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Co-Infection with Human Immunodeficiency Virus and High-Risk Human Papillomavirus in Women in Mbujimayi, DRC

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Abstract

Introduction: Persistent infection with high-risk human papillomavirus is the main cause of cervical cancer. Women living with human immunodeficiency virus (HIV) are at a higher risk of HPV infection and HPV-related diseases. In the Democratic Republic of the Congo (DRC), the national HIV control program focuses exclusively on the management of tuberculosis-HIV co-infection. This study aims to estimate the prevalence, genotype distribution, and associated risk factors of HIV/high-risk HPV co-infection among women in Mbujimayi, Democratic Republic of the Congo. **Methods:** A cross-sectional analytical study was conducted from January to May 2025 in Mbujimayi, involving 86 women aged 30 to 65 years who tested positive for high-risk HPV (Hr-HPV) by polymerase chain reaction (PCR) on vaginal swabs. HIV infection was investigated using the Determine® test on blood serum, followed by confirmation with the Uni-Gold® HIV test. **Results:** The prevalence of HIV/Hr-HPV co-infection was 13.9%. The age group 40 - 49 was the most affected, comprising the majority (58.3%) of this co-infection. HIV/Hr-HPV co-infection was significantly associated with a history of sexually transmitted infections, increasing the risk sixfold (OR = 6.00, 95% CI [1.21 - 37.4], p = 0.035). **Conclusion:** This study shows a high prevalence of HIV/Hr-HPV co-infection, with a predominance in the 40 - 49 age group. Furthermore, a history of sexually transmitted infections is a factor associated with the risk of

this co-infection. Consequently, organizing effective screening in healthcare facilities, as well as vaccination against HPV in this group, is crucial and justified.

Keywords

HPV, HIV, Associated Factors, Women

1. Introduction

Cervical cancer, the fourth leading cause of cancer death among women worldwide, is caused in more than 90% of cases by high-risk human papillomavirus (Hr-HPV) [1]. Due to its infectious trigger, cervical cancer is largely preventable through primary prevention measures, such as vaccination, and secondary prevention methods, such as regular screenings [2]. Besides Hr-HPV, several other factors could increase the risk of developing cervical cancer, including age, parity, smoking, the use of hormonal contraceptives, and co-infection with the human immunodeficiency virus (HIV). Indeed, immunodeficiency increases the risk of cancers, whether related to acquired immunodeficiency syndrome (AIDS) or not [3]. Consequently, cervical cancer is classified as an AIDS-defining condition and is the most frequently diagnosed cancer in women living with HIV (WLWH) [4]. Over the past four decades, the HIV and AIDS pandemic has emerged and persists as one of the most serious challenges in the world in terms of public health, development, and economics. With approximately 37.7 million (uncertainty range, 30.2 to 45.1 million) people living with HIV (PLHIV) worldwide in 2020, 53% of all people living with HIV were women and girls. HIV/AIDS disproportionately affects people in low- and middle-income countries, where cervical cancer is also extremely common. More than two-thirds of PLHIV live in sub-Saharan Africa. Untreated HIV causes severe impairment of the immune system, increasing the risk of developing opportunistic infections as well as infection-related cancers and other chronic comorbidities that are otherwise rare in people with normal immune function [5]. In the West African region, for example, up to 18% of women with cervical cancer are infected with HIV, with approximately 30,000 cases of cervical cancer recorded each year, resulting in 16,000 deaths. Compared to other regions of the continent, Southern Africa is the region most affected by HPV infection due to the high prevalence of HIV/AIDS infection. Thus, in South Africa, this prevalence has been estimated at 76.3% among Black women in KwaZulu-Natal [6]-[8]. In the Democratic Republic of the Congo (DRC), approximately 214 deaths from cervical cancer occur and are diagnosed each year (estimates for 2020) [9]. Indeed, cervical cancer ranks as the leading cause of cancer death among women who die from cancer in Congo and is the third leading cause of cancer death among women aged 15 to 44, with age-adjusted incidence and mortality rates estimated at 25.2 and 13.0 per 100,000 women, respectively [10]. Furthermore, in the DRC, it is noted that the program to fight cervical cancer exists but is not very operational. Information on cervical cancer and HPV-HIV co-

