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Original article

Human papillomavirus (HPV) genotypes and risk determinants among Nigerian women with cervical lesions

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ABSTRACT

Background: The second most common infection-related cancers worldwide are those associated with human papillomavirus (HPV). HPV is a leading cause of cancer deaths in Nigerian women, however very little is known about its molecular epidemiology in Nigeria. We, therefore, set out to evaluate the circulating genotypes of HPV in women who had abnormal cervical cells and determine the association. **Methods**: Cervical swab samples were collected from 250 consenting women ages 20 - 70 years accessing cervical cancer test facilities at Jos University Teaching Hospital (JUTH), Federal Medical Centre (FMC) Keffi, and the National Hospital, Abuja (NHA), on obtaining ethical approvals. Socio-demographic/risk factors information were obtained through structured questionnaires. Samples were analyzed for the presence of both squamous intraepithelial lesions (SILs) and HPV DNA using the standard Papanicolaou staining ('Pap' smear) and polymerase chain reaction (PCR) respectively. Type-specific primers targeting E6 and E7 oncogenes of the virus were used in nested-multiplex PCR followed by Sanger sequencing of positive amplicons. **Results:** The reported prevalence of HPV infection and abnormal cervical cytology was 35.6% and 15.6% respectively with a mean age ±SD of 44.5±11.9 years. Cervical cytology features identified were ASCUS (48.7%), LSIL (30.8%) and HSIL (20.5%). Women aged 40 - 49 were more likely (4.4%) to have abnormal cervical cells. In addition, 12 HPV genotypes (HPV- 6/11, 16, 18, 33, 35, 39, 42, 45, 51, 58, 59 and 66) were responsible for the abnormal cervical cells with HPV-18 predominating. Moreover, our findings revealed that HPV-18, 16, 33 and 59 were the four most frequently identified genotypes circulating among women with abnormal cytology in the population. **Conclusions:** These findings provided strong molecular evidence on the circulating genotypes of HPV in patients with abnormal cervical cells in Nigeria.

Introduction

Cervical cancer is the second most frequent cause of cancer-related deaths worldwide and the leading cause of cancer-related deaths among women in developing countries [1]. The highest burden of the disease is borne by Sub-Saharan Africa which has an estimated 84 % of all cases [2]. However, Nigeria has the highest prevalence in Sub-Saharan Africa with annual estimates of newly diagnosed cases and deaths among Nigerian women being 12,075 and 7,968 respectively [1]. In addition, the disease is the second leading cause of female cancer deaths in Nigeria next to breast cancer, with up to 80% of women dying who have been diagnosed with the disease [2].

Cervical cancer burden has been largely observed in women within the age range of 15–44 years, and the key aetiologic agent is persistent infection with high-risk human papillomavirus (HPV) whose transmission is primarily by sexual

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contact [3-4]. The conventional assay for HPV detection is the polymerase chain reaction (PCR) followed by Deoxyribonucleic acid (DNA) sequencing for the determination of specific types. Besides, type-specific multiplex primers allow exact typing of HPV and can detect multiple infections together with a broad range of high-risk HPV genotypes. Cytology-based Papanicolaou (Pap) smear can also be used for cervical cancer screening [5].

The abnormal cells identified with 'Pap' tests are classified into atypical squamous cell of undetermined significance (ASCUS), Low squamous cell intraepithelial lesion (LSIL), High squamous cell intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) based on the transformation zone of the cervix [6]. There are 14 high-risk HPV types out of the over 200 types. These include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73 [4].

Several studies have reported that diverse high-risk serotypes that vary by geographical location are associated with approximately 99% of cervical cancer cases, and integration of the responsible genotypes to the host genome results in recognizable dysplastic variations that are markers for the incidence of cervical cancer [7,8]. HSILs are known to have a high tendency for malignant transformation and this transformation is caused by high-risk HPV types [8].

Moreover, above 80% of cervical cancers in Sub-Saharan Africa are detected in late stages as a result of public unawareness of cervical cancer prevention services including early detection [9]. Prevention and control of cervical cancer are directly correlated with the identification of pre-cancer cases through early screening and, in developed countries, such screening programs are routinely organized by public health authorities, with the result being an estimated 50% decrease in morbidity and mortality in certain settings [10].

It is concerning that, other than the 'Pap' staining procedure, HPV genotyping is not a routine practice in Nigeria. Consequently, there is a near dearth of data on HPV genotype distribution in the country. This makes the identification of high-risk variants known for their strong association with cervical cancer difficult, with attendant implications for prevention.

Therefore, we hypothesized that we would find HPV genotypes that contributed to driving the incidence of the disease in Nigeria. Hence, we aimed to determine the prevalence of different types of abnormal cervical cells among patients who presented for screening and to evaluate HPV genotype distribution in the study population.

Materials and methods

Study population

The study was conducted in three tertiary institutions (Jos University Teaching Hospital (JUTH), Plateau State; Federal Medical Centre (FMC) Keffi, Nasarawa State and National Hospital Abuja (NHA) between December 2020 and November 2021. Abuja is the capital city of Nigeria, the three locations are all located in North-Central Nigeria. These hospitals are tertiary health institutions owned by the federal government of Nigeria and they offer special care for cervical cancer screening such as Papanicolaou (Pap) tests and colposcopy. The hospitals serve as referral centers for other neighboring hospitals owned by the government or private sectors. NHA has an estimated patient attendance of three per week, FMC Keffi has 2 and JUTH has 6 per week.

