UNIVERSITY OF RWANDA

PREVALENCE OF HIGH RISK ANAL HPV AMONG RWANDAN WOMEN LIVING WITH HIV: A CROSS-SECTIONAL STUDY DESIGN AT RWANDA MILITARY HOSPITAL

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PREVALENCE OF HIGH RISK ANAL HPV AMONG RWANDAN WOMEN LIVING WITH HIV: A CROSS-SECTIONAL STUDY DESIGN

AT RWANDA MILITARY HOSPITAL

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Kigali-Rwanda, 2025.

DECLARATION

I, KABILA Elyse Sandra, hereby declare that this research project submitted to the University of Rwanda, Rwanda for the degree Master of Science in Biotechnology is my own original work and has not been submitted before to any Institution by myself or any other person in fulfilment of the requirements to the award of any degree or any other qualification.

KABILA Elyse Sandra

Signature: 

Date: 01th August 2025

DEDICATION

With gratitude I dedicate this research project to:

God Almighty for His abundant grace;

To my supervisor, whose insights guided this work.

To my family, for unwavering encouragement

And to my friends, for their constant belief in me

ACKNOWLEDGMENT

I extend my deepest gratitude to the Almighty God for His grace and guidance throughout the course of this academic journey. I am profoundly thankful to my supervisors, Assoc. Prof.MUTANGANADieudonné, Prof.LeonMUTESA, and Mr. Faustin KANYAMIBWISHA, PhD(c), for their steadfast mentorship, insightful feedback, and continuous support during the development of this research.

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LIST OF ABBREVIATIONS AND ACRONYMS

- **AIDS :**Acquired Immune Deficiency Syndrome
- **AIN :**Anal Intraepithelial Neoplasia
- **ART :** Antiretroviral Therapy
- **CD4 :**Cluster of Differentiation 4 (a glycoprotein on the surface of immune cells)
- hr-HPV :High-Risk Human Papillomavirus
- **HIV :**Human Immunodeficiency Virus
- HPV :Human Papillomavirus
- **RMRTH :** Rwanda Military Referral and Teaching Hospital
- **SPSS :**Statistical Package for the Social Sciences
- **WHO :** World Health Organization
- **WLWHIV :** Women Living with HIV

ABSTRACT

Background: Persistent high-risk HPV infection is a leading cause of anal cancer, with women living with HIV at higher risk due to impaired viral clearance. Despite Rwanda's HPV vaccination program, the epidemiology of anal hr-HPV in WLWHIV remains poorly studied. This study aimed to determine the prevalence, genotype distribution associated with anal high-risk HPV infections in WLWHIV in Rwanda.

Methods: Archived anal swab samples from 300 WLWHIV, aged 22 to 33, and were analyzed at Rwanda Military Referral and Teaching Hospital Research Laboratory. Samples were processed using the Atila AmpFire HPV High-Risk Genotyping Kit, employing real-time isothermal amplification to detect hr-HPV genotypes. Statistical analysis using SPSS assessed hr-HPV prevalence, genotype distribution, and associations with demographic factors like age and vaccination status.

Results: The prevalence of high-risk anal HPV was 39.5%, with HPV56 being the most prevalent genotype, followed by HPV35, HPV18, and HPV53. Co-infections with multiple high-risk HPV types were common, particularly HPV39 with HPV51 and HPV39 with HPV66 as the most frequent combinations. No significant relationship was observed between hr-HPV positivity and age, vaccination status, suggesting that immunosuppression may have a more prominent role in the persistence of infection.

Conclusion: This study underscores the significant burden of high-risk anal HPV infections among women living with HIV in Rwanda, with a unique genotype distribution, including a high prevalence of non 16 and18 types. It highlights the need for targeted public health interventions, such as regular anal HPV screening and broader spectrum HPV vaccines, to ensure long-term prevention.

Keywords: *High-risk HPV, HIV, anal cancer, genotype distribution, co-infections, immunosuppression, Rwanda, women living with HIV, vaccination, screening*

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Human papillomavirus (HPV) is the most prevalent sexually transmitted infection globally. Among its diverse strains, high-risk oncogenic types, particularly HPV 16 and 18, are the primary causes of more than 90% of anal and cervical cancers (Dahlstrom *et al*., 2023). According to the World Health Organization (WHO), HPV infection is widespread, with high-risk types playing a critical role in the development of anogenital and oropharyngeal cancers worldwide. The impact of these infections is significantly amplified in individuals living with HIV (PLHIV), as their weakened immune systems hinder the clearance of the virus. This results in an increased likelihood of persistent infections, which can lead to the progression of precancerous lesions and ultimately cancer (Binagwaho et al., 2012).

