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Cytological Aspects of Cervico-Uterine Smears from HIV/HPV HR Coinfected Women Treated with Antiretrovirals

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ABSTRACT

Introduction: Women living with HIV are at high risk of developing persistent HPV-HR infections. This favors the development of precancerous lesions that may progress to cervical cancer. With a view to improving management and taking preventive action against cervical lesions and cervical cancer in women living with HIV, our study aimed to describe cervical abnormalities and establish a relationship between lesion severity and HPV-HR genotypes in HIV/HPV-HR co-infected women.
Methodology: This was a descriptive cross-sectional study conducted at the CTAB from March 2021-December 2022. Of 122 women living with HIV screened HPV HR positive 90 presented for FCU. Cervico-uterine smears were read after Papanicolaou staining by two cytopathologists at the anatomy-cytology-pathology laboratory of the university hospital center. Results were rendered according to the 2014 Bethesda system classification, and data processing was performed using SPSS 25 software.
Results: 90 patients co-infected with HIV/HPV-HR were included, with a mean age of 44.16±10.08 years, ranging from 20 to 70 years. The women in our study were all on triple therapy, and most had a controlled viral load and CD4 count above 200mm3. Cytological abnormalities accounted for 38.9%, with a predominance of atypical squamous cells of undetermined significance (ASCUS) 51.43%, followed by low-grade squamous intraepithelial lesions (LSIL) 37.14% and 11.43% of atypical squamous cells for which a high-grade lesion could not be excluded (ASC-H). Triple therapy had a protective impact in women against progression to precancerous states, although no statistically significant association was observed between cervical lesions and HR-HPV genotypes (p=0.819).
Conclusion: Our study reveals a high proportion of ASCUS lesions in women living with HIV and co-infected with HPV HR. Triple therapy has a protective effect against progression to severe lesions. To this end, screening for precancerous lesions in this population should include diagnosis of the P16 protein by Histoimmuno chemistry.

Key words: FCU, HR- HPV, Women, HIV, ARV, Congo.

INTRODUCTION

Cervical lesions are abnormal cells in the cervix that may develop into cervical cancer, a significant public health issue that contributes to women mortality worldwide [1].

Cervical cancer is a preventable malignancy of the cervix caused by persistent oncogenic

infection with the human papillomavirus (HPV) [2]. It is the fourth most common cancer in women worldwide and one of the leading causes of cancer death in sub-Saharan Africa[3]. In the Congo, cervical cancer is the second most common cancer in women after breast cancer [4].

Persistent infection with high-risk human papilloma virus (HR HPV) is necessary for the progression from precancerous stages to invasive cancer [5]. In individuals living with HIV, immunosuppression leads to the reactivation of latent HPV infection, resulting in decreased viral clearance and prolonged virus persistence. As a result, immunosuppressed patients are more prone to benign lesions, dysplasia, and HPV-related cancers [6, 7]. Additionally, HIV infection is a contributing factor to carcinogenesis associated with high-risk HPV infections [8]. Studies from the early 90s have shown that precancerous lesions and invasive cervical cancer progress more rapidly and are more prevalent in HIV-infected patients [9].

Prospective studies have reported that the incidence of HPV is higher in HIV-positive women than in HIV-negative women [10,11]. The prevalence of HR HPV infections in HIV-positive women ranges from 30% to 50%, depending on the study, and is correlated with immunosuppression. In HIV-positive women, the prevalence of abnormal cervical smears is 69.3% when the smear shows squamous cell atypia of undetermined significance and a low-grade intraepithelial lesion (ASC-US/LSIL), and 84.1% when it shows a high-grade intraepithelial lesion (HSIL) [12]. A study conducted in France found that HIV-infected women had a much higher frequency of abnormal cervical smears during screening compared to the general population: 42% vs. 5%, $p < 0.001$ [10]. Two American studies have shown that the risk of cervical cancer increases by a factor of 2 to 22 in the presence of HIV infection, and this risk is dependent on CD4 cell count [11, 13]. Additionally, HIV/HPV co-infected women have a higher risk of cancer progression than the general population [12].

In Congo, a biopsy study by Peko et al in 2009 showed that 38.1% of asymptomatic HIV-positive women had precancerous lesions on the cervix [14]. Control strategies for reducing the extent of HPV infection and cervical cancer are based on HPV vaccination and early detection of benign or precancerous lesions [15].

The aim of our study was to describe cervical abnormalities and assess the relationship between lesion severity and HR HPV genotypes in HIV/HPV co-infected women. This is with the objective of improving management and taking preventive action against cervical lesions and cervical cancer in women living with HIV.

