



■ Original Research Article

**Prevalence and distribution of high-risk human papillomavirus genotypes in HIV positive and HIV negative women with Abnormal cervical cytology**

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**Abstract**

**Objective:** To evaluate HR- HPV prevalence and genotype distribution among women with abnormal cervical cytology.  
**Methods:** A cross sectional study among 75 HIV positive and 75 HIV negative women with abnormal cervical cytology using 21 HPV Geno array method carried out between July to December 2018 at the cervical cancer screening unit. Chi square test was performed to compare the association of specific HR HPV types between HIV-

positive and HIV negative women. Logistic regression was used to determine predictors of HR HPV and a P value of less than 0.05 considered statistically significant.

**Results:** Sixty-six (44%) were positive for HR-HPV, with 43 (57.3%) being HIV positive and 23(30.7%) HIV negative (OR 3.1 [95%CI:1.6-6.2, p < 0.001]). Among the HIV positive women 20(27%) had multiple HR- HPV infections compared to 9(12%) in HIV negative women (OR 3.7 [95%CI:1.5-9.5, p = 0.038]). The most prevalent HR- HPV types among women without HIV were 31(16%), 18(9.3%), 58(8%), 16(6.6%), 35(2.6%) and among women with HIV were 31(22.7%), 58(16%), 18(12%), 16(8%), 45 & 52(6.6%). **Conclusion:** Women with HIV had a significantly higher prevalence of HR HPV. Our study confirms the presence of the most common HR HPV types covered in bivalent and quadrivalent vaccines, but the nonavalent vaccine would be needed to protect women against other HPV subtypes (such as 31 and 58) which were also found in this population. Thus, we recommend that preventive measures for cervical cancer should take into cognizance other HPV genotypes present and the HIV status of the population

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**Conflict of Interest:** Nil

### Introduction

HPV is one of the most common sexually transmitted infections worldwide and most sexually active people will get it in their life time. <sup>[1]</sup> High risk (HR) HPV DNA has been shown to be present in 99.7% of cervical cancers worldwide. <sup>[2]</sup> HR HPV types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,68,73 and 82 and LR HPV types include 6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81. <sup>[3]</sup> To date, several meta-analyses have confirmed the five most prevalent strains in women with normal cytology and cervical neoplastic diseases to be HPV 16, 18, 31, 52, and 58. <sup>[4,5]</sup> HPV 16 and 18 account for approximately 70% of global cervical cancer cases, <sup>[6]</sup> but this differs in women from Africa and women infected with HIV, in whom HPV 58 was reported to be the second most dominant strain behind HPV 16. <sup>[7,8]</sup>

HPV infection coexisting with HIV is a subject of concern because the immunocompromised women are less likely to clear the virus, are more susceptible to a wide range of

HPV viruses, and even the less frequently reported HPV genotypes are more common. This makes such women vulnerable to and at a higher risk of developing cervical dysplasia and cancer. <sup>[9]</sup> Cervical cancer is also considered an AIDS defining condition by the Center for Disease Control and WHO added invasive cervical cancer (ICC) to the stage 4 HIV/AIDS classification of its clinical staging and case definition of HIV for resource-limited settings. <sup>[10]</sup>

Several countries have adopted the bivalent and quadrivalent HPV vaccines that protects against HPV genotypes 6,11,16 and 18, <sup>[11,12]</sup> but currently there is Gardasil 9 HPV vaccine that protects against additional HPV types 6,11,16,18,31,33,45,52 and 58. These vaccines have the potential to protect against 90% of the HPV types associated with invasive cancer in the world. <sup>[13]</sup> We currently do not have a comprehensive data on the most prevalent HPV genotypes in Nigeria. This information is critical for guiding HPV vaccination policy. In this study we sought to determine the prevalence and distribution of the most common HR HPV

genotypes in HIV positive and HIV negative women with abnormal cervical cytology and whether the types differ by HIV status.

## Materials and Methods

**Study setting and Design:** This was a cross sectional study at the cervical cancer screening unit of the general Gynaecological department and the APIN/Harvard PEPFAR HIV clinic.