The prevalence of high-risk anal HPV infections among women living with HIV is notably high worldwide. A systematic review and meta-analysis found a pooled prevalence of 46.1% for any anal HPV and 34.2% for high-risk anal HPV in HIVpositive women (Murenzi et al., 2021)(. As a result, the incidence of anal cancer is rising, particularly among PLHIV. Women living with HIV face an approximately 20 to 30 times higher risk of developing anal cancer compared to the general population(Wolf, 2023) . Despite these concerning figures, anal HPV infection and its potential for malignancy are often under recognized and inadequately screened for, particularly in low and middle-income countries (LMICs)(Dahlstrom *et al.*, 2023).

Sub-Saharan Africa bears a disproportionate burden of both HIV and HPV infections. The region accounts for nearly 70% of the global population of people living with HIV. Studies across the continent have reported high-risk anal HPV prevalence rates in HIV-positive women ranging from 45% to over 70%, highlighting the urgency of addressing the issue(Brotons *et al*., 2018) . The significant immunosuppression seen in HIV infection further amplifies the risk of persistent high-risk HPV and the development of anal intraepithelial neoplasia (AIN) and invasive cancer. Unfortunately, routine screening for anal HPV and its precursors is virtually nonexistent in most African public healthcare systems, contributing to late-stage diagnoses and reduced treatability (Xiao *et al* . , 2025).

In Rwanda, the adult HIV prevalence is 2.6%, with women disproportionately affected, representing 3.3% of the female adult population .The country has made significant strides

in public health, particularly through its national HPV vaccination program for adolescent girls, which has greatly reduced the future burden of cervical cancer . Although significant research has been conducted on HPV, it has predominantly focused on the cervical form at the expense of the anal form. Consequently, a critical knowledge gap exists regarding the prevalence of high-risk anal HPV in a particularly vulnerable population adult, HIV positive women who were ineligible for vaccination and thus remain at high risk.

Rwanda Military Referral and Teaching Hospital is a major referral center providing comprehensive HIV care to a large cohort of women living with HIV. The absence of local epidemiological data on high-risk anal HPV infection in this specific population prevents the design and implementation of evidence-based screening, prevention, and management strategies tailored to their needs.

1.2 PROBLEM STATEMENT

Rwanda's successful implementation of a national HPV vaccination program has significantly contributed to the prevention of cervical cancer. However, a critical gap remains in addressing anal cancer risk, particularly among women living with HIV (WLWHIV). HIV induced immunosuppression heightens susceptibility to persistent high-risk (hrHPV) infections, increasing the likelihood of progression to anal cancer (Binagwaho et al., 2012).

Currently, Rwanda lacks national public health initiatives focused on the screening, prevention, and management of anal hr-HPV infections. This oversight is primarily due to the absence of comprehensive epidemiological data on the prevalence and genotype distribution of anal hr-HPV among WLWHIV. While a pioneering study has provided initial insights, the data remain insufficient to inform public health strategies or justify resource allocation for widespread screening (Murenzi et al., 2022).

The absence of robust epidemiological evidence hampers evidence-based policymaking, leaving health professionals unable to determine appropriate screening protocols, diagnostic methods, or public health messaging. Consequently, anal cancer may silently affect one of Rwanda's most vulnerable populations without detection and intervention.

This research aims to address this significant gap by providing detailed data on the prevalence and genotype distribution of high-risk anal HPV infections among women living with HIV in Rwanda. The results will contribute to the creation of targeted prevention measures, helping to protect these women from a preventable and potentially life-threatening condition.

1.3 RESEARCH OBJECTIVES

1.3.1 GENERAL OBJECTIVE

To determine the prevalence and genotype distribution of high-risk anal human papillomavirus (hr-HPV) infection among Rwandan women living with HIV.

1.3.2 SPECIFIC OBJECTIVES

1. To assess the prevalence of high-risk anal HPV infection among Rwandan women living with HIV attending care at Rwanda Military Referral and Teaching Hospital.