Ethical consideration and consent

Before the commencement of the study, ethical approvals were obtained from the Health Research Ethics Committee (HREC) of the participating healthcare institutions with the following reference numbers: JUTH/DCS/ ADM /127/XXVIII/1343, FMC/KF/HREC/244/18 and NHA/ADMIN/236/V.VII. In addition, informed consent was obtained from all subjects before they participated in the study. Prospective participants were properly informed about the purpose of the study and relevant questions were asked to determine if they met inclusion criteria. All consenting women who had not undergone a hysterectomy, were not menstruating and were not pregnant at the time of sample collections were enrolled to participate.

Determination of sample size

A statistical formula described by [11] was used to determine the sample size for this study at a 95% confidence level and a reported 18.6% prevalence of human papillomavirus infection among women in the Southwestern part of Nigeria [12]. A total of 250 women from age 18 years and above were enrolled. These included women who presented for cervical cancer screening ('Pap' smear) in any of the three tertiary institutions either

been referred by doctors or healthcare workers following complaints of symptoms suggestive of either genital tract infection or cervical cancer or based on their personal decision.

Data/ sample collection

The socio-demographic information of each participant was obtained using an interviewer-administered questionnaire. With the help of a gynaecologist, cervical swab samples were collected. A sterile vaginal speculum was inserted into the vagina to part the vaginal walls to expose the cervix. Excess mucus was removed from the cervix and surrounding mucosa using a cleaning swab. A cytobrush (Rovers Medical Devices, Netherlands) was used to collect cervical swab samples from the ectocervix of each participant by inserting it at the squamocolumnar junction and rotating at 360˚ for 10 to 15 seconds to ensure adequate sampling. This was then smeared on a labeled clean glass slide and the smear was immediately fixed with a cytospray, allowed to air-dry, and kept in a slide box for further analysis. The cytobrush, containing the remaining cervical cells was placed into a labelled screw-capped vial containing 10ml preservative fluid, (Liqui-PREP by LGM International, Inc, Melbourne, FL, USA) and was transported to the laboratory on ice packs and was stored at -80ºC until further analysis for HPV DNA PCR and genotyping.

Sensitivity/specificity test

The sensitivity, specificity and positive predictive value were calculated using the formulae TP/TP+FN, TN/TN+FP and TP/TP+FP respectively; (TP: True Positive; TN: True Negative; FP: False Positive and FN: False Negative).

Laboratory investigations

The cervical smears were analyzed for the presence of both squamous cells intraepithelial lesions (SILs) and HPV DNA using the standard Papanicolaou stains and PCR respectively.

Cervical cytology

To evaluate the presence of cervical abnormalities (intraepithelial lesions or SILs), the standard Papanicolaou method was used to stain the cervical smears obtained from each participant. The abnormalities were analyzed microscopically depending on the morphologic appearance of the cells and were classified by a cytopathologist according to the 2001 Bethesda classification scheme [6].

HPV detection and typing

DNA extraction of the viral genome from the cervical swab samples was carried out using a column-based commercially available viral RNA+DNA extraction kit (JENA Bioscience, Jena, Germany) following the manufacturer's instructions. Briefly, about 100 µl supernatant was transferred into a 1.5 ml microtube and 250 µl of lysis buffer was added to it. The mixture was vortexed for 15 seconds and then incubated at room temperature for 10 minutes. About 250 µl of ethanol (96-99%) was added and well mixed by vortexing gently. The above mixture (lysate-ethanol mixture) was then transferred into the spin column and centrifuged at 13,000 rpm for 1 minute and the flow-through in the collection tube was discarded. About 500 µl of washing buffer A was added to the spin column and centrifuged at 13,000 rpm for 1 minute and the flow-through was discarded afterwards. Then 500 µl washing buffer B was added to the spin column and centrifuged at 13,000 rpm for 1 minute and the flow-through was discarded. The spin column was then centrifuged at 13,000 rpm for 1 minute to dry the membranes further. The DNA was eluted into a clean eppendorf tube by adding 50 µl of elution buffer onto the membrane of the spin column. It was incubated at room temperature for 1 minute and then centrifuged at 13,000 rpm for 1 minute. The eluted HPV DNA was subjected to nested PCR amplification of its consensus region using primers which target the E6/E7 gene region [one forward primer (GP-E6-3F) and two back primers (GP-E7-5B and GP-E7-6B)] for the first amplification followed by a second amplification reaction using type-specific primer pairs as described [13]. The cycling conditions for PCRs with GP-E6/E7 consensus primers were subjected to an initial denaturation at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 47°C for 60 seconds and extension at 65°C for 60 seconds. The last cycle was followed by a final extension at 72°C for 10 minutes [13]. The sequences for GP-E6/E7 consensus primers are shown in **Table (1)**.

The amplicons from the first round of PCR were re-amplified in the second round of PCR using type-specific primer pairs for identifying high-risk genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52,56, 58, 59, 66 and 68 and low-risk genotypes 6/11, 42, 43 and 44. To reduce the number of nested PCRs necessary to discriminate among a broad spectrum of different HPV genotypes, nested primers were

arranged in multiplex PCR primer cocktails. The primers were used in four cocktails, each containing four to five different primer pairs as described [13] **(Table 2)**.