**Study Population:** Between July to December 2018, we recruited HIV positive and HIV negative women with abnormal cervical cytology who had cervical cancer screening using the Papanicolaou technique. Inclusion criteria were: (1) age between 30-65years;(2) Abnormal cervical cytology (ASCUS, LSIL, HSIL); (3) signed informed consent. Those with previous hysterectomy, chemotherapy, radiotherapy and pregnant women were excluded from the study.

**Sample size determination:** Sample size was determined using appropriate formula for comparing two proportions,<sup>[14]</sup> where we used confidence interval of 95%, with a reference prevalence of HR HPV among HIV positive and HIV negative women in a study conducted in Abuja, Nigeria.<sup>[15]</sup> This gave a minimum sample size of 74 for each group. A total of 150 participants were recruited for the study.

For the HIV negative women there was already a well-established cervical cancer unit where an average of 200-250 women are screened per month with about 20-25 having an abnormal result and over 20,000 women have been screened in total. So, there was a data base with results of all women, so it was easier to identify those with abnormality and recruit them for the study and is also a referral center where women with abnormal cervical cytology were referred to. So, participants were recruited as they present until adequate sample size was achieved. Two hundred and sixty-nine (326) HIV positive women were screened to obtain 75 women with abnormal Pap smears for this study

**Data collection:** A detailed questionnaire was administered to determine the sociodemographic, sexual and reproductive history of the participants. The Gynaecologist /reproductive health nurses performed a detailed pelvic examination and

cervical smear samples were collected from the squamocolumnar junction by a 360° rotator movement using a Digene HPV cervical sample collection device (Digene Corporation, Gaithersburg, USA). The tip of the brush was cut and inserted into Qiagen specimen collection medium and stored at -80°C for HPV genotyping.

The samples were transported in dry ice for HPV detection and typing using HPV Geno Array test kits (HybriBio Biochemical Company Limited, China) for 21 HPV genotypes which included high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, low risk types 6, 11, 42, 43, 44, and 81 and probable high risk types 53 and 66. The assay was performed according to the manufacturer's protocol.<sup>[16]</sup>

**HIV test:** HIV test was performed in all women with unknown HIV status using the Alere Determine HIV 1/2 Ag/Ab combo test to detect both HIV1/2 Antibodies. Reactive specimen was confirmed by trinity Biotech Unigold Recombigen HIV test. All participants who tested positive were referred for HIV treatment, care and support.

**Statistical analysis:** We created a data base which included sociodemographic, sexual and reproductive variables of all the participants. HIV status, results of cytology screening and result of HPV test were also included. All analysis was performed using R version 3.6.0.

Descriptive statistical analysis included frequencies with percentages with median and interquartile range used in view of the fact that the assumption of normality was not fulfilled. The main outcome or dependent variable was HR HPV infection, and the independent variables were HIV infection, age, parity, number of sexual partners, age at sexual initiation. Prevalence of HR HPV was determined in the general study population and based on HIV status. Chi square test was performed to determine the association of the different sociodemographic variables by HIV and HPV infections and logistic regression was used to estimate the crudes odds ratio as a measure of the strength of the association of HPV infection. A 95% confidence was used in the study with a P value of less than 0.05 considered statistically significant. In these analysis HPV 16,18,31,33,35,39,45,51,52,53,56,58,59,66, and 68

were considered HR HPV. While HPV 6,11,42,43,44 and 81 were considered LR HPV.

**Ethical consideration:** The study was approved by the ethical review board. All participants gave their signed informed consent. All participants with a positive HR HPV, abnormal cytology or both were informed about their results and referred for appropriate treatment to the gynaecological oncology unit.

## Results

**Study population:** During the study period (July - December 2018), a total of 150 women (75 HIV negative and 75 HIV positive women) were enrolled into the study. The median age and interquartile range (IQR) were 40.0(35-45) years (95% CI: 39.1-43.7) for HIV positive women and 49.5(41.4-55.0) years (95% CI: 41.4-55.0) for HIV negative women (p-value < 0.001). Forty-three (57.3%) of the HIV negative women were 50 years of age or higher compared to 19 (25.3%) of the HIV positive women (p < 0.001), there were no differences in other sociodemographic variables between HIV positive and HIV negative women. Details of age group distribution, educational level, marital status and smoking characteristics of participants are presented in Table 1.