2. To identify which specific types of high-risk anal HPV are most common in Rwandan women living with HIV.

1.4 RESEARCH QUESTIONS

1. What is the prevalence of high-risk anal HPV infection among women living with HIV at Rwanda Military Referral and Teaching Hospital?

2. What are most hr HPVgenotypes distribution among women living with HIV at Rwanda Military Referral and Teaching Hospital?

CHAPTER 2: LITERATURE REVIEW

2.0. INTRODUCTION

This chapter reviews key literature on human papillomavirus (HPV) with a focus on anal high-risk HPV (hr-HPV) among women living with HIV. It begins with an overview of HPV biology, including its genome structure, proteins, and replication cycle. The discussion then shifts to anal HPV infection, its transmission, and major risk factors, particularly in HIV-positive women who face higher rates of persistence and progression due to immune suppression. Epidemiological evidence from global and African studies is presented to highlight prevalence patterns and co-infections. The chapter concludes by identifying research gaps, especially the lack of data from Rwanda, and outlines the need for targeted studies to guide screening, vaccination, and prevention strategies in high-risk populations.

2.1. HUMAN PAPILLOMAVIRUS

Human Papillomavirus (HPV) consists of over 200 distinct viruses that primarily infect the skin and mucous membranes in humans (Wolf, 2023). It is primarily transmitted through sexual contact, including vaginal, anal, and oral sex, as well as through direct skin to skin contact in the genital area. HPV infects the basal cells of the skin, mucosa, often entering through tiny breaks , microabrasions (Wolf, 2023). Most HPV infections cause no symptoms and are cleared naturally by the immune system within one to two years. However, some infections persist, especially those caused by high-risk types such as HPV 16 and 18, which can lead to precancerous changes and eventually cancers of the cervix, anus, penis, vulva, vagina, and oropharynx if not detected and treated (Brotons et al., 2018). Low-risk HPV types mainly cause benign conditions like genital warts. Prevention includes vaccination against the most harmful HPV types and regular screening, particularly cervical Pap tests, to detect abnormal changes early and reduce the risk of cancer development. HPV is highly prevalent, with nearly all sexually active individuals exposed to it at some point, but effective prevention and early detection strategies significantly reduce its health impact.

2.2. HPV GENOME AND PROTEIN

HPV is a member of the Papillomaviridae family and falls under the Alphapapillomavirus genus. HPV is a non-enveloped virus characterized by its circular double-stranded DNA genome, roughly 8,000 base pairs long. HPV consists of eight key proteins organized into two main genomic regions early region, which includes six proteins (E1, E2, E4, E5, E6, and E7) vital for the virus replication process, and the late region, which contains L1 and L2, which are essential for the assembly of new virions (Uwamungu et al., 2023).

The early proteins primarily have regulatory roles and are involved in various cellular processes during an HPV infection. Throughout an HPV infection, the majority of these early proteins remain expressed; however, their levels may decrease as the infection progresses(Hamid et al., 2009) . The E4 protein is expressed in the early transcript but accumulates and plays a critical role during the advanced stages of an infection(Doorbar, 2013).