Each cocktail PCR reaction mixture consisted of 20 µl premix of PCR buffer, dNTPs, Magnesium chloride, and Taq polymerase enzyme in optimized concentration (2x Mastermix, JENA Bioscience Ruby hot start pol), 1 µl (10pmol) each of forward and reverse primers in the individual cocktail, 5 µl DNA template and 5 µl sterile nuclease-free water to make up a total reaction volume of 40 µl. PCR amplification was carried out in an Applied Biosystem 2720 Thermocycler. The nested-multiplex PCRs were performed under the following conditions: denaturation at 95℃ for 2 minutes; followed by 35 cycles of denaturation at 95℃ for 30 seconds, annealing at 56℃ for 60 seconds and extension at 72℃ for 60 seconds and the last cycle was followed by a final extension at 72°C for 10 minutes. Following amplification, the PCR products were detected by electrophoresis on a 2% agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0) and examined under a blue light transillumination for expected amplicons (Cleaver Scientific, UK). The size of the PCR products that were generated with GP-E6/E7 consensus primers was 630bp while the length of the products amplified with type-specific primer pairs ranged from 151bp to 457bp. Some of the amplicons were sequenced and further analyzed for genotype identification. A commercially available PCR purification kit (Jena Bioscience, Germany) was used to purify the PCR products according to the manufacturer's instructions. The sequencing was performed commercially (INQABA BIOTEC, South Africa) on an ABI Prism 3130 genetic analyzer (Applied BioSystems) using Sanger's sequencing method. The same consensus primers that were used for the PCR were also used to perform the sequencing but only one of the back primers (either GP-E7-5B or GP-E7-6B) together with the forward primer (GP-E6-3F) was used to sequence the DNA samples. The sequences were deposited in Genbank and were assigned the accession numbers 0Q701530-0Q701535.

Statistical analysis

The socio-demographic/risk factors information generated from questionnaires were compared using IBM SPSS version 21 software. Chi-square statistics were used to estimate the strength of the association between variables with ***p*** values of < 0.05 considered statistically significant.

Results

Prevalence of cervical abnormality in relation to socio-demographic/risk factor characteristics of the participants

The prevalence of cervical abnormalities in relation to socio-demographic/ risk factors of the participants showed that the age group 40 - 49 years had the highest prevalence 11 (4.4%) though the association with abnormal cervical cytology was not significant (***p***=0.703). Educational status and age at first coitarche are the only factors that were significantly related to abnormal cervical cytology (***p***=0.037, r=0.52, χ2=32.57 and 0.027, r= 0.64, χ2=42.58) respectively. All other factors were not significantly related **(Table 3)**.

Cytology results

Cytology results revealed that out of the 250 cervical swab samples, 211 (84.4%) samples were normal while 39 (15.6%) were abnormal. Out of those with abnormal cytology results, the prevalence of HSIL, LSIL and ASCUS is 20.5%, 30.8% and 48.7% respectively **(Figure 1)**.

Result of HPV DNA test (HPV infection)

HPV infection results showed that out of the 250 cervical swab samples, 89 were HPV DNA positive giving an HPV infection prevalence of 35.6%. Also, 55 (26.1%) of the 211 women with normal cytology had a positive HPV DNA test and 34 (87.2%) of the 39 women with cytological anomalies were also HPV DNA positive **(Table 4)**.

Association between abnormal cytology and HPV infection

All the women (100%) with HSIL and LSIL were also positive for HPV DNA while 14 (73.9%) of the 19 women with ASCUS had HPV-positive DNA. The distribution of cervical cytology types amongst subjects in relation to HPV infection is shown in **figure (2)**.

The remaining 50 cases out of the 89 cases that were HPV DNA positive had been diagnosed as negative by Pap smear. Following the 39 cytology-detected samples, a sensitivity, specificity and positive predictive value of 43.8%, 96.9% and 88.6% respectively were calculated (***p***-value: 0.000; < 0.05).

HPV prevalence and genotypes distribution in relation to cervical cytology status

Twelve HPV genotypes: HPV- 6/11 (66.7%), 16 (90.9%), 18 (100%), 31 (33.3%), 33 (50.0%), 35 (33.3%), 39 (25.0%), 42 (66.7%), 45 (44.5%), 58 (30.0%), 59 (50.0%) and 66 (33.3%) were associated with abnormal cytology out of which ten were high-risk types. Among the high-risk types, HPV-18 had the highest abnormal cytology (100.0%) followed by HPV-16 (90.9%) and HPV 33 and 59 (50.0%) **(Table 5)**.

HPV-16 (9.1%), 33 (50.0%), 35 (33.3%), 39 (25.0%), 45 (25.9%), and 66 (20.0%) were the genotypes associated with ASCUS. HPV-16 (45.5%), 18 (100.0%), 45 (3.7%), 59 (25.0%), and 66 (2.2%) were equally associated with HSIL while HPV-16 (36.45%), 31 (33.3%), 45 (14.8%), 58 (30.0%), 59 (25.0%), and 66 (11.1%) were associated with LSIL **(Figure 3)**.

