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Prevalence of high-risk HPV types 16 and 18 in relation to immune status and cervical cytological profile of HIV-infected women on antiretroviral therapy in northcentral Nigeria

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Abstract:

Background: Human papillomavirus (HPV) is a well-established causal agent of cervical cancer, and the first group of viruses to have been acknowledged to prompt carcinogenesis. They are linked with cancers of the uterine cervix, anogenital tumours, and head and neck malignancies. Cervical cancer is by far the most common HPV-related disease, with about 99% of cervical cancer cases caused by persistent genital high-risk (HR) HPVs, especially types 16 and 18.
Methodology: A hospital-based descriptive analytical study of 300 consenting HIV-infected women on anti-retroviral therapy (ART), selected from the three senatorial districts of Plateau State, Nigeria, was conducted over a period of 24 months (November 2018 to November 2020). Blood and cervical specimens were collected from each participant. HIV status was confirmed by standard rapid test on serum sample, $CD4^+$ cell count was determined by flow cytometry and HIV viral load estimation was done by GeneXpert nucleic acid amplification technique. Cervical cytology was performed by Papanicolaou (Pap smear) on the cervical specimen and reported according to the 2004 Bethesda system classification. HPV antigen was first detected on the cervical specimen using ELISA, and samples positive for HPV antigen were then subjected to multiplex PCR amplification of E6 and E7 genes to detect HR-HPV (16 and 18) and other HPV types. Standard questionnaires were administered to obtain information on biodata, risk factors and clinical presentations. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0, and significance level was determined at $p<0.05$.
Results: Of the 300 participants, 84 were positive for HPV of any type, giving an overall prevalence of HPV infection of 28.0%. The prevalence of HPV-16 and HPV-18 types were 5.0% (15/300) and 5.3% (16/300) respectively. Cytological analysis showed that 36.3% (109/300) of the participants had cervical abnormalities ranging from low-grade to high grade squamous intraepithelial lesions and cervical intraepithelial neoplasia. HPV prevalence of 46.8% (51/109) in women with cervical abnormalities was significantly higher than 17.3% (33/191) in women with normal cervical cytology (OR 4.2, $p<0.0001$). HPV prevalence was higher in women with AG-US (100.0%), ASC-US (78.8%), AC-US (66.7%), ASC-H (33.3%), HSIL (33.3%), HSIL (23.8%), and LSIL (41.2%) compared with women with normal cervical cytology ($p<0.001$). Aside educational level ($p=0.03$), none of the analyzed sociodemographic characteristics or risk factors for cervical cancer was significantly associated with HPV infection in the study ($p>0.05$).
Conclusion: This study showed high prevalence of HPV infections among HIV-infected patients on ART in Plateau State, north-central Nigeria including detection of high-risk HPV types 16 and 18, which are major risk factors for progression of cervical intraepithelial neoplasia to cervical cancer.

Keywords: High-risk HPV; Cervical cytology; Pap smear, HIV; ART; Multiplex PCR

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Prévalence des types de VPH 16 et 18 à haut risque en relation avec le statut immunitaire et le profil cytologique cervical des femmes infectées par le VIH sous traitement antirétroviral dans le centre-nord du Nigeria

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Résumé:

Contexte: Le virus du papillome humain (VPH) est un agent causal bien établi du cancer du col de l'utérus et le premier groupe de virus à avoir été reconnu comme pouvant provoquer la cancérogenèse. Ils sont liés aux cancers du col de l'utérus, aux tumeurs anogénitales et aux tumeurs malignes de la tête et du cou. Le cancer du col de l'utérus est de loin la maladie liée au VPH la plus courante, avec environ 99 % des cas de cancer du col de l'utérus provoqués par des VPH génitaux persistants à haut risque (HR), en particulier les types 16 et 18.
Méthodologie: Une étude analytique descriptive en milieu hospitalier portant sur 300 femmes consentantes infectées par le VIH sous traitement antirétroviral (TAR), sélectionnées dans les trois districts sénatoriaux de l'État du Plateau, au Nigeria, a été menée sur une période de 24 mois (novembre 2018 à novembre 2020). Des échantillons de sang et de col de l'utérus ont été prélevés sur chaque participant. Le statut VIH a été confirmé par un test rapide standard sur un échantillon de sérum, le nombre de cellules $CD4^+$ a été déterminé par cytométrie en flux et l'estimation de la charge virale du VIH a été effectuée par la technique d'amplification de l'acide nucléique GeneXpert. La cytologie cervicale a été réalisée par Papanicolaou (test Pap) sur l'échantillon cervical et rapportée selon la classification du système Bethesda 2004. L'antigène HPV a été détecté pour la première fois sur l'échantillon cervical à l'aide d'ELISA, et les échantillons positifs pour l'antigène HPV ont ensuite été soumis à une amplification PCR multiplex des gènes E6 et E7 pour détecter le HR-HPV (16 et 18) et d'autres types de HPV. Des questionnaires standard ont été administrés pour obtenir des informations sur les données biologiques, les facteurs de risque et les présentations cliniques. Les données ont été analysées à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 26.0 et le niveau de signification a été déterminé à $p < 0,05$.
Résultats: Sur les 300 participants, 84 étaient positifs pour le VPH de tout type, ce qui donne une prévalence globale de l'infection au VPH de 28,0%. La prévalence des types HPV-16 et HPV-18 était respectivement de 5,0% (15/300) et 5,3% (16/300). L'analyse cytologique a montré que 36,3% (109/300) des participants présentaient des anomalies cervicales allant de lésions intraépithéliales squameuses de bas grade à haut grade et d'une néoplasie intraépithéliale cervicale. La prévalence du VPH de 46,8% (51/109) chez les femmes présentant des anomalies cervicales était significativement supérieure à 17,3% (33/191) chez les femmes présentant une cytologie cervicale normale (OR 4,2, $p<0,0001$). La prévalence du VPH était plus élevée chez les femmes atteintes d'AG-US (100,0%), d'ASC-US (78,8%), d'AC-US (66,7%), d'ASC-H (33,3%), de HSIL (33,3%), de HSIL (23,8%) et LSIL (41,2%) par rapport aux femmes présentant une cytologie cervicale normale ($p<0,001$). Hormis le niveau d'éducation ($p=0,03$), aucune des caractéristiques sociodémographiques analysées ou des facteurs de risque de cancer du col de l'utérus n'était significativement associée à l'infection par le VPH dans l'étude ($p>0,05$).
Conclusion: Cette étude a montré une prévalence élevée d'infections par le VPH chez les patients infectés par le VIH sous TAR dans l'État du Plateau, au centre-nord du Nigeria, y compris la détection des types 16 et 18 du VPH à haut risque, qui sont des facteurs de risque majeurs de progression de la néoplasie intraépithéliale cervicale vers le col utérin cancer.