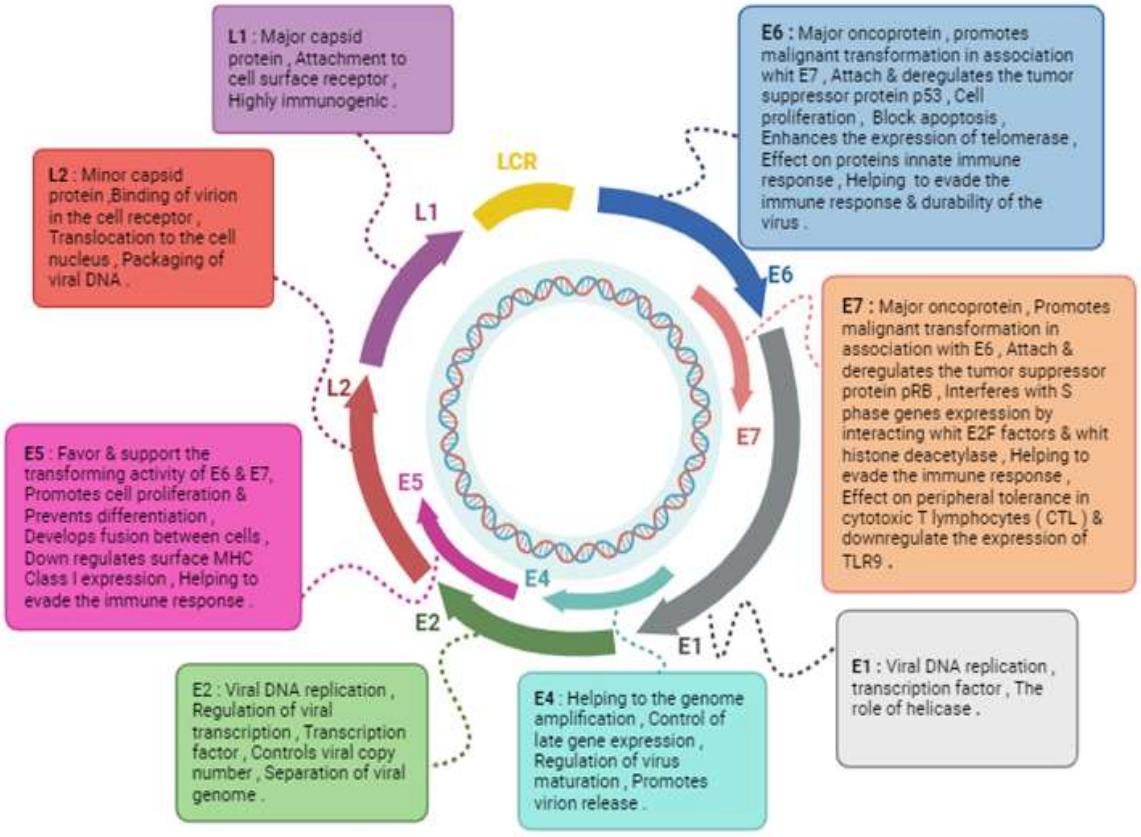

Figure 1: HPV Genome and Protein. Adapted from Nikmanesh et al 2024

2.3. HPV ENTRY AND REPLICATION CYCLE

High-risk human papillomavirus (hr-HPV), known for its role in cervical and other anogenital cancers, gains entry into host cells primarily through microabrasions in the epithelium. At the cervix, hr-HPV targets the vulnerable single-layered squamous-columnar junction between the endocervix and ectocervix (Graham, 2017). The virus begins the attachment process when its L1 capsid protein interacts with cellular receptors, like heparan sulfate proteoglycans (HSPGs), found on the basement membrane and surface of basal cells epithelial cells. HPV is internalized via endocytosis, a process closely resembling micropinocytosis, and is subsequently trafficked through membrane-bound cytoplasmic compartments, including the trans-Golgi network. Using a tubulin-mediated transport pathway, the viral genome is delivered into the nucleus either through nuclear pores or by gaining access during the breakdown of the nuclear membrane during mitosis (Graham, 2017).

Once inside the nucleus, HPV enters its early phase of replication. Early transcription is initiated, leading to the production of viral proteins that prepare the host cell for viral genome replication. The E2 protein binds to E1, facilitating E1's attachment as a hexametric complex at the viral origin of replication, which in turn recruits the host's DNA replication machinery. During this initial phase, the viral genome amplifies to produce approximately 50–100 episomal copies per nucleus (Doorbar, 2013). During the late phase of replication, the virus undergoes vegetative DNA replication and the formation of new virions. This phase is characterized by the activation of the viral major late promoter in the E7 gene region, which leads to enhanced expression of E1, E2, and additional viral proteins including E4 and E5(Graham, 2017). The rolling circle mechanism likely supports the amplification of thousands of viral genome copies.Virions assembly occurs within the nucleus following capsid protein production and genome amplification. The final stage involves maturation of virions in the superficial keratinocytes and release of infectious particles as these terminally differentiated cells are shed from the epithelial surface(Graham, 2017).

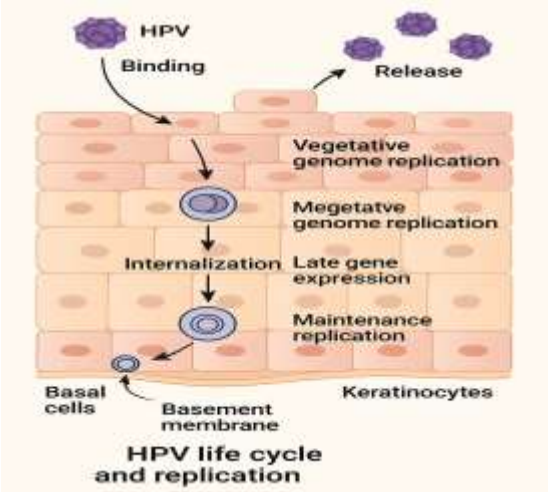

Figure 2: HPV Entry and Replication Cycle. Adapted from Bergant M et al, 2009.