Out of the 19 ASCUS, 11 were multiple infections while 8 were single infections; of the 8 HSILs, 5 were single infections comprising four HPV-16 and one HPV-18, while the 3 double infections were a combination of HPV-16 and 18. The 12 LSILs comprise 9 single and 3 double infections. Hence, the prevalence of multiple and single infections among women with abnormal cytology is 23 (59.0%) and 11 (28.2%) respectively.

Analysis of ASCUS cases revealed that the highest prevalence of HPV was within the age range of 30 - 39 years 7 (36.8%) as the prevalence decreased with age. The highest prevalence rate of HSIL and LSIL was found in ages 20 - 29 years 3 (37.5%) and 4 (33.3%) respectively which also decreased with an increase in age **(Table 6)**.

From the 86 (34.4%) high-risk HPV infection and 39 (15.6%) SIL, HPV infection and SIL in the 3 locations are strongly associated both in the combined (***p***=0.001) and individual group data (***p***=0.002, ***p***=0.004 and ***p***=0.003) for Plateau, Abuja and Nasarawa respectively **(Table 7)**.

Table 1. Sequences of GP-E6/E7 consensus primers [13].

Primer	Sequence
GP-E6-3F	5' TGG WGK KAC TGA AAT CGG T 3'
GP-E6-5B	5' CTG AGC TGT CAR NTA ATT GCT CA 3'
GP-E6-6B	5' TCC TCT GAG TYG YCT AAT TGC TC 3'

Table 2. Sequences of type-specific nested PCR primers used in this study

Primer cocktail	HPV genotype	Amplicon (bp)	Sequence (5'-3')	Position (bp)
I	16	457	CAC AGT TAT GCA CAG AGC TGC	141–161
			CAT ATA TTC ATG CAA TGT AGG TGT A	597–573
	18	322	CAC TTC ACT GCA AGA CAT AGA	170–190
			GTT GTG AAA TCG TCG TTT TTC A	491–470
	31	263	GAA ATT GCA TGA ACT AAG CTC G	137–158
			CAC ATA TAC CTT TGT TTG TCA A	399–378
	59	215	CAA AGG GGA ACT GCA AGA AAG	159–179
			TAT AAC AGC GTA TCA GCA GC	373–354
	45	151	GTG GAA AAG TGC ATT ACA GG	82–101
			ACC TCT GTG CGT TCC AAT GT	232–213
II	33	398	ACT ATA CAC AAC ATT GAA CTA	172–192
			GTT TTT ACA CGT CAC AGT GCA	569–549
	6/11	334	TGC AAG AAT GCA CTG ACC AC	201–220
			TGC ATG TTG TCC AGC AGT GT	534–515
	58	274	GTA AAG TGT GCT TAC GAT TGC	297–317
			GTT GTT ACA GGT TAC ACT TGT	570–550

	52	229	TAA GGC TGC AGT GTG TGC AG	178–197
			CTA ATA GTT ATT TCA CTT AAT GGT	406–383
	56	181	GTG TGC AGA GTA TGT TTA TTG	294–314
			TTT CTG TCA CAA TGC AAT TGC	475–455
III	35	358	CAA CGA GGT AGA AGA AAG CAT C	157–178
			CCG ACC TGT CCA CCG TCC ACC G	514–493
	42	277	CCC AAA GTA GTG GTC CCA GTT A	85–106
			GAT CTT TCG TAG TGT CGC AGT G	361–340
	43	219	GCA TAA TGT CTG CAC GTA GCT G	102–123
			CAT GAA ACT GTA GAC AGG CCA AG	320–298
	44	163	TAA ACA GTT ATA TGT AGT GTA CCG	248–271
			TAT CAG CAC GTC CAG AAT TGA C	410–389
IV	68	333	GCA GAA GGC AAC TAC AAC GG	4049–4068
			GTT TAC TGG TCC AGC AGT GG	4381–4362
	39	280	GAC GAC CAC TAC AGC AAA CC	213–232
			TTA TGA AAT CTT CGT TTG CT	492–473
	51	223	GAG TAT AGA CGT TAT AGC AGG	319–339
			TTT CGT TAC GTT GTC GTG TAC G	541–520
	66	172	TTC AGT GTA TGG GGC AAC AT	353–372
			AAA CAT GAC CCG GTC CAT GC	520–501

Table 3. The Prevalence of Cervical Abnormalities in Relation to Socio-demographic/ Risk Factors of the Participants

Variables	No. Examined	No. Positive	Prevalence (%)	p-value	Correlation (r)	Chi-square (X^2)
Age (Year)						
< 20	0	0	0	0.703	0.32	13.57
20 -	34	9	3.6			
30 - 39	66	7	2.8			
40 - 49	80	11	4.4			
50 - 59	38	7	2.8			
60 - 69	30	5	2			
≥ 70	2	0	0			
Marital Status						
Single	20	3	1.2	0.985	0.45	11.86
Married	151	24	9.6			
Divorced	35	6	2.4			
Widowed	44	6	2.4			
Educational Status						
Primary	40	12	4.8	0.037	0.52	32.57
Secondary	66	7	2.8			
Tertiary	129	15	6			
None	15	5	2			
Employment Status						
Housewife	126	18	7.2	0.718	0.09	12.53
Unskilled	56	12	4.8			
Skilled/Professional	61	8	3.2			
Others	7	1	0.4			
Parity						