Mots clés: VPH à haut risque; Cytologie cervicale; Test Pap; VIH; ART; PCR multiplexe

Introduction:

Human papillomavirus (HPV) is the most common sexually transmitted pathogen, and it cause cervical lesions and cancers in both males and females. Accumulating epidemiological evidence supports a strong association between HPV and genital warts, as well as cancer of the cervix, vulva, anus, and penis (1). Cervical cancer is the most common genital tract malignancy among women in developing countries, including Nigeria accounting for more than 266,000 deaths annually (2).

Globally, there are over 528,000 new cases of cervical cancer with 85% of them occurring in developing countries (3). Although most infections with HPV show no symptoms, persistent genital HPV infection results in cervical cancer and virtually all cases (approximately 99%) of cervical cancer are associated with genital infection with high-risk (HR) HPV infection as reported in a previous study by Ajang et al., (4).

In sub-Saharan Africa, cervical cancer is the leading cause of cancer death among women as previously reported by Garcia-Espinosa et al., (5). Current data indicate that cervical cancer ranks as the second most common cancers among Nigerian women, with 7,968 deaths annually (6). There is a significant difference in terms of cervical cancer mortality and morbidity between developing and developed countries, with significant reduction in cervical cancer cases and associated deaths in develo-ped countries due to implementation of effective screening programs and HPV vaccination (7). In the United States, cervical cancer has decreased in incidence and mortality since the mid-19th century primarily due to screening programs (8). However, even with the intro- duction and widespread use of the pap smear test, cervical cancer still ranks among the top ten cancers diagnosed in the US within minority populations, which include Blacks, Indian Americans and Hispanics (8).

Cervical cancer is both preventable and curable. This is because, cervical cancer has a relatively long lead time and in general

precancerous lesions slowly progress through identifiable and recognizable stages before transforming into invasive disease. If the disease could therefore be identified before progressing to advanced stages, it can effectively be regarded as curable (7). Accumulating evidence suggests a strong correlation between HPV and genital warts as well as cancers of the cervix, vulva, vagina, anus and penis (1).

Human papillomaviruses are broadly categorized into high-risk (HR) and low-risk (LR) HPV types, depending on their association with malignancy. While the HR types e. g. types 16 and 18 are associated with different forms of malignancy, the LR types such as types 6 and 11, are associated with genital warts. HPV is considered as one of the viral infections associated with cancers and other diseases worldwide as reported by Tulay and Serakinci (9). Persistent HR HPV infections, specifically types 16 and 18, has been strongly linked to the development of cervical cancer, anogenital cancers, and oropharyngeal cancers (8).

Genital HPV is highly prevalent among women of reproductive age because they are mostly sexually active. Due to biological and physiological differences in their cervical epithelium with columnar or plastic epithelium as against squamous epithelium in older adults, young women are more vulnerable to HPV infections (10). However, most HPV infections regress spontaneously, and only in a small proportion of cases, the infection persists as low-grade intraepithelial lesion (LSIL), progressing to high-grade intraepithelial lesions (HSIL), and ultimately to invasive cervical carcinoma (10). It is now established that persistent infection with HR HPV is a necessary but not-sufficient cause of cervical cancer (10). The central factor in progression to cervical cancer is persistent HPV infection. Persistence in this case refers to detecting a similar HPV genotype in the same person twice or more within 6 months to one year.

HPV type 16 is the most common HPV infection in invasive cervical cancers but HPV type 18 has been shown to play a more significant role in the development of cervical adenocarcinoma, with a prevalence of nearly 40% in these tumours (2). Although HPV 16 is still the most prevalent HPV infection in adenocarcinoma, infection with HPV 18 confers a higher risk of development of adenocarcinoma. In younger women, HPV 18 has been found in up to 34% of cervical adenocarcinoma and 35% of cervical adenosquamous carcinomas (2).

HPV 6 and 11 are HPV types that cause 90% of all anogenital warts and most cases of oropharyngeal papillomatoses. Most early HPV infections, especially LR types, are self-limiting and often do not result in clinical disease. The HPV types contained in the quadrivalent vaccine (HPV 16, 18, 6, and 11), are implicated in 30% of all cervical intraepithelial neoplasia (CIN) 1 disease. While uncommon in early CIN, HPV 16 and 18 are found in 50%–60% of CIN 2 and CIN 3 disease (2).

Women living with HIV are at increased risk for HPV infection and HPV-related tumours, including CIN2/3 and invasive cervical carcinoma (11). The prevalence of HPV and CIN has been reported to increase with increase in immunosuppression (11). Combination antiretroviral therapies (cART) against HIV have greatly reduced the incidence of opportunistic infections, Kaposi's sarcoma, and non-Hodgkin's lymphoma, but not the incidence of HPV-associated cervical and anal carcinomas (12). This is perhaps not surprising as HPV-associated carcinomas have a long latent phase. A favorable effect of cART on HPV infection and cervical precancerous lesions has been shown in some but not all studies (13-16). In 2006, the United States Food and Drug Administration (FDA) approved HPV vaccine, *Gardasil*, against HR-HPV types 16 and 18 as well as LR types 6 and 11, for all females aged 9-26 years. In 2009, the FDA also approved the use of this vaccine in males aged 9-26 years. Another HPV vaccine, *Cervarix*, which aims to protect against HR-HPV types 16 and 18 only, was approved by the FDA in the Fall of 2009 for females aged 10-25years.

The oncogenic potential of a particular HPV type highlights the importance of detecting and genotyping HR HPV types, especially types 16 and 18. The results of HPV testing may have significant therapeutic and prognostic implications, providing clinicians with valuable information for deciding the most appropriate course of action for each patient. The information could also provide data necessary for implementation of vaccination programs against cervical cancer (1). The objectives of this study therefore are to determine the prevalence of HR-HPV types 16 and 18 in relation to immune status and cervical cytological profiles of selected HIV-infected women on antiretroviral therapy in Plateau State, north-central Nigeria.

Materials and method:

Study setting and design:

This study was conducted in six major hospitals comprising teaching, specialist and general hospitals across the three senatorial zones of Plateau State, northcentral Nigeria. It is a hospital-based descriptive analytical design among HIV-infected women aged 15-74 years on ART irrespective of background and socio-economic status attending the selected hospitals during the study period (Nov 2018 to November 2020).