2.4. ANAL HPV INFECTION

HPV is one of the most common sexually transmitted infections worldwide, affecting both men and women (Dahlstrom *et al*., 2023). While HPV is most often associated with cervical disease, it can also infect other sites, including the anus. Most anal HPV infections are transient and are cleared by the immune system within one to two years. However, persistent infection, especially with HPV-16, can lead to the development of AIN, which is classified into low-grade (AIN 1) and high-grade (AIN 2 and 3) lesions. High-grade AIN is considered a precursor to invasive anal cancer. The risk of progression is significantly higher among HIV-positive individuals, who have reduced ability to clear the virus. The latency period between initial infection and the development of cancer can span several years or decades(Arens *et al*., 2020).

Anal HPV infection, particularly with high-risk genotypes such as HPV-16 and HPV-18, is a well-established cause of anal intraepithelial neoplasia (AIN) and anal cancer. The burden of anal HPV infection is disproportionately high among certain populations, including men who have sex with men (MSM), individuals living with HIV, and women with a history of genital HPV infections (Lum *et al*., 2020). Despite its clinical significance, anal HPV infection remains under-recognized, particularly in low- and middle-income countries, where data are scarce and screening programs are limited.

2.5. TRANSMISSION AND RISK FACTORS FOR ANAL HR-HPV AMONG WOMEN LIVING WITH HIV

Ana hr-HPV spreads mainly through anal sex, but it can also pass through touching the genital area and then the anus, or from the genitals directly to the anal area(Imran O, 2019). HIV-positive women are especially vulnerable because their weakened immune systems, often measured by low CD4 counts, make it harder to clear the virus. In a study of 150 HIV-positive women, those with CD4 counts below 200 had eight times higher odds of having anal HPV16, and smoking or recent genital herpes also raised the risk. Across different studies, between 44% and 77% of HIV-positive women had anal hrHPV, and many had more than one type at the same time. Around one in three of these women continued to have hr-HPV in follow-up compared to about one in four among HIV-negative women(Xiao *et al*., 2025). Multiple high-risk strains like HPV16, 18, 33, 35, 58, and 59 are more common in anal infection than cervical infection in women with HIV. Effective antiretroviral therapy (ART) and keeping CD4 counts above 200 help reduce the risk of infection and persistence (Xiao et al., 2025).

2.6. EPIDEMIOLOGY OF ANAL HPV IN HIV-POSITIVE WOMEN

Studies from Europe, North America, and Latin America have reported anal hr-HPV prevalence among HIV-positive women ranging from 25% to 85%. For instance, in the Women's Interagency HIV Study (WIHS) cohort in the United States, over 60% of HIV-positive women had detectable anal hr-HPV (Ba *et al*., 2016). In Italy, prevalence was 41% among women on long-term antiretroviral therapy (ART). Although ART partially restores immune function, it does not fully eliminate HPV risk.

Sub-Saharan African data are limited but consistent with global estimates. South African studies have shown anal hr-HPV prevalence ranging from 43% to 60% in HIV-infected women. Factors strongly associated with infection include CD4 counts below 200 cells/mm^3, detectable HIV viral load, prior cervical HPV infection, and a history of receptive anal intercourse (Swase *et al*., 2025).

2.7. PREVALENCE OF ANAL HIGH-RISK HPV IN WOMEN LIVING WITH HIV

Anal hr-HPV infection is increasingly recognized as a significant health concern in HIV-positive women, yet it remains under-studied, especially in sub-Saharan Africa. HIV-related immune suppression makes it harder for the body to clear HPV infections, leading to a higher risk of persistent hr-HPV infection (Wolf, 2023). Persistent infection with oncogenic HPV types, particularly HPV16 and HPV18, is a major cause of anal intraepithelial neoplasia (AIN) and anal cancer (Swase *et al*., 2025).