0	30	3	1.2	0.157	0.43	14.92
1 - 2,	45	3	1.2			
≥ 3	175	33	13.2			
Lifetime sexual partner						
0	0	0	0	0.113	0.28	12.45
1	77	9	3.6			
2 - 3,	97	23	9.2			
≥ 3	76	7	2.8			
Spouse with multiple sexual partners						
Yes	159	28	11.2	0.571	0.43	15.23
No	71	8	3.2			
N/A (Singles)	20	3	1.2			
Use of contraceptive						
Hormonal drugs	40	11	4.4	0.39	0.28	16.12
Condom	41	6	2.4			
IUCD	83	11	4.4			
None	86	11	4.4			
Cigarette Intake						
Yes	3	1	0.4	0.498	0.06	17.31
No	247	38	15.2			
Alcohol Intake						
Yes	45	7	2.8	0.994	0.24	10.36
No	205	32	12.8			
History of Sexually Transmitted infection						
HIV	56	10	4	0.732	0.09	12.05
Hepatitis	26	4	1.6			
Others	2	1	0.4			
None	166	24	9.6			
Menopause						
Yes	42	7	2.8	0.859	0.21	11.87
No	208	32	12.8			
Age at First Coitarche						
< 10	0	0	0	0.027	0.64	42.58
10 -14,	7	0	0			
15 -19	136	31	12.4			
≥ 20	107	8	3.2			
Age at Menarche						
< 9	3	0	0	0.454	0.34	14.98
10 - 14,	156	28	11.2			
> 15	91	11	4.4			
Age at First Pregnancy						
< 20	25	7	2.8	0.224	0.35	15.72
20 - 24	96	18	7.2			
≥ 25	92	11	4.4			
N/A	37	3	1.2			
Family History of Cervical Cancer						
Yes	10	2	0.8	0.743	0.26	12.87
No	240	37	14.8			
Total	250	39				

Table 4. Normal and abnormal cytology in relation to HPV infection

Normal Cytology (N=211)		Abnormal Cytology (N=39)	
HPV DNA Pos	HPV DNA Neg	HPV DNA Pos	HPV DNA Neg
55 (26.1)	156 (73.9)	34 (87.2)	5 (12.8)

Table 5. Frequency distribution of HPV genotypes and abnormal cytology among the examined women

HPV Genotype	Normal cytology (%)	Abnormal Cytology (%)	Total
HPV-6/11	1 (33.3)	2 (66.7)	3
HPV-16	1 (9.1)	10 (90.9)	11
HPV-18	0 (0.00)	2 (100.0)	2
HPV-31	2 (66.7)	1 (33.3)	3
HPV-33	2 (50.0)	2 (50.0)	4
HPV-35	2 (66.7)	1 (33.3)	3
HPV-39	3 (75.0)	1 (25.0)	4
HPV-42	1 (33.3)	2 (66.7)	3
HPV-45	15 (55.6)	12 (44.5)	27
HPV-58	7 (70.0)	3 (30.0)	10
HPV-59	2 (50.0)	2 (50.0)	4
HPV-66	30 (66.7)	15 (33.3)	45

Table 6. Association between age and abnormal cervical cytology

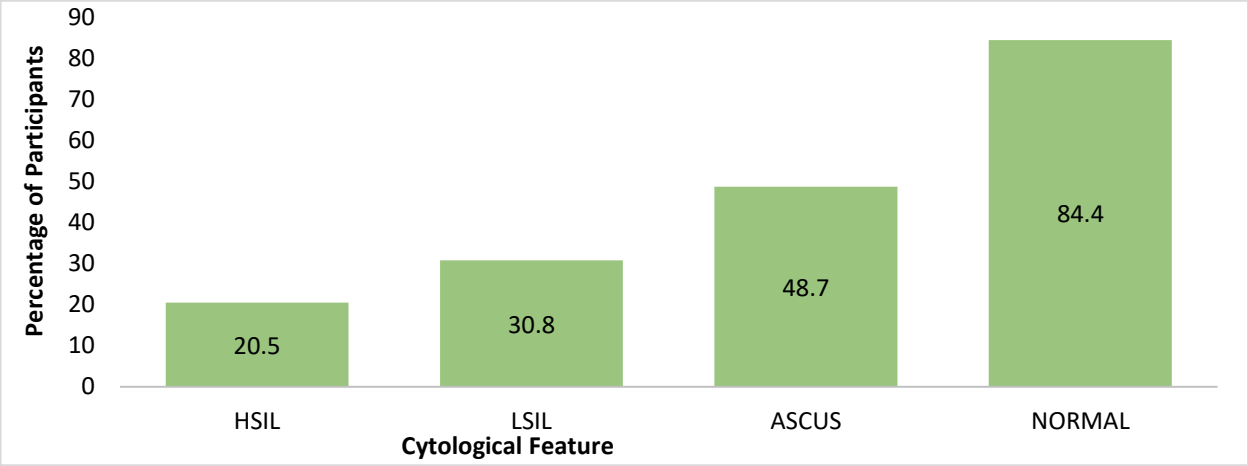
Age (Year)	ASCUS (%)	HSIL (%)	LSIL (%)	Total
<20	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
20 - 29	4 (21.1)	3 (37.5)	4 (33.3)	11 (28.2)
30 - 39	7 (36.8)	2 (25.0)	3 (25.0)	12 (30.8)
40 - 49	4 (21.1)	1 (12.5)	3 (25.0)	8 (20.5)
50 - 59	3 (15.8)	1 (12.5)	0 (0.0)	4 (10.3)
60 - 69	1 (5.3)	1 (12.5)	2 (16.7)	4 (10.3)
≥70	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)
Total	19 (100.0)	8 (100.0)	12 (100.0)	39 (100.0)