Study population and participants:

The study population comprised of HIV

positive women on ART who are undergoing blood sampling for $CD4^+$ cell count and viral load estimations, and those coming for routine cervical screening test. A total of 300 HIV-positive females within reproductive age were recruited as the participants into the study.

Ethical consideration:

The approval for the study was obtained from the Institutional Review Board (IRB) of Jos University Teaching Hospital and the Ethical Committee of Plateau State Specialist Hospital, while informed consent was obtained from all participants and/or their legal guardians.

Sample size determination:

The sample size was determined by using the formula described by Naing et al., (17); $n=Z^2 p(1-p)/d^2$, where 'n' is the sample size, 'p' is the prevalence from a previous study of 15.0% (0.150) by Musa et al., (18), 'Z' is the standard normal distribution at 95% confidence interval (1.96) and 'd' is the absolute desired precision at 5% (0.05). Therefore, the calculated sample size was 195.9, however, in order to account for attrition, the sample size was adjusted for 10% attrition, to give a total of 300 samples.

Sampling method and data collection:

Systematic random sampling method was used to select the 300 HIV-positive participants at the various sampling points across the three senatorial zones of Plateau State, Nigeria. A structured questionnaire containing both closed and open-ended questions was interviewer-administered on each participant to obtain relevant information on socio-demographic data, socioeconomic status, behavioral and sexual habits, clinical presentations and risk factors of HPV infections and cervical cancer.

Sample collection, transportation and storage:

Blood and cervical swab samples were collected from each participant. Swab samples were collected from the endocervix using liquid-based cytology technique after exposing the cervix with Cusco speculum by an experienced cytopathologist. First, excess mucus was removed from the cervix and surrounding mucosa using cleaning swabs, and thereafter, a collection swab was first used to obtain samples for cytology before using a cytobrush to collect swabs for HPV detection. This was done by turning the cytobrush clockwise for approximately 15 seconds to ensure adequate sampling. The head of the cytobrush containing the swab sample was then dropped off inside the sample tube containing the liquid preservative and transported to the histopathology unit of Jos University Teaching Hospital for analysis.

Blood samples collected into sterile specimen bottles were centrifuged and serum separated. Together with the cervical samples, they were stored at -4^0C until analysis for HIV detection, $CD4^+$ profiling and viral load estimations, HPV detection, and cervical cytological analysis

HIV detection by rapid test:

The serum sample of each participant was tested for HIV antibodies using the rapid diagnostic test in accordance with the national (serial) algorithm for HIV testing in Nigeria. This required the use of a first line test kit (Determine®) and confirming with a second line test kit (Unigold®) before finally tie breaking with the third test kit (Stat pak®) in cases where there are discrepancies in the results between the first and the second test kits.

The test procedure involved applying 10 ml of serum sample on the test pad of the kit and allowing it to flow through the chromatographic pad and the results read after 10 minutes and interpreted as positive, negative or invalid.

$CD4^+$ count profiling and viral load estimation:

The flow cytometry (Sysmex cyflow counter) method was used for $CD4^+$ count and the results expressed as cells/mm^3. The viral load of each HIV positive participant was estimated using GeneXpert technology and the results expressed as virus copies/ml.

Cytological analysis:

The standard Pap smear procedure for cervical cells profiling was performed on all the participating women to assess any cytological changes likely associated with HPV infection. The slides were prepared, fixed and stained using the Papanicolaou (Pap smear) technique. The stained smears were examined, and the results reported accordingly to the 2004 Bethesda system classification as reported by Musa et al., (17). Except for participants with normal cytology results, all others were referred to the gynaecology clinic for further evaluation.

Viral antigen detection using ELISA:

All the cervical specimens were tested for the presence of HPV antigens and further characterized using type-specific ELISA for HR HPV (HPV 16 and 18) using commercially available enzyme immune assay (Diagnostic Automation/Cortez Diagnostics Inc., USA) ELISA kits. The assay was performed according to the manufacturer's instructions to determine HPV positive samples.

DNA extraction and PCR assay:

The DNA extraction, pre-amplification procedures and HPV genotyping were perfor-

med using standard methods. The DNA preparation kit (Inqaba biotech, South Africa) was used to extract DNA, according to the manufacturer's instructions at the AIDS Prevention Initiative in Nigeria (APIN) laboratory in Jos, Nigeria. PCR was conducted on the extracted DNA using multiplex PCR detection and genotyping kit (MaxyGene Gradient Thermal Cycler Canada).

Polymerase chain reaction assay:

Multiplex PCR amplification of E6 and E7 genes was done for all 84 identified HPV positive samples, as described by Shahi et al., (19), and following the manufacturer's instructions, using primers (forward and reverse) and conditions outlined in Table 1. During the amplification phase all the PCR reagents were spun out and a mixture of PCR master mix and DNA Taq polymerase were prepared per PCR reaction tube. One microlitre of DNA template was then added to each PCR tube. The solution was centrifuged for a few seconds and placed in the thermal cycler for DNA amplification.

Each PCR was carried out in the thermal cycler (MaxyGene Gradient Thermal cycler, USA) with the following conditions: initial step at 95^0C for 15 min, 10 cycles of 30s at 94^0C, 90s at 65^0C, and 90s at 72^0C, followed by 30 cycles of 30s at 94^0C, 90s at 63^0C, and 90s at 72^0C, with a final extension at 72^0C for 10 min.

Gel electrophoresis of PCR amplicons:

The PCR products were resolved by gel electrophoresis on a 2% agarose gel stained with ethidium bromide, and the band sizes were estimated by comparison with 100bp molecular weight marker (GeneRuler 100bp DNA ladder, Fermentas International, Canada). The gels were photographed in a UV transilluminator (UVP, USA) with a Canon PowerShot A60 digital camera.

HPV types were adequately assigned based on the amplification pattern. However, in cases where band amplification was not clear, an additional PCR amplification with specific primers was performed to confirm the HPV type.

Statistical analysis:

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 26.0. Significant differences between variables were tested using Chi-square test (for categorical variables) and student's *t*-test (for comparing means). Statistical significance was determined at $p<0.05$ with 95% confidence interval. Results were presented in frequency tables, ratios and percentages.