The median number of total life sexual partners of the HIV positive women was statistically higher at 2 (IQR 1, 5) while in the HIV negative women was 1 (IQR 1, 2) (p= 0.004). At univariate analysis (Table 2) showed a greater percentage of HIV positive women (OR:3.0;95%CI:1.5-5.90), were infected with HR HPV compared to HIV negative women. Women who had sexual initiation less than 24years and had at least 2 children had an increased risk of HPV infection even though it was not statistically significant.

**Prevalence of HPV:** A total of 15 different HPV types were identified in HIV positive women while a total of 11 different HPV genotypes were identified in HIV negative women consisting of both HR and LR genotypes. Twelve women were positive for LR genotypes 42(1),43(1) and 81(10).

All the LR HPV genotypes occurred with a HR HPV type.

Overall, the prevalence of HR HPV was 44% (95% CI: 35.98-52.32). The prevalence was higher in the HIV positive women 57.3% (95%CI: 45.39-68.51) compared to the HIV negative women having a prevalence of 30.7% (95% CI: 20.81-42.52) (p-value = 0.001). The odds of having any HR HPV were higher in HIV positive women (OR 3.1 95% CI:1.6-6.2).

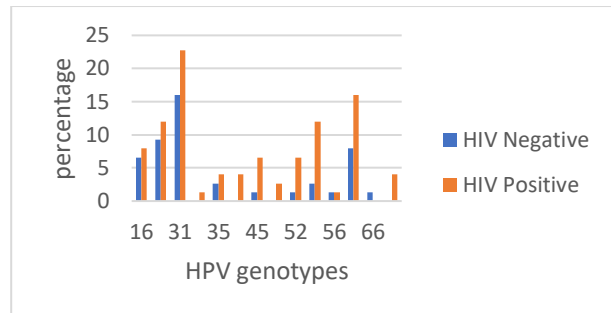


Fig 1. Distribution of HPV genotypes by HIV status

Single HR HPV genotypes were more prevalent in HIV positive women 56% (37/66), compared to HIV negative women 44% (29/66) in the overall population with odds of 2.7(95%CI:1.2-6.2). Among the HIV positive women 27% (20/75) had multiple HR HPV infections compared to 12% (9/75) in HIV negative women (OR 3.7 95%CI:1.5-9.5). Of those that had multiple types a combination of 2 to 7 HPV types were found in both HIV positive and HIV negative women.

**Type specific HPV genotypes:** The most common high-risk HPV genotypes in HIV negative women were 31 (16%), 18 (9.3%), 58 (8%), 16 (6.6%), 35, & 53 (2.6% each), 45, 52, 56 & 66 (1.3% each) while in HIV positive women were 31 (22.7%), 58(16%), 18 & 53 (12% each), 16 (8%), 45 & 52 (6.6% each), 35, 39, 68 (4%each). The HR HPV types 33,39,51 and 68 were found to be present in only HIV positive women, all other HPV types were found to be higher in HIV positive women, but this was not found to be statistically significant.