PATIENTS AND METHODS

The study was conducted at the Centre de Traitement Ambulatoire (CTA) in Brazzaville, at the Laboratoire National de Santé Publique and at the anatomie cytologie pathologique laboratory of the Centre Hospitalier Universitaire de Brazzaville (CHUB).

The CTA is a health facility specializing in the prevention and management of HIV infection. It is located within the Centre Hospitalier Universitaire de Brazzaville. It offers day hospital services as part of its comprehensive care program.

The Laboratoire National de Santé Publique (LNSP) is the reference laboratory for molecular analyses in Congo, and was used to detect HR-HPV DNA.

The pathological anatomy and cytology laboratory at Brazzaville University Hospital was used to analyze cervico-uterine smears.

This was a descriptive and analytical cross-sectional study conducted over a 20-month period from April 2021 to December 2022.The target population consisted of women living with HIV, cared for at the Brazzaville CTA and on ARVs.

We included women living with HIV women living never vaccinated against HPV infection or tested for HRHPV, treated with ARVs, aged over 18, who came to the CTA

either for the supply of antiretroviral drugs, for a periodic follow-up visit or for a health problem, and who consented to take part in the study after signing the form.

We did not include HIV-positive women on ARV treatment at the Brazzaville CTA with a history of total hysterectomy, pregnant women, menstruating women or women who had not given their consent. Based on these criteria, 276 women aged between 20 and 70 years were recruited for the study.

Blood samples were taken for CD4 count using *BD FACS Presto TM* and HIV-1 plasma viral load using *CEIPHED's GeneXpert* Viral-load HIV-1 system at the CTA laboratory.

Cervico-uterine samples were used for genotyping at the national public health laboratory. Real-time PCR using *Abbot Realtime* System m2000rt technology from the *ABBOT RealTime High Risk HPV kit (Abbott RealTime High Risk HPV Assay, m2000sp, m2000rt, m24sp*) was used for simultaneous detection and genotyping of HPV 16, HPV 18 and pooled detection of 12 otherHR -HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) [16]. HR-HPV infection was defined as the presence of at least one high-risk HPV genotype in the samples tested.

Cervico-uterine smears were taken according to a standardized protocol, then stained and light-microscopically read by two cytopathologists from the pathological anatomy and cytology laboratory of the Centre Hospitalier Universitaire de Brazzaville. Results were written up according to the 2014 Bethesda classification: ASCUS or LSIL (atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesions), atypical squamous cells for which a high-grade lesion cannot be excluded (ASC-H), atypical glandular cells of undetermined significance (AGUS), or HSIL (high-grade squamous intraepithelial lesions).[17]

Study variables were age, aspects of cytological lesions, therapeutic aspects, immunological (CD4) and virological (HIV-1 plasma viral load and HR-HPV genotypes) parameters.

Data analysis

The data collected were entered into a Microsoft Excel 2016© spreadsheet. Data processing and analysis were performed using SPSS version 12.0.

Qualitative variables were expressed as frequencies. Quantitative variables were expressed as mean, standard deviation and median. Comparisons were made using the Fisher test. Odds ratios were used to estimate the strength of association. The threshold of significance was set at a p-value of less than 0.05. Logistic regression was used to estimate associations between antiretroviral treatment and HIV-related factors, CD4, viral load on the one hand, and cytological aspects of uterine cervical smears on the other, and different genotypes and cytological aspects of cervical-uterine smears.

Ethical considerations

The study was approved by the Health Sciences Research Ethics Committee (CERSSA) under number 228/MRSIT/IRSSA/CERSSA on September 20, 2019. All women included in the study provided free, informed, and written consent. The study was conducted in strict compliance with ethical rules for human health research.

RESULTS

Of the 276 women living with HIV never vaccinated against HPV, 122 tested positive for HPV HR. Among them, 90 had returned for a cervical smear. (Figure :1)