Table 1: Primer sequences used for the study

Name	Forward primer sequence (5'-3')	Reverse primer sequence (3'-5')	Lane	Size bp
16-1/F	TTAGGCAGCACTTGGCCAACCA	TAATCCGTCCTTTGTGTGAGCT	2	207
16-2/R	ACTGCAATGTTTCAGGACCCAC	CGAAGCGTAGAGTCACACTTGC	1	661
18-1/F	TCGCGTCCTTTATCACAGGGCGA	TGCCCAGGTACAGGAGACTGTG	2	536
18-2/R	TCCGTGGTGTGCATCCCAGCAG	CACTTGTGCATCATTGTGGACC	7	274
β-globin (internal control)	GAAGAGCCAAGGACAGGTAC	CAACTTCATCCACGTTCACC	7	286

Results:

Sociodemographic and clinical characteristics of the study participants:

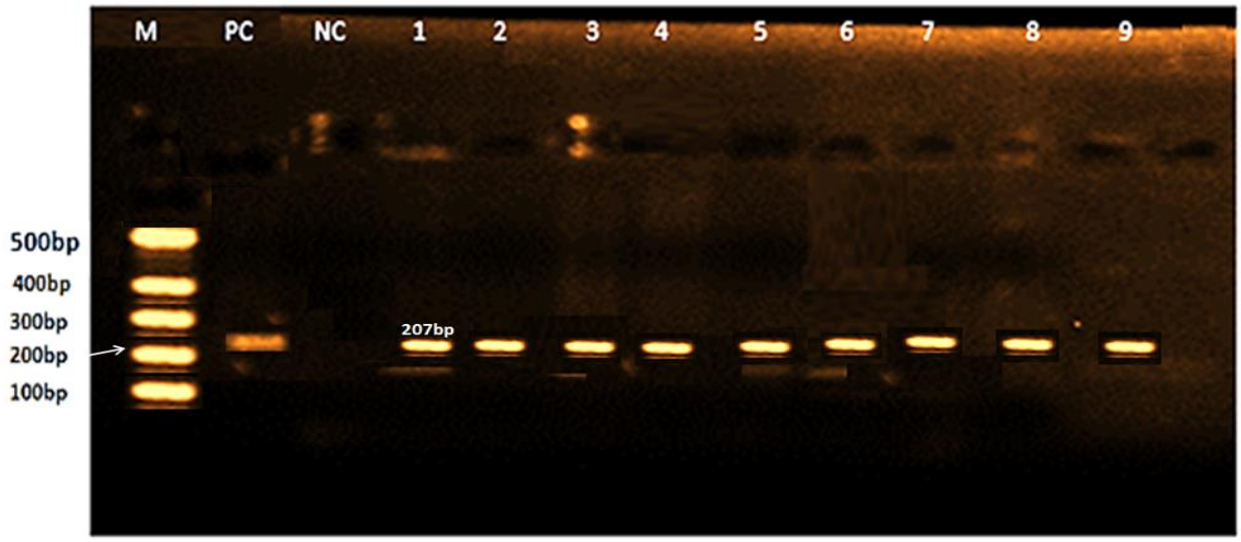
Table 2 shows socio-demographic and clinical characteristics including risk factors of the study participants. Most of the participants are married (42.0%), with 30.7% being widowed, 17.3% single and 10.0% divorced/separated. Majority (189, 63%) are in monogamous marriage. Most (109, 36.3%) had secondary and tertiary education each while only 10 (3.3%) had no formal education.

Most occupational groups among the participants are civil servants (27.7%) followed by traders (26.3%), full housewives (14.0%), farmers (8.7%), artisans (8.3%), students (5.0%), applicants (2.3%), retires (2.0%) and teachers (2.0%).

Table 2: Sociodemographic characteristics of HIV-infected women with respect to HPV infection in Plateau State, Nigeria

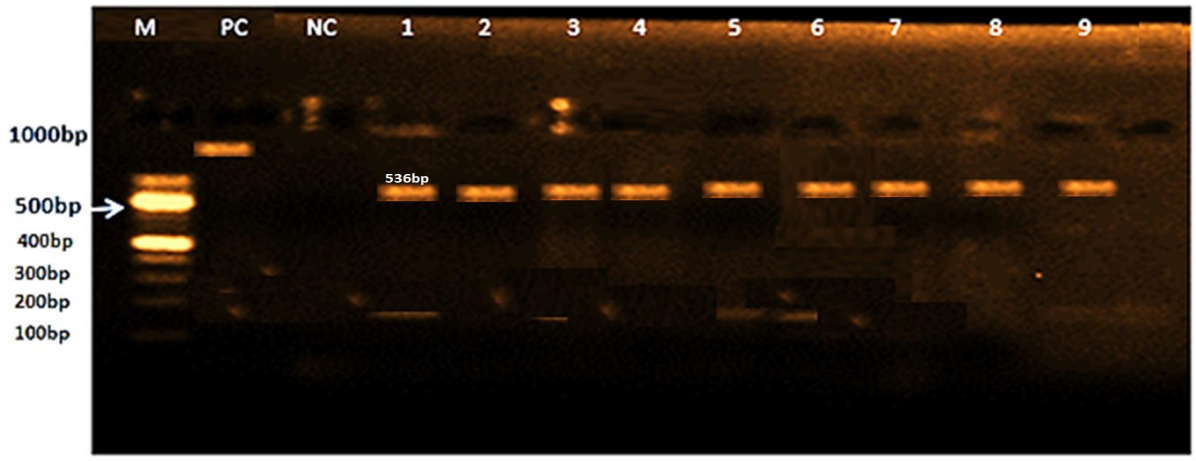
Socio-demographics	No. screened (n=300)	No positive for HPV (n=84, 28.0%)	*p*-value
Marital status			
Single	52	15 (28.8)	0.914
Married	126	33 (26.2)	
Divorced/separated	30	8 (26.7)	
Widow	92	28 (30.4)	
Type of family			
Monogamy	189	58 (30.7)	0.525
Polygamy	55	11 (20.0)	
Others	56	15 (26.8)	
Level of education			
No formal education	10	0	0.03*
Primary	72	25 (34.7)	
Secondary	109	24 (22.0)	
Tertiary	109	35 (32.1)	
Occupation			
Applicants	7	0	0.313
Civil servants	83	26 (31.3)	
Farmers	26	05 (19.2)	
Housewives	42	11 (26.2)	
Artisans	25	10 (40.0)	
Retirees	06	2 (33.3)	
Students	15	1 (6.7)	
Tailors	11	4 (36.4)	
Teachers	06	1 (16.7)	
Traders	79	24 (30.4)	

* = significant at p≤0.05, % = percentage, No = number, HPV= human papillomavirus



Lane M: 100-1000bp DNA ladder. Lane PC: Positive control; Lane NC: Negative control; Lanes 1-9: Samples' labels.

Plate 1: Agarose gel electrophoresis of PCR products after amplification of HPV 16 gene at 207bp



Lane M: 100-1000bp DNA ladder. Lane PC: Positive control; Lane NC: Negative control; Lanes 1-9: Samples' labels.

