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

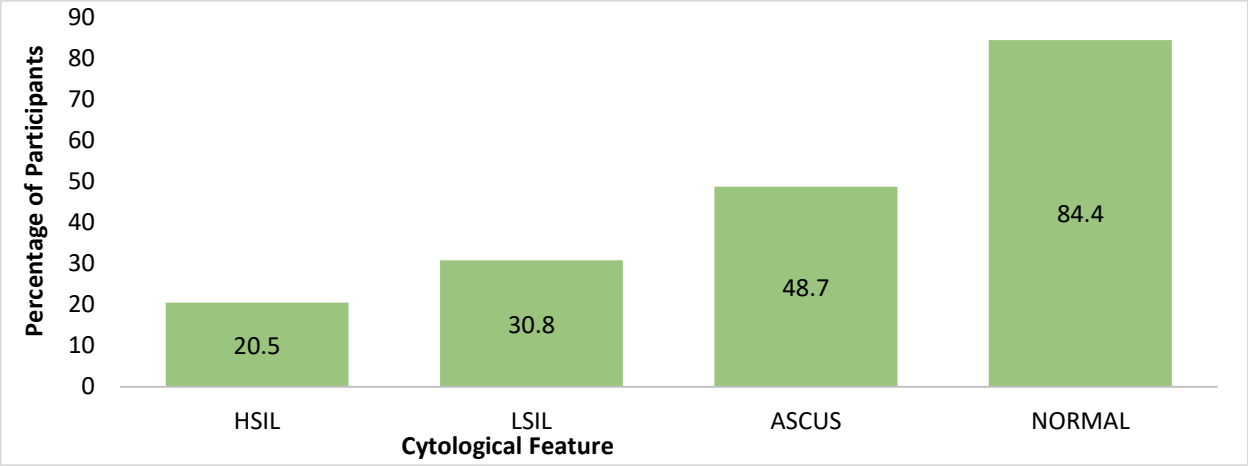
Age (Year)	ASCUS (%)	HSIL (%)	LSIL (%)	Total
<20	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
20 - 29	4 (21.1)	3 (37.5)	4 (33.3)	11 (28.2)
30 - 39	7 (36.8)	2 (25.0)	3 (25.0)	12 (30.8)
40 - 49	4 (21.1)	1 (12.5)	3 (25.0)	8 (20.5)
50 - 59	3 (15.8)	1 (12.5)	0 (0.0)	4 (10.3)
60 - 69	1 (5.3)	1 (12.5)	2 (16.7)	4 (10.3)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

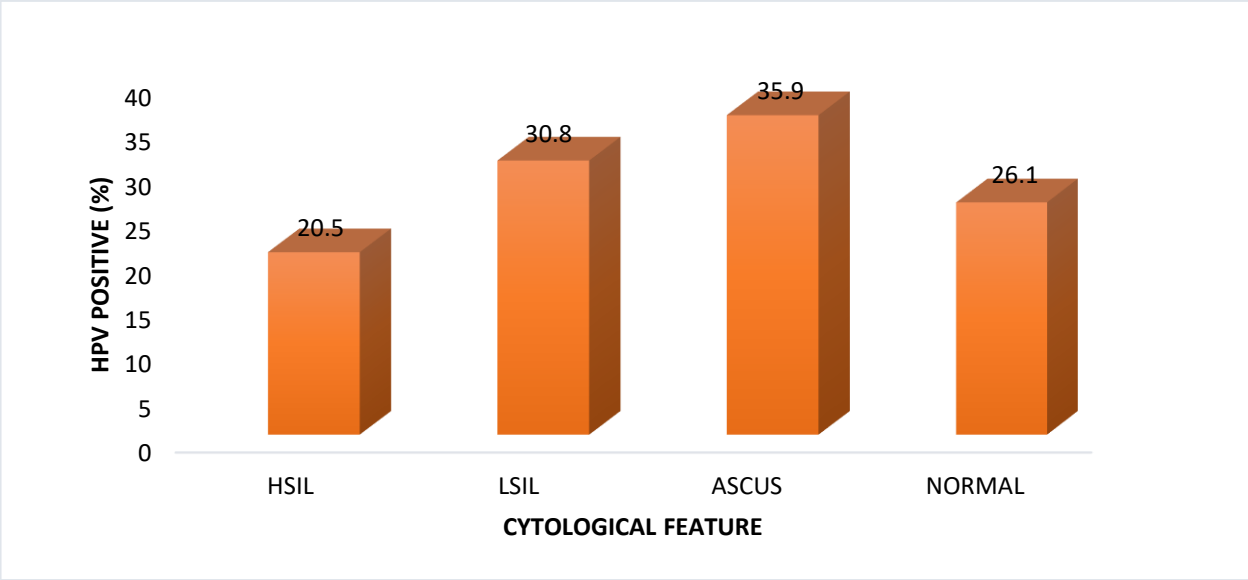
Location	Combined (%)	Plateau (%)	Abuja (%)	Nasarawa (%)	p-value
High-risk HPV	86/250 (34.4)	60/194 (30.9)	10/33 (30.3)	16/23 (69.6)	0.014
SIL	39/250 (15.6)	26/194 (13.4)	3/33 (9.1)	10/23 (43.5)	0.003

Figure 1. Distribution of cytological features observed amongst the participants



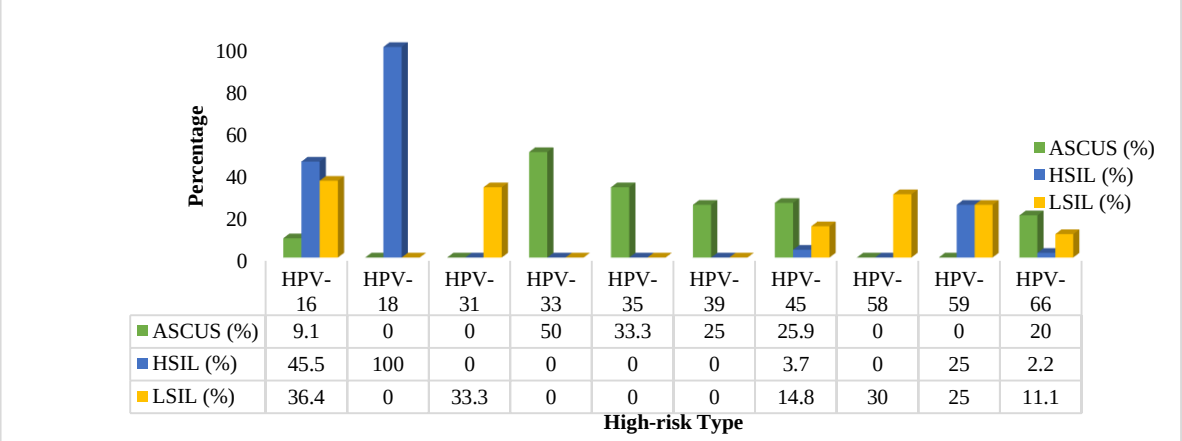
Key: HSIL (high squamous cell intraepithelial lesion), LSIL (low squamous cell intraepithelial lesion), ASCUS (atypical squamous cell of undetermined significance) ***p***=0.023; $\chi 2$=214.82) (***p***<0.05 Statistically Significant)

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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