Table 1. Baseline Sociodemographic Characteristics by HIV Status

Variable	HIV +ve n=75 n (%)	HIV -ve n=75 n (%)	P value
Age	40.0(35.0-45.0)*	49.5 (41.5-55.0)*	<0.001
Age category, y			
<b>30-39</b>			
<b>40-49</b>	30(40.0)	8(10.7)	<0.001
<b>50-59</b>	26(34.7)	24(32.0)	0.730
<b>&gt;60</b>	19(25.3)	36(48.0)	0.004
	0(0.0)	7(9.3)	0.007
Education			
<b>None</b>	6(8.0)	5(6.0)	0.515
<b>Primary</b>	13(17.3)	14(18.7)	0.832
<b>Secondary</b>	20(26.7)	11(14.7)	0.070
<b>Tertiary</b>	36(48.0)	45(60.0)	0.142
Lifetime smoking status			
<b>Yes</b>			
<b>No</b>	1(1.3)	0(0.0)	1.000**
	74(98.7)	75(100.0)	1.000
Age at sexual Debut, y	19(16.0-22.0)*	20(17.3-22.0)*	0.506
<b>&lt;15</b>	5(6.7)		
<b>15-24</b>	63(84.0)	8(10.7)	0.386
<b>≥25</b>	7(9.3)	58(77.3)	0.303
		9(12)	0.598
Lifetime partners	<b>2(1-5)*</b>	<b>1(1-2)*</b>	<b>0.004</b>
<b>1</b>			
<b>2-4</b>	26(34.7)	38(50.7)	0.076
<b>≥5</b>	31(41.3)	29(38.7)	0.740
	18(24.0)	8(10.7)	0.032
Parity	<b>2.0(1-3.5)*</b>	<b>4.0(34.75)*</b>	<b>&lt;0.001</b>
		5(6.7)	
<b>0-1</b>	23(30.7)	44(58.7)	<0.001
<b>2-4</b>	31(52.0)	26(34.7)	0.034
<b>≥5</b>	13(17.3)		0.016
Contraceptive use			
<b>Yes</b>			
<b>No</b>	54(72.0)	58(77.3)	0.454
	21(28.0)	17(22.7)	0.454

\*Median (IQR),\*\*Fishers test

Table 2. Logistic regression of the factors predicting HR HPV

Characteristics	HR HPV Positive n = 66	HR HPV Negative n = 84	P value	COR (95% CI)
<b>Age, y</b>				
<b>30-39</b>	18(26.2)	20(23.8)	0.629	5.1(0.55-46.65)
<b>40-49</b>	21(32.3)	29(34.5)	0.728	4.3(0.48-38.38)
<b>50-59</b>	26(40.0)	29(34.5)	0.540	5.3(0.60-47.69)
<b>≥60</b>	1(1.5)	6(7.1)	-	1(ref)
<b>Education</b>				
<b>None</b>	5(6.1)	6(7.1)	0.920	1.15(0.33-4.09)
<b>Primary</b>	13(19.7)	14(16.7)	0.633	1.28(0.54-3.08)
<b>Secondary</b>	14(21.2)	17(20.2)	0.884	1.14(0.49-2.62)
<b>Tertiary</b>	34(51.5)	47(56.0)	-	1(ref)
<b>Lifetime smoking status</b>				
<b>Yes</b>	1(1.5)	0(0.0)	0.440	0.00-14.93
<b>No</b>	65(98.5)	84(100)	-	1(ref)
<b>Age at sexual Debut, y</b>				
<b>&lt;15</b>	2(3.0)	11(13.1)	0.030	0.4(0.06-2.52)
<b>15-24</b>	59(89.4)	62(73.8)	0.017	2.0(0.68-6.38)
<b>≥25</b>	5(7.6)	11(13.1)	-	1(ref)
<b>Number of lifetime partners</b>				
<b>1</b>	26(39.4)	38(45.2)	0.474	1.2(0.58-2.43)
<b>2-4</b>	27(40.9)	33(39.3)	0.841	1.5(0.58-3.65)
<b>≥5</b>	13(19.7)	13(15.5)	-	1(ref)
<b>Parity</b>				
<b>0-1</b>	11(16.7)	17(20.2)	0.579	0.9(0.34-2.50)
<b>2-4</b>	39(59.1)	44(52.4)	0.016	1.3(0.59-2.78)
<b>≥5</b>	16(24.2)	23(27.4)	-	1(ref)
<b>HIV status</b>				
<b>Positive</b>	43(65.2)	32(38.1)	0.001	3.0(1.5-5.90)
<b>Negative</b>	23(34.8)	52(61.9)	-	1(ref)

## Discussion

In this study, we found the prevalence of HR HPV infection among the study participants to be high (44%) and this was significantly higher in HIV positive (57.3%) women than HIV negative women (30.7%). This finding is similar to previous studies where majority reported a higher prevalence of HR HPV in HIV positive women.<sup>[17,18,19]</sup> Previous studies in Jos reported a prevalence of 44.9% for HR HPV among HIV positive women with normal cervical cytology.<sup>[20]</sup> The variation in the prevalence reported in the various studies is likely due to differences in ages of the participants, presence of normal or abnormal cytology and the HPV evaluation method used. The high prevalence in this study may be related to the proportion of participants with abnormal cervical cytology included in this study.