Evidence from global and regional studies shows that women living with HIV are at a much greater risk of anal hr-HPV infection compared to HIV-negative women. A meta-analysis has shown that women living with HIV are 3 to 5 times more likely to have persistent anal hr-HPV infections. In sub-Saharan Africa, data are limited, but available studies highlight a substantial burden. For instance, a study in South Africa reported an anal hr-HPV prevalence of 60% among HIV-positive women(Matjie *et al.*, 2020).

The co-occurrence of cervical and anal hr-HPV is common in this group due to shared risk factors such as unprotected sex and multiple partners and the close anatomical relationship between the cervix and anus. This pattern suggests that integrated screening for both cervical and anal HPV-related diseases may be important for effective prevention and early detection (Castle, 2021).

Given these findings, there is an urgent need for more research, especially in low-resource settings like Rwanda, to better define the true burden of anal hr-HPV, its genotype patterns, and its contribution to anal cancer risk. Such data are essential for designing appropriate prevention, screening, and treatment strategies for HIV-positive women at high risk of anal cancer(Hernandez *et al.*, 2006).

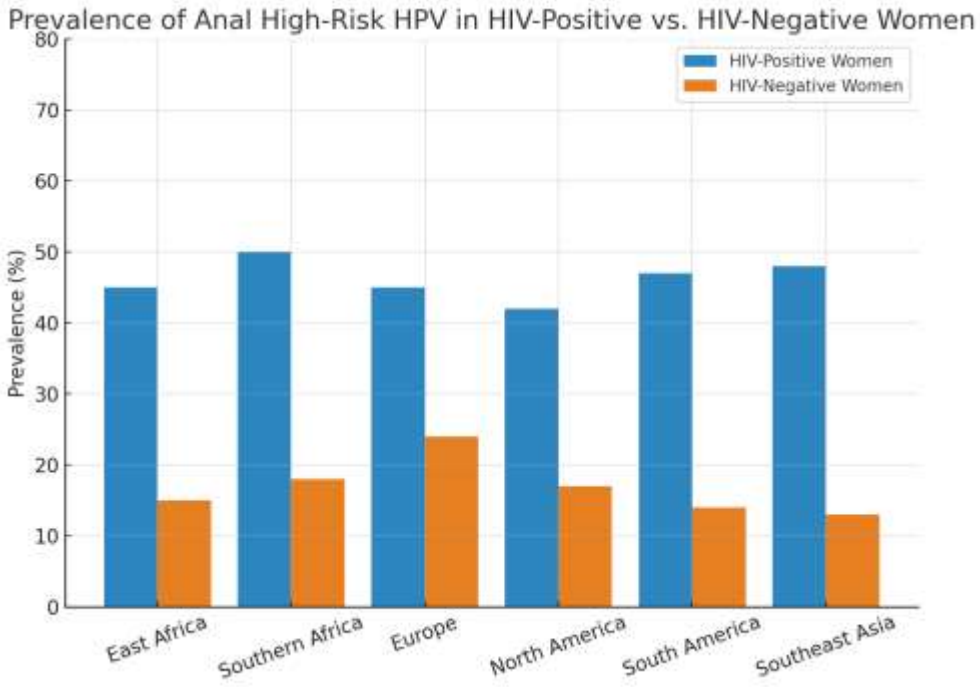

Figure 3: Prevalence of anal high-risk HPV in HIV-positive women across selected studies, Adapted from Lin, C *et al.*, 2016.

2.8. IMMUNE SUPPRESSION AND HPV PERSISTENCE

Persistent hr-HPV infection, rather than transient acquisition, is the major risk factor for progression to intraepithelial neoplasia and invasive cancer. In HIV-positive women, the immune suppression caused by HIV impairs viral clearance, increasing both the duration and multiplicity of hr-HPV infections. Lower CD4+ T cell counts, detectable HIV RNA, and shorter duration of ART have been associated with increased HPV persistence (Burchell *et al.*, 2022).

HIV infection increases the risk of getting and keeping anal hr-HPV infections. This is mainly because HIV weakens the body's immune system, especially by lowering the number of CD4 cells which help fight infections. When the immune system is weak, it is harder for the body to clear HPV, allowing the virus to stay in the body for a long time. Long-lasting (persistent) hr-HPV infections are the main cause of changes in anal cells that can lead to anal cancer (Swase *et al*., 2025).