Plate 2: Agarose gel electrophoresis of PCR products after amplification of HPV 18 gene at 536bp

Prevalence of HPV and high-risk HPV 16 and 18 infections:

Of the 300 participants, 84 were HPV-positive by PCR, giving an overall prevalence of HPV infection of 28.0%. The prevalence of high-risk HPV types 16 and 18 was 10.3% (31/300), with HPV-16 (5.0%) and HPV-18 (5.3%). Plate 1 is the agarose gel electrophoresis of HPV-16 PCR amplicon size of 207bp while plate 2 is that of HPV 18 with amplicon size of 536bp.

HPV infection also occurred more frequently among civil servants, traders and full-time housewives than among artisans, tailors and teachers, students and retirees as shown in Fig 1. HPV-16 infection occurred more frequently among HIV-infected women of age group 45-54 years and least or no infection observed among early sexual debutants (15-24 years old) and among the elderly (65-74 years) as presented in Table 3.

For HPV-18 infection, the highest prevalence (14.3%) was recorded among the age group 55-64 years and 0% among the very elderly (65-74 years) although the association between age group and HPV-16/18 infections was not statistically significant (p>0.05).

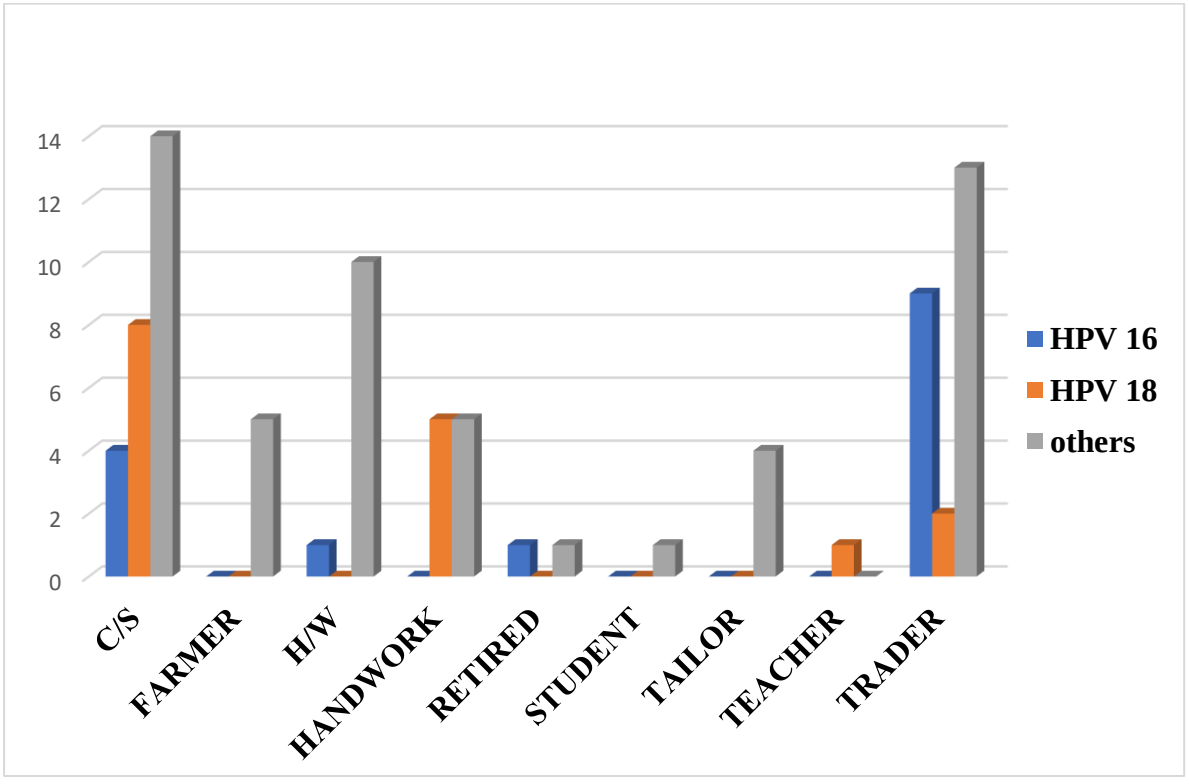


Fig. 1: Frequency distribution of HPV infections with respect to occupations of HIV-infected women in Plateau State, Nigeria

Sociodemographic characteristics and risk factors of HPV infections:

There only sociodemographic characteristic significantly associated with HPV infection in the study was the participant's level of education, with lower HPV prevalence (0%) among those with no formal education compared to those with primary (34.7%), secondary (22.0%) and tertiary education (32.1%) levels (p=0.03). There was no statistically significant associations (p>0.05) between marital status, type of family and occupation, with HPV infections (Table 2), as well as with the age group of participants (Table 3).

Of all potential risk factors for cervical cancer analyzed among the study participants, no significant statistical association (p>0.05) was established between HPV infections and the analyzed risk factors both in the bivariate analysis (Table 4) and in the multiple logistic regression analysis (Table 5).

HPV infection and immune status ($CD4^+$ cell count):

Analysis of immune status of the parti cipants in relation to HPV infection showed that robust immune system (as determined by $CD4^+$ cells) was associated with lower HPV prevalence. In other words, the HPV prevalence of 31.9% (53/166) was higher in HIV-infected women with lowest $CD4^+$ count of 0-200 cells/mm³ compared to HPV prevalence of 25.0% (6/24) in those with $CD4^+$ cell count of >600 cells/mm³ (Table 6). However, the difference is not statistically significant (p=0.383).

HPV infection and cervical cytology:

The cytological analysis showed that a total of 109 (36.3%) participants had cervical abnormalities, 51 (46.8%) of whom had HPV infections while 33 of 191 (17.3%) women with normal cervical cytology had HPV infections. The HPV prevalence was highest in women with advanced forms of cervical abnormalities such as AGUS endocx (100.0%, 1/1) and AGUS endomet favor neoplastic (100.0%, 1/1) (p<0.001). On the other hand, women with milder forms of cytological abnormality such as LSIL, HSIL and HSIL susp for invasion, have lower HPV prevalence of 41.2%, 33.3% and 23.8% respectively, compared to those with advanced cervical abnormality, while women with harsher forms of cervical abnormalities such as ASC-H, AC-US, and ASC-US have low to high HPV prevalence of 33.3%, 66.7% and 78.8% respectively. Conversely, women with normal cervical cytology have lower HPV prevalence of 17.3% (33/191), which shows significantly lower HPV prevalence compared to women with abnormal cervical cytology (OR 4.2, p<0.0001) (Table 7).