(***p***= 0.014; χ^2=384.23) (***p***<0.05 Statistically Significant)

Table 7. Prevalence of high-risk human papillomavirus and squamous intraepithelial lesion

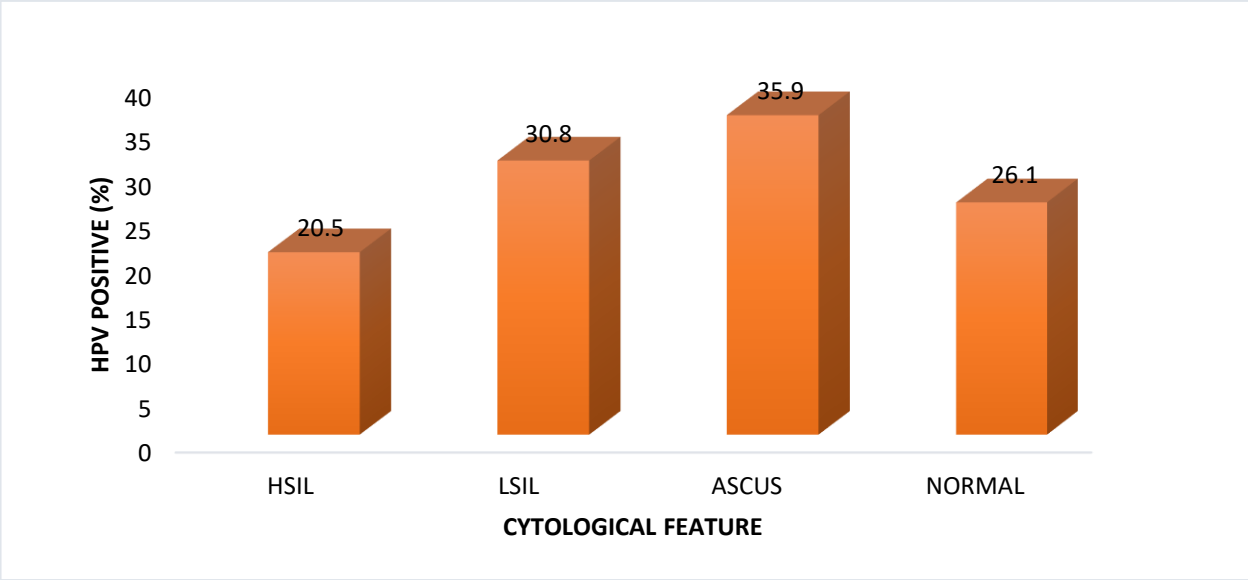
Location	Combined (%)	Plateau (%)	Abuja (%)	Nasarawa (%)	p-value
High-risk HPV	86/250 (34.4)	60/194 (30.9)	10/33 (30.3)	16/23 (69.6)	0.014
SIL	39/250 (15.6)	26/194 (13.4)	3/33 (9.1)	10/23 (43.5)	0.003

Figure 1. Distribution of cytological features observed amongst the participants



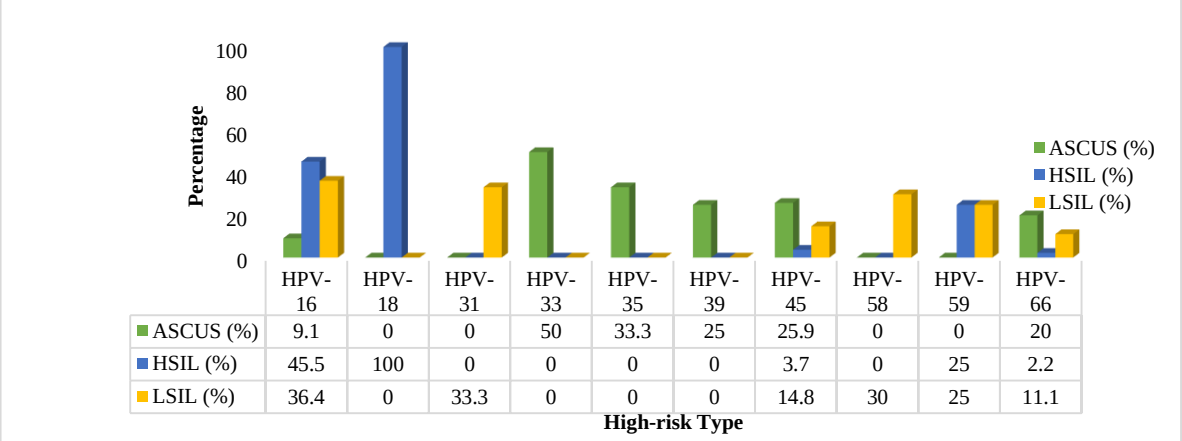
Key: HSIL (high squamous cell intraepithelial lesion), LSIL (low squamous cell intraepithelial lesion), ASCUS (atypical squamous cell of undetermined significance) ***p***=0.023; $\chi 2$=214.82) (***p***<0.05 Statistically Significant)