Studies have shown that the risk of persistent anal hr-HPV infection is strongly linked to how low the CD4 count is and how high the HIV viral load is. People with very low CD4 counts are more likely to have ongoing hr-HPV infection and to develop serious (high-grade) anal cell changes that can become cancer (Murenzi *et al*., 2021).

HIV-positive women often have both cervical and anal hr-HPV infections at the same time. This happens because both areas share similar risk factors such as having multiple sexual partners or unprotected sex and because the cervix and anus are close together, making it easier for the virus to spread from one area to the other (Morhason-Bello, 2021).

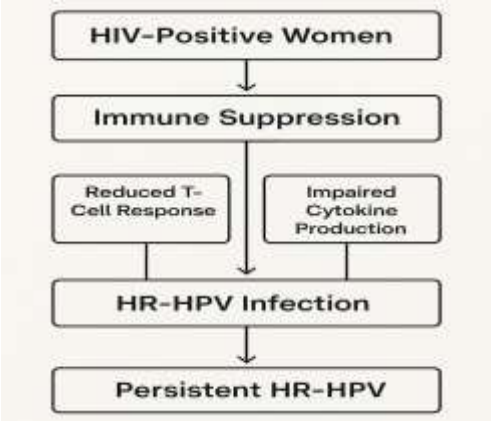

Figure 4: Immune Suppression and HPV Persistence, Adapted from Hernandez *et al.*, 2006.

2.9. RELATED WORK

Recent studies across sub-Saharan Africa have consistently shown that HIV positive women are at substantially higher risk for anal hr-HPV infection. This increased susceptibility is due to HIV induced immunosuppression, which impairs the body's ability to clear oncogenic HPV types such as HPV16, HPV18, and HPV35. Persistent hr-HPV infection is a well-established precursor to anal intraepithelial neoplasia (AIN) and invasive anal cancer (Kaba *et al.*, 2023).

In Rwanda, data remain limited. However, a study by Schifra *et al.*, 2021 involving HIV seroconcordant heterosexual couples found that 24% of anal swab samples from HIVpositive women tested positive for hr-HPV. This study provided the first nationally representative insight into anal HPV burden in Rwanda.

Another study from South Africa showed even higher prevalence (60%) among HIV positive women, emphasizing the regional variability and severity of the issue.Meta-analyses from broader sub-Saharan African settings reveal hr-HPV prevalence among HIV-positive women ranging from 40% to 75%, often with multiple concurrent infections(Okoye *et al.*, 2021) . These include not only globally recognized oncogenic types like HPV16 and HPV 18, but also regionally dominant types such as HPV 35, HPV 52, and HPV 56 many of which are not fully covered by current vaccines. This highlights the urgent need for genotype-specific surveillance and vaccine re-evaluation (Okoye *et al.*, 2021).

2.10. RESEARCH GAPS AND FUTURE DIRECTIONS

There is a pressing need for national epidemiologic studies that focus specifically on anal hr-HPV among Rwandan women living with HIV. Data on genotype distribution, persistence, co-infection with cervical HPV, and associated risk factors are critical for designing targeted interventions. Studies should also explore cost-effectiveness and feasibility of anal screening tools like anal cytology and self-swabbing for HPV detection.

Importantly, most current HPV prevention programs in Rwanda including the national vaccination program initiated in 2011 target adolescent girls and focus almost exclusively on cervical cancer. HIV positive women, particularly adults, are often excluded from vaccination campaigns and do not receive routine anal HPV screening or follow-up care for AIN or other HPV related anal lesions.

Recent global guidelines recommend anal cytology, high-resolution anoscopy for high-risk populations, including HIV-positive individuals. However, these practices are not yet integrated into Rwanda’s HIV care protocols, creating a blind spot in cancer prevention. The absence of anal cancer surveillance data further complicates early intervention.

There is thus a clear need for local studies to define genotype prevalence, persistence patterns, and co-factors of hr-HPV progression in Rwandan women living with HIV. Such studies would guide national policy on HPV screening and vaccination, especially in HIV care settings where anal HPV disease is currently overlooked.

CHAPTER 3: METHODOLOGY

3.1. INTRODUCTION

This chapter describes the research methodology used to achieve the study objectives. It covers the research design, study setting, target population, sample size, inclusion and exclusion criteria, sampling approach, data collection techniques, laboratory and analytical procedures, data management practices, and ethical considerations.