infection is fragmented and poorly documented. A study conducted in 2015 in Kinshasa revealed that the prevalence of HPV infection was higher among HIV-positive women compared to HIV-negative women, at 31.3% and 3.9%, respectively [11]. In 2021, Munkana *et al*. in Lubumbashi reported a significantly higher frequency of HPV infection among women infected with HIV compared to women not infected with HIV (81.08% versus 52.63%). Although vaccination has contributed to a global decrease in HPV infections, the circulating genotypes vary from one population or sub-population to another due to several factors such as screening and vaccination rates, immune status, behavioral habits, etc. This underscores the need for continuous surveillance in order to determine the most effective vaccination and screening strategy for each population [12] [13].

Understanding the burden of HPV among people living with HIV is crucial for improving the screening and treatment of precancerous lesions in this group and ultimately reducing cervical cancer-related morbidity and mortality.

The aim of this study is to estimate the prevalence, genotypic distribution, and factors associated with HIV/Hr-HPV co-infection among women aged 30 - 65 in Mbujimayi, Democratic Republic of the Congo. The results of this study will provide essential epidemiological data for the prevention and control of HIV/Hr-HPV co-infection in the DRC.

2. Methods

2.1. Framework, Type, and Study Period

The study was conducted in Mbujimayi, the capital of the Kasaï-Oriental province in central DRC, at the Mbujimayi Pediatric and Maternity Clinic. The study was cross-sectional and analytical in nature and took place over a period of 5 months, from January 10, 2025, to May 14, 2025. This period included data collection and the analysis of biological samples using PCR.

2.2. Study Population

This study included non-pregnant women aged 30 to 65 years without a history of invasive cervical cancer or hysterectomy.

2.3. Data Collection

The data collection was carried out in the context of the project for screening precancerous cervical lesions in the city of Mbuji-Mayi. HPV testing was organized in three health facilities (Valentin Disashi Hospital, Megumi Hospital Center, and the Pediatric and Maternity Clinic of Mbujimayi) from January to May 2025. The data were collected through interviews using a semi-open questionnaire combining several methods: interview, gynecological examination, and biological and molecular analysis. At the same time, all data were entered daily using the KoboCollect application installed on the investigators' phones and compiled in real time on the KoboToolbox platform (https://kf.kobotoolbox.org/). Continuous monitoring was established to detect and correct data entry errors or inconsistencies. Finally, at the

end of the survey, the database was exported to Excel format (Microsoft Office 2010, USA), checked, corrected, and consolidated into a single final database.

2.4. Cervical Sample Collection

After providing their informed consent to participate, all eligible women underwent a gynecological examination. While the woman was lying in the lithotomy position on the examination table, a trained healthcare provider inserted a Collin speculum into the vagina to visualize the cervix. The cervical sample was collected using the Cervex-Brush® (Rovers Medical Devices) with gentle rotational movements clockwise and then counterclockwise. Then the head of the Cervex-Brush® was transferred into a vial with 20 ml of PreservCyt® (Hologic, Marlborough, MA) and stored for 24 hours at room temperature between 15˚C and 30˚C, according to the manufacturer's protocol. All collected samples were sent within 24 hours to the provincial public health laboratory for HPV testing.

2.5. Hr-HPV Screening Test

The HPV screening test was carried out using the Xpert HPV IV Cepheid® assay. The Xpert HPV Cepheid® test is a qualitative in vitro test designed to detect the E6/E7 region of the viral DNA genome of high-risk human papillomavirus (HPV) in patient samples. The test performs multiplex amplification of the target DNA through a real-time polymerase chain reaction (PCR) for 14 types of HPV in a single analysis. The Xpert HPV test specifically identifies HPV types 16 and 18/45 in two separate detection channels and reports 11 other high-risk types (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68) in a pooled result.