HPV prevalence typically peaks around age 25 years in women, and starts to decline afterwards, as the immune systems clears the virus from the cervical tissues. HPV prevalence also varies between women with normal and abnormal cervical cytology, and this may account for the difference seen in these studies. The high prevalence of HPV in HIV positive women could be attributed to increased

susceptibility of HIV positive women to HPV infections, decrease ability to clear infection and frequent reactivation of latent HPV infections.<sup>[21]</sup>

We found a high prevalence of multiple HPV infections in HIV positive women compared to HIV negative women and this is consistent with previous findings.<sup>[16,21,22]</sup> The higher prevalence of multiple HPV infection in HIV positive women has been attributable to the effect of HIV induced immunosuppression, common mode of transmission of HPV and HIV, persistence of HPV and reactivation of latent HPV infections in HIV positive women. In this study we used a method that has a high sensitivity and specificity of greater than 95% with adequate reproducibility and with ability to detect multiple infections. The analytical sensitivity for the detection of HPV 16 and 18 is as low as 10 to 50 copies, with better detection of HPV52.<sup>[14]</sup>

In this study, we found a wide range of HR HPV types, with HPV types 31,58,18 and 16 being the four most common HR HPV genotypes detected. The diversity in the prevalence of HR

HPV genotypes found between HIV positive and HIV negative women was not significant. These results are consistent with findings from other studies that showed high prevalence of non-HPV 16 and 18 types in Africa and especially within the HIV positive population.<sup>[16,20,23,24]</sup> These differ from the worldwide meta-analysis that reported HPV 16 and 18 to account for 70% of the oncogenic HPV types found in preinvasive and invasive cervical cancer. In this study we found a high prevalence of HPV 31 and HPV 58 both in HIV positive and HIV negative women with premalignant lesion of the cervix.

The high prevalence of HPV-58 and its contribution to development of high-grade dysplasia and squamous cell carcinoma has been reported in East Asia.<sup>[26,27]</sup> In Korean women, HPV-58 was found to be the second most common type of HPV in women with abnormal cervical cytology.<sup>[2]</sup> In this study we also found a high prevalence of HPV 31 and 58, so further studies are needed to determine the potential risk of these HPV genotypes in causing invasive cervical cancer.

The Currently available bivalent and quadrivalent HPV vaccines in Nigeria will still be relevant especially that there are now reports on cross-protection against HPV 45 and 31. However, the newer nonavalent vaccines that target nine HPV types (6,11,16,18,31,33,45,52,&58) has been shown to provide better coverage and is effective in targeting additional cancer causing genotypes which account for 15% of cervical cancer, 25% of precancer and more than 95% of persistent HPV 31,33,45,52,58 infections associated cervical, vulva and vaginal disease.<sup>[28,29]</sup>

This study has some strength also in that we were able to recruit both HIV positive and HIV negative women with abnormal cervical cytology, the HPV detection method was able to detect both single and multiple HPV infections and it has added to knowledge about HPV infections in North central Nigeria.

We acknowledge some limitations in this study. First, it was a hospital-based study, so the findings might not be generalizable to the entire population of women in north central Nigeria. However, being a teaching hospital with one of the largest HIV clinics in the country providing care and support to almost the entire north central region of the country, the data is likely to be a true estimate of prevalence and types of HR-HPV found in the population of women with or without HIV in North

central Nigeria. Secondly, the cross-sectional design allowed for presentation of only baseline information and did not allow us to assess which genotypes will persist and cause incident invasive cancer.

Our next step will be to perform a longitudinal study that will identify the HPV genotypes that are present in women with normal, abnormal, and invasive cervical cancer and know which ones are likely to persist.

### **Conclusion**

The prevalence of HR HPV genotypes is higher in women with HIV and other genotypes of HPV are also common apart from genotypes 16 and 18 within this group of participants. These findings suggest that using a polyvalent vaccine like Gardasil 9 which offers protection against HPV31 and HPV58 found to be more prevalent in both groups of women would be more effective in this population.

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