Figure 2. Distribution of cervical cytology features amongst subjects in relation to HPV infection



Key: HSIL (high squamous cell intraepithelial lesion), LSIL (low squamous cell intraepithelial lesion), ASCUS (atypical squamous cell of undetermined significance) (***p***= 0.001; $\chi 2$=120.37) (***p***<0.05 Statistically Significant)

Figure 3. Association of high-risk HPV genotypes and abnormal cervical cytology



	HPV-16	HPV-18	HPV-31	HPV-33	HPV-35	HPV-39	HPV-45	HPV-58	HPV-59	HPV-66
ASCUS (%)	9.1	0	0	50	33.3	25	25.9	0	0	20
HSIL (%)	45.5	100	0	0	0	0	3.7	0	25	2.2
LSIL (%)	36.4	0	33.3	0	0	0	14.8	30	25	11.1

Key: HSIL (high squamous cell intraepithelial lesion), LSIL (low squamous cell intraepithelial lesion), ASCUS (atypical squamous cell of undetermined significance). (***p***= 0.018; $\chi 2$=132.70) (***p***<0.05 Statistically Significant)

Discussion

The mean age of 44.5 years recorded in this study showed that the majority of the women who screened for cervical cancer in North-Central Nigeria are older women. A study by [7] reported that pre-malignant lesions peak in the late 20s. The mean age in this study was past the peak age of pre-malignant lesions. HPV the aetiologic agent of cervical cancer mainly being transferred sexually explains why the peak incidence of HPV will be shortly after sexual debut. The precursor lesions, CIN also peaks a decade after the peak incidence of HPV infection hence, cervical cancer screening is suggested to start in the early 20s. Women within the age group 40 - 49 years have the highest prevalence of abnormal cytology though the association is not significant. This was in disparity with the study carried out by [14] where women with abnormal cytology who are less than 31 years old had the highest prevalence.

Educational status and age at first coitarche were found to be significantly associated with abnormal cervical cytology in this study. Study [15] reported that the level of education is the most important limitation affecting Pap smear tests. The study reported low education status increases the risk of abnormal cytology and vice versa. Another study reported similar findings but found no effect using the regression analysis [16]. This study found that abnormal cervical cytology is more predominant among those with tertiary education. This could be a result of the total number of women examined, 129 compared to 40 and 15 with primary school and no formal education respectively.

Age at first coitarche is an important risk factor for HPV infection and cervical cancer. Study [17] reported that HPV infection was higher among those who had their first coitarche before the age of 20 years (26.8%), compared to those who had their first coitarche after the age of 20 years (7.6%). Studies [18-19] provide evidence that an early age at first sexual intercourse (coitarche) may be seen as a predictor of an early age at first exposure to HPV and other STIs.

HPV prevalence of 87.2% was found among participants with abnormal cytology results and 26.1% was found in participants with normal cytology results (26.1%). Similar findings were obtained from the result of past studies [20-22] and a slightly lower prevalence (15.3%) was reported by a study [14]. The variations in the HPV prevalence rates that were detected could be indicative of the impact of geographical variations, a standardized and suitable volume of body fluids, methods of DNA extraction and the diagnostic performance of HPV detection protocols [23].

About 211 (84.4%) of the 250 women who participated in this study had normal cytology (they were negative for squamous cell intraepithelial lesion), while 39 (15.6%) women had abnormal cytology. The prevalence of abnormal cytology among the participants in this study is moderately high (15.6%) with ASCUS having the highest prevalence (48.7%) followed by LSIL (30.8%) and HSIL (20.5%).

The highest HPV prevalence in ASCUS cases was between ages 30 - 39 years (36.8%) while the prevalence decreases with increasing age. The same trend follows for HSIL and LSIL with ages 20 - 29 years being the age group with the highest prevalence in both (37.5% and 33.3%) respectively. Consistent with our findings, a decrease in the prevalence of HPV among all grades of abnormal cytology with increasing age was also observed by study [24] in women ages 30 - 64 years. A study conducted by [25] also reported similar findings in perimenopausal women where HPV prevalence decreased with an increase in age. A study carried out by [26] reported that HPV testing in older women with LSIL anomalies detected by cytology can help to differentiate between true infections that carry a risk for progression to cervical pre-cancer and other morphologic changes that are not linked with the risk of cancer. This current study found that HPV infection was more recurrent among women of childbearing age who are sexually active and patients diagnosed with LSIL had the oldest age followed by patients with ASCUS. Women ≤30 years have an improved capability to clear HPV infection compared to women above 30 years infected [27]. Based on that, women older than 30 years are more likely to develop a persistent HPV infection which progresses to cervical cancer [15]. In consonance with this study, study [28] reported a high prevalence of HPV infection in women of childbearing age. This is attributed to the absence of a screening program in Nigeria as HPV infection may not show symptoms.