For the analysis of the samples, upon arrival at the laboratory, cervical samples in PreservCyt® solution were recorded; the processing of the samples was carried out in batches of 4 samples following these steps: first, examine the test cartridge to ensure it is not damaged, then open its lid; vortex the samples for 5 seconds at medium speed continuously. Next, transfer a 1 ml aliquot of the cervical sample directly into the Xpert cartridge while ensuring there are no air bubbles in the pipette. The Xpert Dx gene was used according to the manufacturer's recommendations. The final categorical results were recorded as follows: "CAE; Primary" for the sample adequacy control, "HPV 16; Primary" for HPV 16, "HPV 18 - 45; Primary" for the grouped result of HPV types 18 or 45, "P3; Primary" for the grouped result of HPV types 31, 33, 35, 52, or 58, "P4; Primary" for the grouped result of HPV types 51 or 59, and "P5; Primary" for the grouped result of HPV types 39, 56, 66, or 68. The presence of a single-copy human gene and an adequate number of human cells were detected to perform a qualitative assessment of HPV status.

2.6. HIV Infection Screening

HIV serology using the Determine® test was performed on blood plasma according to the manufacturer's instructions and was considered positive if both a test line (T) and a control line (C) appeared on the test cassette. To confirm a positive HIV

infection diagnosis, a second confirmatory test, Uni-Gold HIV, was performed on the same blood plasma.

2.7. Statistical Data Analysis

Statistical analyses were conducted using R software version 4.5.1 (https://cran.r-project.org/bin/windows/base/). Categorical variables were summarized as frequencies and percentages, and numerical variables as means with standard deviations. The normality of numerical variables was assessed using the Shapiro-Wilk test. To identify factors associated with the risk of HPV-HR infection, a multivariate logistic regression model was performed. For the interpretation of statistical tests, a p-value ≤ 0.05 was considered statistically significant.

2.8. Ethical Considerations

This study was approved by the Ethics Committee of the University of Mbujimayi under number 001/CEI/UM/2025. Free and informed consent was obtained, and an identifier was assigned to all participants. The study was conducted in accordance with the requirements of good clinical practice and the principles of the Declaration of Helsinki (2013) of the World Medical Association and relevant subsequent amendments.

2.9. Role of the Funding Source

The execution of this project was made possible thanks to the funding from the Royal College of French-speaking Belgian Gynecologists and Obstetricians (CRGOLFB) and the Gynecology Department of the Saint Luc University Clinics.

3. Results

3.1. Prevalence of HIV-HPV Co-Infection among HR-HPV Positive Participants and Socio-Demographic Characteristics

Among the 86 Hr-HPV positive participants, 12 (13.9%) had a positive HIV serology and 74 (86.1%) had a negative HIV serology. The average age was 46.8 ± 8.7 years, and the most represented age group was 40 - 49 years (41.9%) (see **Table 1**).

3.2. Age and Risk of HIV/HPV Co-Infection

The age group of 40-49 years was the most affected by HIV/Hr-HPV co-infection (58.3%); however, the proportion of co-infection did not vary significantly with age (p = 0.446) (**Figure 1(A)**). The analysis of the trend curve of the probability of co-infection shows a slight decrease in risk with age (**Figure 1(B)**).

3.3. The Prevalence of Co-Infection According to Viral Types

Viruses of types 31, 33, 35, and 52, grouped under P3, were more frequent in both groups (HIV+/HPV+ vs HIV−/HPV+) (66.7% vs 46%). Similarly, a higher frequency of different viral types was observed in the co-infection group than in the group infected with HPV alone (**Figure 2**).

3.4. Factors Associated with HIV-Hr HPV Co-Infection

Only a history of sexually transmitted infections was found as a factor associated with HIV+/Hr-HPV+ co-infection, increasing the risk sixfold (OR = 6.00, 95% CI [1.21 - 37.4], p = 0.035) (Figure 3).