The prevalence of HR HPV (HPV16 and 18) infections in women with abnormal cervical cytology was 8.2% (8/109) for HPV-16 and 10.1% (11/109) for HPV-18 while for other HPV types, the prevalence was 28.4% (31/109) (Table 8). These prevalence rates are comparatively lower for women with normal cervical cytology, with rates of 3.1% (6/191), 2.6% (5/191) and 11.5% (22/191) respectively, although the differences are not statistically significant (x^2=0.546, p=0.760).

HPV co-infections with other sexually transmitted infections:

There were more co-infections of HPV with bacterial vaginosis and other yeast infections than with *Candida* and herpes simplex virus (HSV) infections as shown Fig 2.

Table 3: Prevalence of HPV infections in relation to age of HIV-infected women on ART in Plateau State, Nigeria

Age group (years)	No of women tested	No positive (%) HPV-16	No positive (%) for HPV-18	No positive (%) for other HPV types	*p*-value
15-24	10	0	1 (10.0)	0	0.450
25-34	46	0	1 (2.2)	10 (21.7)	
35-44	131	8 (6.1)	6 (4.6)	19 (14.5)	
45-54	82	6 (7.3)	4 (4.9)	16 (19.5)	
55-64	28	1 (3.6)	4 (14.3)	7 (25.0)	
65-74	3	0	0	1 (33.3)	
Total	**300**	**15 (5.0)**	**16 (5.3)**	**53 (18.3)**	

% = percentage, No = number, HIV=human immunodeficiency virus, ART=antiretroviral therapy, HPV=human papillomavirus

Table 4: HPV infection in relation to risk factors of cervical cancer among HIV-infected women in Plateau State, Nigeria

Variable	No of sample (n=300)	No positive for HPV (%) (n=84, 28.0%)	X^2	*p*-value
History of immunisation				
Yes	17	6 (35.3)	0.476	0.490
No	283	78 (27.6)		
If yes, number of doses				
One	14	4 (25.2)	1.570	0.210
Two	3	26 (31.7)		
Heard of cervical cancer?				
Yes	291	82 (28.2)	0.032	0.844
No	8	2 (25.0)		
Heard of pap smear test?				
Yes	276	77 (27.9)	0.018	0.894
No	24	7 (29.2)		
Have you been screened?				
Yes	115	33 (28.7)	0.045	0.832
No	185	51 (27.6)		
Parity				
0 – 5	241	67 (27.8)	0.024	0.877
6 – 10	59	17 (28.8)		
Vaginal herbs/douching?				
Yes	67	21 (31.3)	0.478	0.489
No	233	63 (27.0)		
If yes, how long? (years)				
1 – 5	28	8 (28.6)		
6 – 10	27	8 (29.6)	0.667	0.881
11 – 15	5	2 (40.0)		
>15	7	3 (42.9)		
Do you use contraceptives?				
Yes	166	40 (24.1)	2.809	0.094
No	134	44 (32.8)		
Had STI?				
Yes	220	66 (30.0)	1.637	0.201
No	80	18 (22.5)		
Name of STI				
Others	80	18 (22.5)	10.480	0.313
Bacterial vaginosis	81	30 (37.0)	9.177	0.164
Candidiasis	20	7 (37.0)		
Gonorrhoea	1	0		
HSV	3	1 (33.3)		
Yeast	115	28 (24.3)		

x^2 = Chi square, % = percentage, No = number, STI=sexually transmitted infections, HSV=Herpes simplex virus

Table 5: Multiple regression analysis of independent risk factors for HPV infection in the study participants

Risk factors	Odd Ratio	95% CI	*p*-value
No of sex partners	0.19	(0.01-1.32)	0.342
Condom usage	0.95	(0.52-1.72)	0.855
Cigarette smoking	1.33	(0.52-3.42)	0.555
Alcohol intake	0.95	(0.52-1.72)	0.855
Immune status	1.56	(0.87-2.81)	0.136
Immunization status	1.43	(0.51-4.01)	0.492
Vaccine Dose	1.43	(0.51-4.01)	0.492
Parity	1.01	(0.23-4.23)	0.111
Other STIs	1.48	(0.81-2.69)	0.202
Age at sexual debut	1.21	(0.628-2.13)	0.139

CI = Confidence interval; % = percentage, No = number, STI = Sexually transmitted infections, HPV=Human papillomavirus

Table 6: Relationship between HPV infection and immune ($CD4^+$) status of HIV-infected women

$CD4^+$ count (cells/mm^3)	**Number tested**	**No of HPV positive (%)**	***p*-value**
0-200	166	53 (31.9)	0.383
201-400	70	15 (21.4)	
401-600	40	10 25.0)	
≥ 601	24	6 (25.0)	
Total	**300**	**84 (28.0)**	

% = percentage, No = number, HIV=human immunodeficiency virus, ART=antiretroviral therapy, HPV=human papillomavirus

Table 7: Prevalence of HPV in relation to cervical cytology of HIV-infected women in Plateau State, Nigeria

Cytological changes	**No examined**	**No of HPV positive (%)**	***p*-value**
AGUS, endocx	1	1 (100)	<0.001*
AGUS, endomet favor neoplastic	1	1 (100)	
ASC-US	33	25 (78.8)	
AC-US	6	4 (66.7)	
ASC-H	3	1 (33.3)	
HSIL	6	2 (33.3)	
HSIL, susp for invasion	42	10 (23.8)	
LSIL	17	7 (41.2)	
Negative for intraepithelial lesion or malignancy	191	33 (17.3)	
Total	**300**	**84 (28.0)**	

* = significant at $p \leq 0.05$, % = percentage, No = number, AGUS=Atypical glandular cells of undetermined significance, ASC-US=Atypical squamous cells of undetermined significance, HSIL=High-grade squamous intraepithelial lesion, LSIL=Low-grade squamous intraepithelial lesion, ASC-H= Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, AC-US=Atypical cell of undetermined significance.