The prevalence of high-risk HPV in relation to SIL showed that HPV DNA was found in 14 of the 19 women who had ASCUS and in all 8 and 12 women who had HSIL and LSIL

respectively. This suggests that the possibility of detecting HPV DNA in a cervical lesion increases with the severity of the lesion. It is advised that a repeat pap smear be administered to high-risk HPV-infected ASCUS women within 6 - 12 months while immediate colposcopy and consequent treatment be administered to high-risk HPV-infected HSIL or LSIL women respectively [29].

As observed in this study, the low sensitivity (43.8%) and higher specificity (96.9%) in the usage of cervical smear cytology for the detection of cervical HPV infection compared to the gold standard (PCR) was anticipated [30]. The probable reason for this could be that only about one-third of women with HPV infections detectable by DNA testing have known cytopathology [31]. Several studies have emphasized the importance of HPV DNA testing in cervical cancer screening with promising results [32-35]. The introduction of self-sampling methods and the use of other body fluids such as urine in detecting HPV made the efficacy and correctness of its DNA testing in cervical cancer screening have better prospects [36]. Socio-cultural and/or religious matters may have caused some hindrances to cervical cancer screening thereby reducing the tolerability of the current conventional screening method. Lately, it has been hypothesized that screening for cervical cancer using HPV DNA testing might surpass conventional cytology and will also be less expensive even though it provides better protection [37].

About 12 HPV genotypes were associated with abnormal cytology in this study. HPV-18 (100.0%), HPV-16 (90.9%) and HPV-33 and 59 (50.0%) were the four most common HPV genotypes. The prevalent genotypes found in abnormal cytology were only reported in four geopolitical zones of Nigeria (North-central, North-west, North-east and South-west). They include HSIL (HPV-16, 35, 31, 18, 45, 52, 33, 51 and 58) and LSIL/ASCUS (HPV-31, 51, 52, 35, 58, 16, 56, 18, 39 and 59) in descending order [14]. HPV-16, 18, 45, 59 and 66 were associated with HSIL in this study. Three out of the 5 genotypes associated with HSIL in this study were also found to be associated with HSIL from the reported study in Nigeria. HPV-16, 31, 45, 58, 59 and 66 were associated with LSIL while HPV-16, 33, 35, 39, 45, and 66 were associated with ASCUS in this study. A low prevalence of HPV infection in women with normal cervical cytology in the Middle East and North Africa was reported by a study [38] which agrees with this study. The prevalence of high-risk HPV in women with abnormal cytology was 76.9%. This confirms the possibility of harbouring high-risk HPV DNA by most women having cervical anomalies. Therefore, a follow-up test and immediate treatment for these women are strengthened. The prevalence of high-risk HPV obtained in this study is higher than the prevalence reported by [39] in a study carried out in Osun State, South-West Nigeria but less than that reported by [40] in a study conducted in Kaduna State, North-West Nigeria which recorded 22.7% and 82.4% respectively.

There were more multiple infections (59.0%) than single infections (28.2%). This supports the findings of [41] in a study conducted on Iranian women with 56% multiple infections belonging to the high-risk group. Another previous study which is in agreement with this study was carried out in Burkina Faso by [42]. Alternatively, studies [43-44] reported a higher prevalence of single infections (85.9%) and (64.1%) and a lower prevalence of multiple infections (14.1%) and (35.9%) respectively in studies conducted on women from North-East Brazil and Turkey respectively. The high prevalence of multiple HPV infections observed in this study is expected as a result of the type-specific primers (TS-PCR) used in genotyping [30]. More genotypes have been detected using sequencing compared to TS-PCR this signifies the need to combine both methods for optimum yield [45]. The low prevalence of low-risk HPV genotypes shown in this study despite the use of TS-primers could be attributed to the fact that low-risk HPV genotypes are more common in males than females and also usually less in cervical specimens [46]. A greater risk of developing cervical cancer is posed by infection with multiple high-risk HPV types. HPV testing can be affected by these multiple infections particularly when the assay used is unable to detect other types present in multiple infections. This possibly could bring about underreporting of HPV type-specific prevalence leading to difficulty in achieving adequate protection against HPV infection where the existing vaccines are only able to give protection against some HPV types leaving others circulating in the population.

Conclusion

We found that 12 HPV genotypes namely; HPV- 6/11, 16, 18, 33, 35, 39, 42, 45, 51, 58, 59 and 66 were responsible for the abnormal cervical cells

with HPV-18 predominating. Our findings further revealed that HPV-18, 16, 33 and 59 were the most frequently identified genotypes among women with abnormal cytology in the population. These findings provide strong molecular evidence on the circulating genotypes of HPV in patients with abnormal cervical cells in Nigeria. Adequate epidemiological data could target vaccine research and development. Therefore, the data obtained here could provide a standard for evaluating the effect of a newly introduced vaccination programme in the future.

Authors' contributions

Abigail William Zakka designed the research plan, was involved in the collection of the cervical swab, administered the structured questionnaires to the participating population and was principal in the write-up of the manuscript. Christianah Idowu Ayolabi was involved in the statistical analysis of the research, participated in designing the methods used and also participated in the preparation of the manuscripts. Babatunde Adebiyi Olusola was involved in the utilization of molecular techniques and also participated in designing the methods used. All authors gave their approval for the submission of the article.

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