Table 1. Socio-demographic characteristics of participants positive for Hr-HPV.

Characteristics	N	%
Marital Status		
Single	0	0
Common-law union	6	7
Divorced	12	14
Widow	15	17.4
Married	53	61.6
Level of education		
None	0	0
Primary	30	34.9
Secondary	52	60.5
University	4	4.6
Profession		
Housewife	23	26.7
Non-liberal	4	4.7
Liberal	59	68.6
Age (year)		
30 - 39	18	20.9
40 - 49	36	41.9
50 - 59	24	27.9
≥ 60	8	9.3
Mean* ± *Standard deviation **46.8 ± 8.7**		
Parity		
Nulliparous	**2**	2.3
Primiparous	3	3.5
Paucipare	10	11.6
Multiparous	23	26.7
Grand multiparous	48	55.8

Continued

Age at first birth (in years)		
Before 18 years	28	33.3
From 18 years old	56	66.7
Number of partners in a lifetime		
1 partner	39	45.3
More than one partner	47	54.7

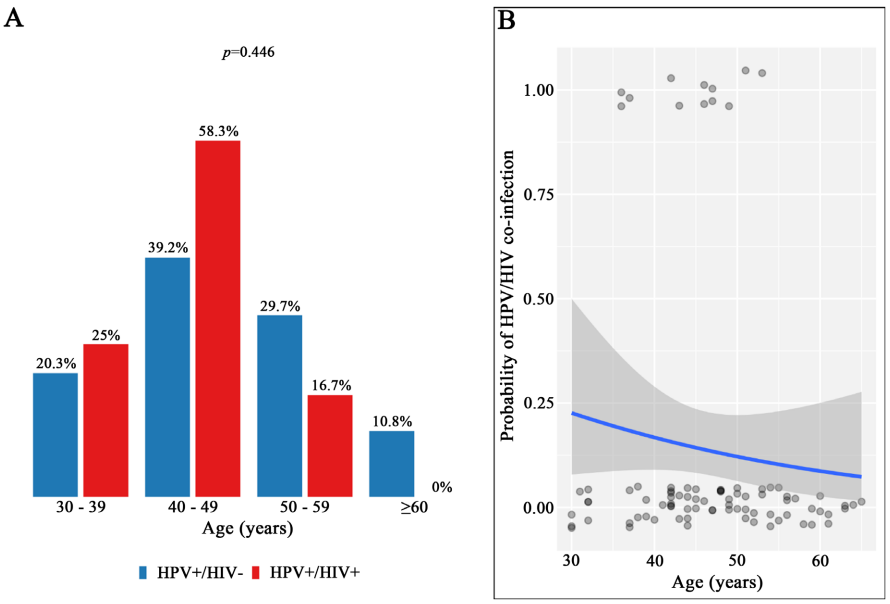
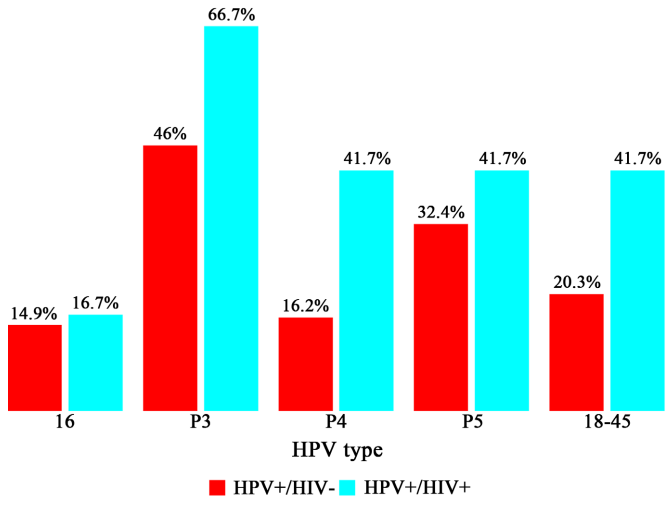


Figure 1. Age and risk of HPV/HIV co-infection. (A) Prevalence of co-infection according to age groups; (B) Regression curve of Hr-HPV infection according to age.