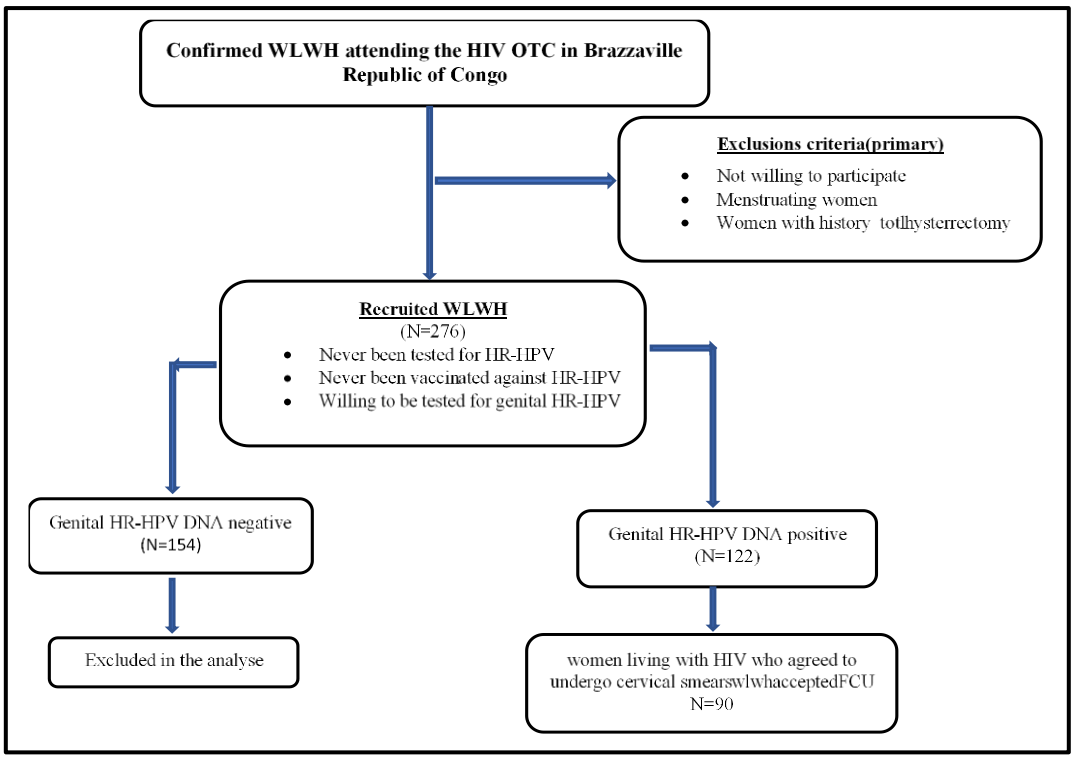

Figure 1: Flow diagram of the study population.

1-Demographic characteristics

The sample size consisted of 90 women living with HIV who tested positive for high-risk HPV. The mean age of women co-infected with HIV and HPV HR was 44.16±10.08 years, with extremes ranging from 20 to 70 years. The median was 45 years, with a first quartile of 36.75 years and a third quartile of 51.00 years. Table I shows the age distribution of patients.

Age range (years)	Number	Percentage
<25	2	2.2
25-35	14	15.6
35-45	28	31.1
45-55	37	41.1
55	9	10
Total	90	100

Table I: Patient distribution by age group

2-Description of cytological lesions

Table II shows the distribution of patients according to cytological features.

Of 90 HPVHR-positive women living with HIV, 38.9% (n=35) had cytological abnormalities, of which atypical squamous cells of undetermined significance (ASCUS) were the most predominant (51.43%), followed by low-grade squamous intraepithelial lesions (37.14%), as shown in Table II

Lesions	Number	Percentage
ASCUS	18	51.43
LSIL	13	37.14
ASC-H	4	11.43
TOTAL	35	100

Table II: Distribution of patients according to cervical lesions.

3-Distribution of cytological aspects according to genotypes HR HPV

The majority of cytological lesions are induced by HR HPV other than aHPV-16 and 18. there is a low proportion of cytological lesions induced by HPV-16 and HPV-18, as shown in the table III.

Type of HR HPV	ABSENCE	ASC-H	ASCUS	LSIL
HPV-16	4(66.7)	0	0	2(33.3)
HPV-16+Other HR HPV	3(42.9)	0	2(28.6)	2(28.6)
HPV-18	5(71.4)	0	2(28.6)	0
HPV-18+OtherHR HPV	2(50)	1(25)	0	1(25)
Other-HPV	41(62.1)	3(4.5	14(21.2)	8(12.1)

Table III: Distribution of cytological aspects according to genotypes HR HPV.

4-Immunovirological and clinical characteristics.

All patients were receiving antiretroviral therapy (ARVs). The average duration of treatment was 10.96 ± 4.71 years, ranging from 3 to 21 years. The median duration was 11 years, with the first quartile at 7 years and the third quartile at 15 years. The mean duration of HIV-1 infection was 11.72 ± 4.88 years, ranging from 3 to 22 years; The median duration was 11 years, with the first quartile at 7.75 years and the third quartile at 16 years. The viral load rate had a mean of 587.36 ± 296.17 copies/mL, ranging from 11 to 1,586. The median was 580.50, with the first quartile at 403.25 and the third quartile at 767.75. The CD4 mean was 587 (11-1586), with a median of 580.50 (403.25-767.75). Table IV displays the distribution of patients based on their immunological, virological, and therapeutic parameters.