Table 8: Statistical analysis of HPV infections with cervical cytology of HIV-infected women on ART in Plateau State, Nigeria

Cervical status	**No. examined**	**Total HPV (%)**	**x^2**	**OR (95% CI)**	**p value**	**HPV types**			**x^2**	**p-value**
						HPV 16 (%)	**HPV 18 (%)**	**Other HPV types (%)**		
Normal cervix	191	33 (17.3)	28.534	4.210 (2.475-7.163)	<0.0001*	6 (3.1)	5 (2.6)	22 (11.5)	0.546	0.760
Abnormal cervix	109	51 (46.8)				9 (8.2)	11 (10.1)	31 (28.4)		
Total	**300**	**84 (28.0)**				**15 (5.0)**	**16 (5.3)**	**43 (14.3)**		

χ^2 = Chi square, OR = Odds ratio, CI = Confidence interval, * = Significant at $p \leq 0.05$, % = percentage, No = number, HIV=human immunodeficiency virus, ART=antiretroviral therapy, HPV=human papillomavirus

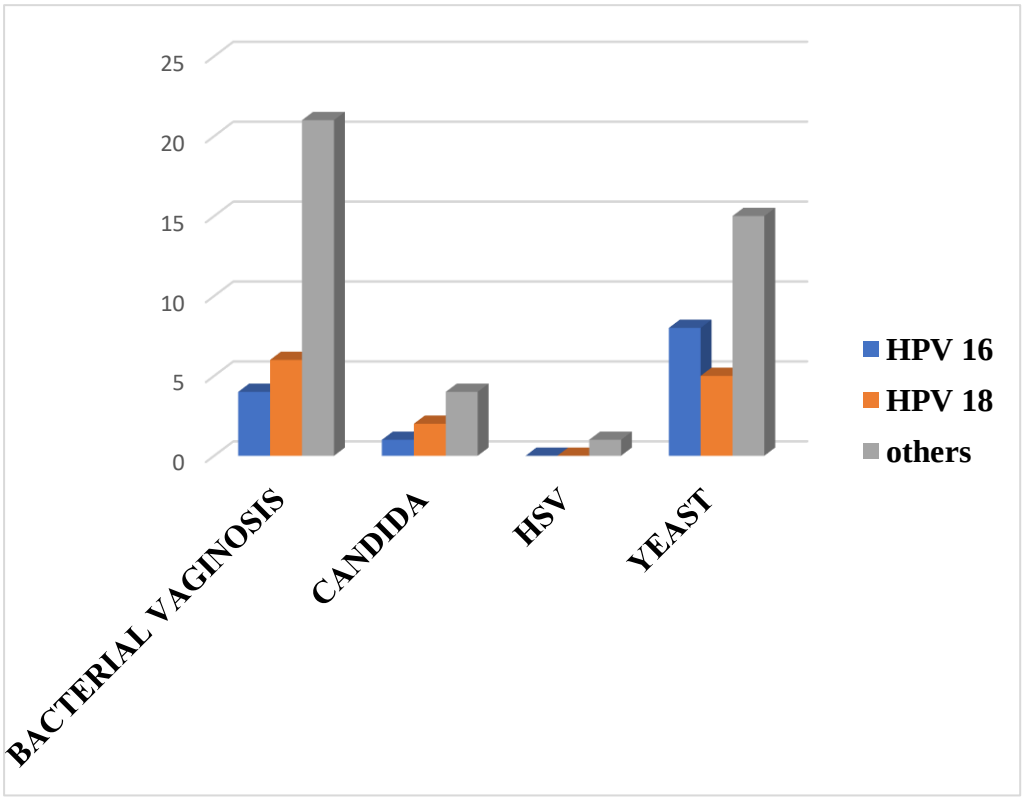


Fig. 2: Co-infection of HPV and other sexually transmitted infections among HIV-infected women in Plateau State, Nigeria

Discussion:

This study was designed to determine the prevalence of high-risk HPV infection, with particular interest in highly oncogenic types 16 and 18 infection and its association with immune status and cervical cell abnormalities and risk of cervical cancer among HIV-infected women in northcentral Nigeria. The population studied are considered at high risk of cervical cancer due to their HIV status. The demographic analysis showed that majority of the participants were married (126/300), and in monogamous family (63.0%). Most of the participants (27.6%) were civil servants and majority (36.3%) had secondary and tertiary education. However, only education was significantly associated with HPV infections (p=0.03) with 0% HPV prevalence in participants with no formal education compared to 34.7%, 22% and 32.1% in those with primary, secondary and tertiary education respectively. This finding implies that higher educational status may increase the odd of acquiring HPV infection.

The low HPV prevalence in HIV-infected women without formal education in our study is in contrast to the findings of a multivariate analysis of previous studies conducted by Stephen et al., (20) that showed strong association of HPV awareness with higher level of education. Part of the reasons for this may be due to the fact the risk of HPV infection is a complex phenomenon where aside educational status, there are biological and psychosocial variables that are equally important determinants of the risk of HPV infection.

Globally, HPV testing for HR HPV especially types 16 and 18 is now acceptable as a viable and validated option in the management of women with abnormal cervical cytology results. Recently, interest is increasingly mounting for the use of HPV testing from cervical samples of asymptomatic women with normal cytology results. This is to enable early identification of different levels of risk for cervical neoplasia especially from high-risk population such as HIV-positive women, due to the close relationship between HPV infection and cervical cancer development. In spite of this need, there are few reliable data on the prevalence of HR HPV among high-risk population in Nigeria.

Our results showed an overall prevalence of 28.0% for HPV infection among HIV-infected women 15 years and older attending various ART and gynecological units in Plateau State, northcentral Nigeria. This is however lower than the 37.0% HPV prevalence in the general population in Nigeria. Our finding is similar to 28.5% HPV prevalence reported among HIV-positive women in Eastern Cape, South Africa, and also agrees with the findings of Sarkar et al., (21) in India who reported the HPV prevalence among HIV-infected females of 32.2%, and Cubie et al., (22) who reported

HPV prevalence of 25.0%. However, the HPV prevalence in our study is much lower than the prevalence of 63.3% reported by Monteiro et al., (23) in Brazil.

The prevalence of 5.0% for HPV-16 and 5.3% for HPV-18 in our study are comparable to the prevalence of 2.7% and 3.1% reported by Ahmed et al., (24) for HPV 16 and 18 in Saudi Arabia, and the prevalence of 7.1% and 10.1% for HPV-16 and 18 in northern Ethiopia by Temesgan et al., (25). The probable reasons for the similarity in HPV prevalence in our study with theirs may be due in part to the common risk factors for HPV infections in the population of these two countries. More so that all the studies included women without apparent cervical cancer risks i.e. no known cervical abnormality. On the other hand, the prevalence in our study are lower than those reported by Xiang et al., (26) in China and Kulkarni et al., (27) in India who reported as high as 18.0% and 22.0% for HPV 16 and 18 respectively. This difference may partly be due to the differences in the population characteristics, as both of these researchers conducted their studies on women known to have abnormal cervical cytology in contrast to our study that did not concentrate on such subpopulation. It may also be due to differences in the diagnostic sensitivities of the detection methods used in the studies.