P3, pooled result of HPV types 31, 33, 35, 52, or 58; P4, pooled result of HPV types 51 or 59; and P5, pooled result of HPV types 39, 56, 66, or 68.

Figure 2. The prevalence of co-infection according to viral types.

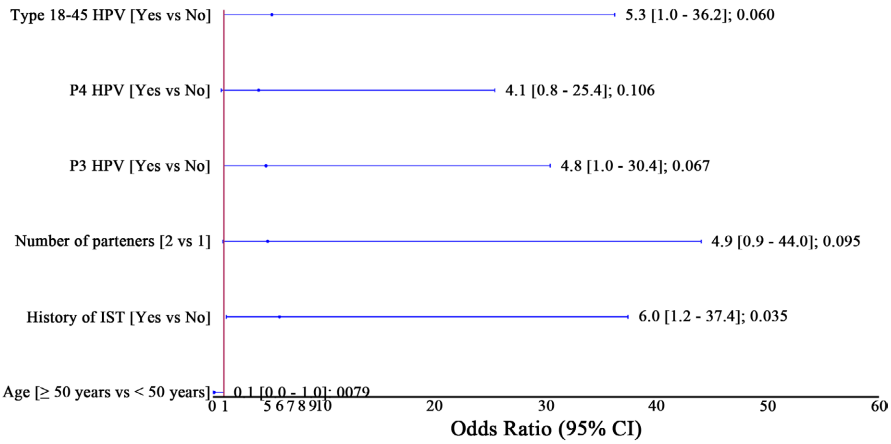


Figure 3. Factors associated with HIV-Hr HPV co-infection.

4. Discussion

The objective of this study was to estimate the prevalence, genotypic distribution, and factors associated with the risk of HIV/Hr-HPV co-infection among women aged 30 - 65 in Mbujimayi, Democratic Republic of the Congo. This is the first study in Mbujimayi focusing on HIV co-infection in women who tested positive for Hr-HPV.

4.1. Prevalence of HIV-HPV Co-Infection among HR-HPV Positive Participants and Socio-Demographic Characteristics

Although the burden of disease associated with HIV and HPV infection is significant, especially in sub-Saharan Africa, there is little research on the link between HIV-HPV co-infection. In the present study, among the 86 participants who tested positive for Hr-HPV, 12 (13.9%) had positive HIV serology, and the mean age of the participants was 46.8 ± 8.7 years, with the most represented age group being 40 - 49 years (41.9%). This prevalence is close to that found by Smith-McCune of 18.1% (88/487) [13]. However, Bertran Auvert in South Africa found 40% (25/62), and Sarah H. Averbach in Zimbabwe 33.2% (148/446) [14] [15]. This difference in prevalence could be explained by the choice of the target population of the study. Bertran Auvert's study focused on female sex workers, while in Sarah H. Averbach's study, women using contraception were the majority. Moreover, in these two previously cited studies, the average age of participants was 24 and 25 years, respectively. According to several literature reviews, having multiple sexual partners, contraception use, and young age are factors associated with the risk of HPV and HIV infection and may explain these high prevalence rates. Indeed, it is now well recognized that high vaginal inflammation, defined by elevated concentrations of cervicovaginal cytokines, is strongly associated with a high risk of contracting HIV. The underlying causes, ranging from bacterial and viral sexually transmitted infections, lead to vaginosis [16]-[18]. Underlying genital infections can independently increase the risk of acquiring HIV by disrupting the integrity of the mucosal barrier, increasing the expression of neutrophil proteases,

and inducing an influx of CD4+ T lymphocyte populations into the genital epithelium and submucosa, the main targets of HIV upon mucosal exposure [19]. This may explain the increasingly rising prevalence of HIV-HPV co-infection.