VARIABLES	Number	PERCENTAGE
Duration of traitement(years)		
<11	41	45.6
>11	49	54.4
Line of treatment		
L1	68	75.6
L2	23	24.4
CD4		
<200	21	23.3
>200	69	76.7
Viral load		
detectable	21	23.3
Undetectable	69	76.7
Type of HRHPV		
HPV-16	6	6.7
HPV-16+other HR HPR	7	7.8
HPV-18	7	7.8
HPV-18 +Other HR HPV	4	4.4
Other HR HPV	66	73.3
Duration of HIV(years)		
<11	33	36.7
>11	57	63.3

Table IV: Distribution of patients according to biological and clinical parameters.

5-Relationship between HR HPV genotypes and severity of cytological lesions in women living with HIV.

The relationship between HR HPV genotypes and the severity of cervico-uterine cytological lesions is shown in table The relationship between HR HPV genotypes and the severity of cervico-uterine cytological lesions is shown in table V.

Table V: Relationship between HR HPV genotypes and the severity of cytological lesions

Variables	Anomalies		OR[IC95%]	p value
	Yes	No		
TYPE HPV-HR				0,819
HPV-16	2(33.3)	4(66.7)	1	
HPV-16+Other HPV-HR	4(57.1)	3(42.9)	2.67[0.28-25.64]	0.592
HPV-18	2(28.6)	5(71.4)	0.80[0.08-8.47]	1.000
HPV-18+Other HPV-HR	2(50)	2(50)	2,00[0,15-26,74]	1.000
Other HPV-HR	25(37.9)	41(62.1)	1.22[0.21-7.15]	1.000

No statistically significant association was found between HPV-HR genotypes and lesion severity.

DISCUSSION

This cross-sectional study involved 90 women living with HIV-1 who consented to participate and were followed at the Brazzaville Outpatient Treatment Centre (ACT). All participants tested positive for the high-risk papillomavirus.

The preferred method for diagnosing precancerous and cancerous lesions is through biopsy. However, to avoid reducing the sample size due to the financial cost and mental distress that biopsies could inflict on these vulnerable women, who are already carrying the heavy burden of HIV, cervical smears were performed instead.

The aim of this study was to identify cytological lesions in women living with HIV, in order to provide early treatment, to establish a correlation between the various

HR HPV genotypes and the severity of the detected lesions.

The mean age of the women in our study was 44.16 years ranging from 20 to 71 years. The presence of 20-year-old women in the study highlights the need for an early detection program. The most common age group was between 45 and 55 years, accounting for 41.1% of the participants. All women in the study were on triple therapy, with most having a controlled viral load and a CD4 count greater than 200mm3. In this study, cytological abnormalities accounted for 38.9%. ASCUS was the most common abnormality, accounting for 51.43%, followed by LSIL at 37.14% and ASC-H at 11.43%.

In Congo, a previous study of biopsies taken from women living with HIV who had not yet received triple therapy observed a 38.1% proportion of precancerous lesions [10].

Previous hospital studies in the general population have reported low rates of cytology abnormalities [18, 19, 20].

The proportion of cytology abnormalities found in our study was 38.9%, which is higher than the rates reported in several previous studies (19%, 21%, and 22.4% respectively) [21, 22, 23].

In contrast, a study conducted in Denmark reported a lower rate of 10.4% [24].

The cytological abnormality profiles observed in our study consisted of 51.43% ASCUS, 37.14% low-grade intraepithelial lesions, and 11.43% ASC-H lesions. A study conducted in Cameroon on untreated women living with HIV reported a predominance of high-grade intraepithelial lesions. Conversely, Rodallec et al. (2018) identified cervical cytology lesions with a predominance of AUC-H at a rate of 13%, which is similar to our findings. Our study found a high rate of ASCUS (51.43%) in women infected with HR-HPV other than HPV-16 and HPV-18. Therefore, we suggest searching for the P16 protein in immunohistochemistry.

Previous studies have reported higher rates of abnormal cytology lesions in HIV-positive women compared to the general population. The methodology and duration of treatment varied among these studies [25]. Several factors can influence the variability of HR-induced cytology abnormalities in women living with HIV on antiretroviral therapy, including the duration of infection, viral load, types of HR-HPV, lifestyle, and immune status. CD4 count is a major determinant in the management of immunosuppression. According to Bammo et al. (2015) and Kabeyene O C et al. (2015), CD4 count of less than 200 cells/mm3 was found to be more predictive of high-grade cytology lesions in women living with HIV. Our series identified two CD4 groups: 4 cases (11.42%) with CD4 count of less than 200 cells/mm3 and 31 cases (88.57%) with CD4 count of 200 cells/mm3 or more. Furthermore, immunosuppression is implicated in increased HPV replication and persistence [22].