The analysis of association of risk factors of cervical cancer with prevalence of HPV infection in our study did not show significant association with any factor (p>0.05). However, there were higher odds for HPV infection among women who debuted sexual relationships at an early age, women co-infected with other STIs, cigarettes smokers and women with low immune status while on the other hand, women with multiple sex partners, who consumed alcohol and who regularly used condoms during sex, had lower odds of HPV infection, although these odds did not attain statistical significance (p>0.05). It is evident that many studies evaluating influence of risk factors on both HPV infections and cervical abnormalities have produced conflicting results as reported by Bahmanyar et al., (28).

Some of the reasons for conflicting findings in studies that investigate influence of risk factors on HPV and cervical abnormality may be due to differences in study designs including the diagnostic sensitivity and specificity of methods used in detecting HPV infection. While some researchers used ELISA technique, others including ours used the more sensitive and specific technique such as PCR assay. Another possible reason maybe the study population involved. For instance, while some studies were based on the general population with perceived low risk, our study was conducted among the most-at-risk-population (HIV-infected women) thereby contributing to the observed differences. However, in agreement with our findings, several other studies have documented significant associations of cigarette smoking, level of education, low immune status and lower age at sexual debut with both HPV infection, cervical abnormality and cervical cancer. Bacterial vaginosis and yeast infections were the commonest STIs co-infecting with HPV among the HIV-infected women in this study while co-infections with others STIs such as *Candida* and HSV occurred less frequently. It is well established that other STIs may increase the risk of persistent HPV infection and invasive cervical cancer.

The implementation of highly active antiretroviral therapy (HAART) among HIV-infected persons results in immune reconstitution, slower progression of HIV disease and decrease in occurrence of opportunistic infections. However, the impact of HAART on cervical HPV infection, clearance, and persistence in high-risk adolescents remains controversial (16). Our study showed that majority (55.3%) of the HIV-infected women had very low $CD4^+$ cells (0-200 cells/µl of blood) while only 8.0% had robust $CD4^+$ cells of >600/µl of blood. Generally, the immune status of HIV-infected persons is measured by the level of their $CD4^+$ cells. Therefore, the level of the immunity as measured by $CD4^+$ cell count shows how effective they will be in resisting any form of infection or minimizing its impact after infection. In the case of HPV infection and by extension cervical cancer, the immune status may not prevent HPV infection, but can go a long way in clearing the virus thereby preventing its persistence and by implication progression to cervical cancer. Adequate immunosurveillance is crucial for viral elimination and preventing disease establishment or persistence. When immunosurveillance is compromised, HPV disrupts critical signaling pathways and apoptosis. resulting in immune evasion (29).

The relationship between HPV infection and immune status showed that the weaker the immune status, the higher the chances of HPV acquisition and persistence. Hence, in our study, women with the lowest immunity ($CD4^+$ count of 0-200 cells/µl) have the highest prevalence of HPV infection (31.9%) and those with the most robust immune system ($CD4^+$ count >600 cells/µl) have correspondingly lower HPV infection (25.0%) although the prevalence difference was not statistically significant (p=0.383). This aligns with literature that the immune status determines vulnerability to HPV infection and other infections as well. Our study also agrees with the findings of others that lower immunity is associated with high incidence of HPV infection, that can lead to CIN and invasive cancer as reported in Aminu et al., (30). Women with low $CD4^+$ cell count and high viral load have elevated risk of

HPV acquisition. Low CD4$^+$ count is also associated with decreased HPV clearance, and a compromised immune response is a pre-requisite for disease progression. One unique feature of HPV infection is that it can affect the immune system in such a way that it presents a much more tolerant state, which facilitates persistent HR HPV infection and cervical lesion progression to cervical cancer (31).

The prevalence of abnormal cervical cytology in our study, in accordance with the Bethesda system of classification, was 36.3%. Cervical smear test is routinely done to screen for cervical cancer since the clinical disease is preventable through early detection and appropriate management of the major precursors (CIN and persistent HR HPV infection). There is abundant evidence to suggest that regular screening of sexually active women confers an overall public health benefit in reducing morbidity and mortality from cervical cancer as reported by Yousif et al., (32). The analysis of the relationship between HPV infection and cervical cytology in our study showed strong association between cervical cytological status and HPV infections ($p<0.0001$). This finding is similar to the study of Jaya et al., (33) who reported strong association between HPV infection and cervical cytological changes. Gui et al., (34) also reported that HIV-positive women have higher risk of acquiring HPV infection, pre-cancerous lesions and cervical cancer. They further showed that HIV infection was associated with higher incidence of and reduced clearance of HPV infection. A similar study conducted by Burd (35) showed that HIV-infected women are 5 times more likely than HIV-negative women to have lower genital tract neoplasia, a precursor of cervical cancer, and Yakub et al., (36) in Nigeria showed that HPV infection correlates with cervical changes in HIV-infected women. The similarities in findings between our study and those of many others in Nigeria and other countries proves that indeed HPV infection significantly affects the integrity of the cervix and the ability to clear the infection.

A relatively large proportion of HIV-infected women in our study had low CD4$^+$ cell counts, suggesting poor adherence to ART guidelines. Expectedly, the same sub-population (low CD4$^+$ counts) also have the highest HPV infection. Also, many of the participants used different forms of contraceptives and other practices such as douching and vaginal herbs as preventive measures against unplanned pregnancies and STIs. These may have contributed to the overall risk of cervical cancer. Majority of the women had also initiated sex at early age and had multiple sex partners, and were involved in unprotected sex. Since HPV infection often occur through sexual intercourse and other sexual behaviors, it clearly shows that HPV infection is influenced by the sexual practices of the participants.

Conclusion:

Our study showed high prevalence of HPV infections among HIV-infected patients on ART in Plateau State, north-central Nigeria, with detection of HR HPV types 16 and 18, which are major risk factors for progression of CIN to cervical cancer. We therefore recommend routine cervical cancer screening among HIV-infected women. Furthermore, we suggest better monitoring of ART regimen to improve the immune status of HIV-infected women. Further prospective study to establish the role of ART on HPV infection and cervical cancer progression in HIV-infected women is recommended.

Contributions of authors:

AAY was involved in research conceptualization, design formulation, methodological activities, and writing of the original draft; EEE was involved in research design, supervision and review of the manuscript; OAO was part of the design, supervision, validation of cytological/histological analysis and review of the manuscript; IE was actively involved in the cytological/histological and molecular analysis; AM was involved in the design, supervision, validation of research data and review of the manuscript. All authors read and approved the manuscript for submission.

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Conflict of interest:

Authors declare no conflict of interest.

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