The age group of 40 - 49 years was the most affected by HIV/Hr-HPV co-infection (58.3%); however, the proportion of co-infection did not vary significantly with age (p = 0.446). Analysis of the trend curve for the probability of co-infection shows a slight decrease in risk with age. This pattern is similar to that observed for cervical HPV infection in immunocompetent women, which is characterized by a peak in young women (*i.e.*, those around 25 years old) followed by a subsequent decline with age (after 45 years) [20] [21]. This similarity could reflect the existence of biological factors (e.g., HPV clearance with age) or behavioral factors (e.g., more responsible sexual behavior with age) on one hand [22], and on the other hand, the vulnerability of the immune system with age could explain poorer HPV clearance, maintaining vaginal inflammation, which could explain a high frequency of HPV in the 40 - 49 age group along with the slight decrease in the co-infection curve [23] [24].

4.2. Prevalence of Co-Infection According to Viral Types

Considering HIV status, HPV genotypes 31, 33, 35, and 52 grouped in P3 were more frequent in both groups (HIV+/HIV−) (66.7% vs. 46%). Similarly, a higher frequency of different viral types was observed in the co-infection group than in the group infected with HPV alone. Munkana, in his study, found a significantly higher prevalence of HPV in women infected with HIV than in women not infected with HIV (81.08% vs. 52.63%) [9]. The high prevalence of HPV among people infected with HIV could be explained by the impairment of immune defenses associated with HIV infection, as latent HPV infections can be reactivated [9] [25]. Moreover, HPV infection and its persistence are more common in people living with HIV due to the shared risk of acquisition between HPV and HIV [9]. In the Bertran Auvert study, the most frequent high-risk viral types in cases of co-infection were types 18, 68, 51, and 39 [14]. This difference in viral types could be explained by the geographic distribution of HPV, which has been known for several years. The high prevalence of HPV genotypes 31, 33, 35, and 52 grouped in P3 in this category would be due to the high prevalence of these viral types in our community.

4.3. Factors Associated with HIV-Hr HPV Co-Infection

Only a history of sexually transmitted infections was found to be associated with HIV+/Hr-HPV+ co-infection, multiplying the risk by six (OR = 6.00, 95% CI [1.21 - 37.4], p = 0.035). This association could be explained by the fact that HIV and HPV are sexually transmitted viruses that share the same transmission route and behavioral factors with other STIs according to the literature review. In the study by Bertran Auvert, HIV acquisition in the presence of HPV infection increased significantly with the number of HR-HPV genotypes and the level of

education, and decreased significantly with age [14]. Higher education level and advanced age may underlie responsible sexual behavior, and thus have a protective effect against HIV+/HPV+ co-infection. However, due to the reactivation of latent HPV infection in the case of HIV, it is possible that the association between the number of Hr-HPV genotypes and HIV is due to HPV reactivation. Although some meta-analyses do not show a difference in the proportion of HIV infection attributable to HPV infection regardless of the associated HPV genotype [26], the meta-analysis by Pascale Lissouba shows that the HPV genotypes associated with HIV acquisition included HPV 52, 31, 58, 70, 16, and 18. No relationship was found for HPV 6 and 11 [27]. In our study, types 18 - 45 and types 31, 33, 35, 52, 58 grouped in P3 were found to be associated factors, although the result was not significant ($p = 0.006$ and 0.067).

5. Conclusion

This study reports a high prevalence of HIV/HPV co-infection, as well as high-risk HPV viral types in cases of co-infection, indicating a high risk of cervical cancer in this category. An appropriate vaccination program, along with regular screening using an effective test, is necessary in cases of HIV/HPV co-infection. We also recommend the integration of systematic HIV screening into cervical cancer screening programs in the DRC.

Disclaimer

The content is the sole responsibility of the authors.

Study Limitations

The small sample size of this study reduces statistical power, which limits the ability to detect significant differences. Consequently, this may also lead to wide confidence intervals for the odds ratios, reducing the precision of the results.

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Conflicts of Interest

The authors: No conflicts of interest have been reported.

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