Among women with abnormal Pap smears, the most common high-risk HPV genotypes were other than types 16 and 18. The distribution of high-risk HPV genotypes by cytological status was as follows: two patients with atypical squamous cells of undetermined significance (ASCUS) had a coinfection of HPV-16 and another high-risk HPV genotype.

Two with ASCUS lesions had HPV-18 genotype, and 14 with ASCUS lesions had high-risk HPV genotypes other than HPV-16 and HPV-18.

The women with low-grade lesions were carriers of HPV-16.

Two others had HPV16 and HPVHR genotypes that were not HPV-16 and HPV-18. Only one had HPV-18 and HRHPV co-infection, while eight carriers had HPV genotypes other than HPV-16 and HPV-18.

Three women with ASCH lesions had HRHPV genotypes other than 16 and 18, and only one had HPV-18 and HRHPV co-infection other than HPV-16 and HPV-18.

Our study found no association between HR-HPV genotypes and cytological abnormalities, suggesting that antiretroviral therapy protects women against the severity of lesions by recovering immunity, which

weakens the action of HR HPV. However, previous work in Ghana has shown a strong association between HPV-35 genotypes and low-grade LSIL lesions [26]. While the causal link between cytological lesions and high-risk papillomavirus genotypes is well established, our study found that treatment had a protective effect and limited progression to severe lesions. In fact, antiretroviral treatment has a doubly positive effect on women living with HIV. In our study, it was found that the intervention improved immunity and prevented the onset of severe precancerous lesions in women living with HIV. The literature has also shown that women living with HIV are at a greater risk of both low- and high-grade precancerous lesions (Kabeyene et al., 2015). Due to HIV-induced immunosuppression, even mildly aggressive serotypes can be highly virulent [22]. In this study, all women were receiving antiretroviral (ARV) therapy, with the majority having restored immunity and a suppressed viral load. This is encouraging because, according to Robinson, ARV treatment may have a positive impact on the progression of cervical lesions in women living with HIV [27].

Furthermore, research has demonstrated that individuals who are HIV+ have an increased risk of abnormal cervical cytology. Additionally, a low CD4 count can increase the risk of cytology lesions. However, regular follow-up, maintaining a controlled viral load, and receiving appropriate antiretroviral therapy appear to limit the risk of cervical lesions and promote their regression [12; 28].

The reduction in the persistence of HPV infections, which is essential for carcinogenesis, may be due to the restoration of immune function through triple therapy. This allows the body to better fight infections and reduce the risk of developing precancerous lesions. Many authors have demonstrated that viral persistence decreases if the plasma HIV-1 viral load is controlled and if the CD4 lymphocyte count is greater than 200/µL [12]. Our study confirms that ART increases the life expectancy of women living with HIV. The mean duration of treatment in our study was 10.96 ± 4.71 years (range: 3-21 years) and the age of the women ranged from 20 to 71 years. No serious lesions were observed.

Cervical cancer can be prevented and cured if diagnosed early. Therefore, it is important to closely monitor HPV-related illnesses in women living with HIV who are on antiretroviral therapy.

One potential limitation of this study is the methodology, as the diagnosis was not confirmed by biopsy and cytological abnormalities were not histologically confirmed. Additionally, women diagnosed with ASCUS-positive were not tested for the p16 protein by immunohistochemistry for differential diagnosis.

CONCLUSION

The study found that 38.9% of women living with HIV who tested positive for high-risk human papillomavirus (HPV) had cytological lesions. These lesions were mainly atypical squamous cells of undetermined significance (ASC-US), followed by low-grade squamous intraepithelial lesions (LSIL) and squamous cell atypia, which did not exclude a high-grade squamous intraepithelial lesion (ASC-H).This study is the first to be conducted among women living with HIV on triple therapy in Congo. It could serve as a basis for large-scale studies.Cervical lesions are mainly induced by high-risk HPV genotypes other than HPV-16 and HPV-18. The study indicates that regular organized screening, based on high-risk human papillomavirus (HPV) tests, colposcopy cytology, histology, and immunohistochemistry testing for the P16 protein for ASCUS, as well as HPV vaccination, is important to reduce the risk of cytology lesions in women living with HIV. This screening programme should be carried out regularly and in conjunction with psychological care.

Declaration by Authors

Ethical Approval: Approved

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