visualization, investigation, rev-iew and editing of manuscript; AOO was invol-ved in supervision, investigation, cytology val-idation and review of manuscript; and FOO was involved in molecular analysis, methodo-logy and data validation. All authors approved the manuscript for submission.

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