3.2. STUDY SETTING

The study was conducted in Kigali at Rwanda Military Referral and Teaching Hospital (RMRTH). RMRTH is a major national referral and teaching hospital that provides healthcare services to a diverse patient population from Kigali and surrounding regions. The study leveraged the molecular diagnostic capacity of the RMRTH Research Laboratory.

3.4. STUDY POPULATION

The target population comprised women living with HIV. Participants were recruited through purposive sampling with complete demographic and clinical data. All participants had previously undergone routine screening during which anal swab specimens were collected and tested for high-risk HPV genotypes using validated molecular diagnostic techniques.

3.5. INCLUSION CRITERIA

Eligible participants were women aged 18 years to 65 with a confirmed HIV Positive status and consented to take part in the research.

3.6. EXCLUSION CRITERIA

Participants were excluded from the study if they had incomplete clinical or laboratory data. Additionally, women with a prior diagnosis of anal cancer or those who had received treatment for HPV-related conditions before the time of sample collection were not eligible for inclusion.

3.7. STUDY DESIGN

This study employed a cross-sectional, where archived anal swab samples were collected and tested for hr-HPV genotypes. The study utilized 300 dry swab samples collected from HIV-positive women at RMRTH's RMRTH Research Laboratory, a facility equipped for high-throughput molecular testing.

3.8 SAMPLE SIZE DETERMINATION

The required sample size was determined using Cochran's formula for estimating a single population proportion. The formula is expressed as:

$$n = [Z^2 * p * (1-p)] / d^2$$

Where n is the required sample size, Z is the Z-score corresponding to the desired confidence level, p is the estimated prevalence of the outcome, d is the desired margin of error (precision).

For this study, a 95% confidence level was selected (Z = 1.96), and the margin of error (d) was set at 6% (0.06). As there was limited prior data on anal hr-HPV prevalence in this specific Rwandan cohort, a conservative prevalence estimate of 50% (p = 0.50) was utilized to maximize the required sample size and ensure sufficient statistical power. These parameters yielded an initial calculated sample size of 268. To account for potential data incompleteness or invalid samples, this figure was adjusted by 10%, leading to a final target sample size of 298, which was rounded up to 300 participants.

3.9 SAMPLE SELECTION

A total of 300 specimens were randomly selected from the repository of samples collected during the original investigation. The randomization procedure was implemented to generate a subset representative of the initial study population, thereby minimizing selection bias and enhancing the generalizability of the findings. This methodological approach safeguarded both the rigor and the integrity of the dataset, ensuring that subsequent analyses were robust and scientifically defensible.

3.10. LABORATORY PROCEDURES

3.10.1 SAMPLE THAWING AND PREPARATION

1. **Sample Retrieval**: 300 anal dry swab samples were retrieved from -80°C freezer.
2. **Thawing**: The samples were allowed to thaw at room temperature for 30-45 minutes, or until completely defrosted.
3. **Solution Removal**: After thawing, the preserved solution was removed from each sample, leaving only the anal dry swabs for further processing.

3.10.2 PREPARATION OF LYSIS BUFFER

The 1X Lysis Buffer was prepared from the 40X concentrated stock supplied with the AmpFire HPV High Risk Genotyping Kit, following the manufacturer's instructions for dry swab samples (1 mL of 1X buffer per sample). The total working volume was calculated using the dilution equation:

$C_1V_1=C_2V_2$

The 300 samples processed, the total working volume was:

$V_2=300\times1.0\ mL=300\ Ml$

Where C_1= 40X, C_2= 1X and V_2=300 mL

The stock volume was calculated as:

$V_1= 1\times300/40 = 7.5\ mL$

This volume of 40X stock was mixed with $300ml-7.5ml=292.5$ mL of nuclease-free water to yield 300 mL of 1X working solution. The buffer was then used to lyse cellular material from the dry anal swabs, releasing nucleic acids for downstream isothermal amplification.

The calculated reagent volumes for the entire process are summarized in Table 1.

3.10.3 INCUBATION AND LYSIS OF SAMPLES

Each tube containing a swab received 1.0 mL of 1X Lysis Buffer. Tubes were vortexed for 10–15 seconds and incubated at 95 °C for 20 minutes to ensure complete lysis.

3.10.4 MASTER MIX PREPARATION

Four master mixes (PM-1 to PM-4) were prepared according to the kit protocol, each targeting a specific subset of high-risk HPV genotypes using a corresponding primer mix. For each mix, the required volumes were calculated as:

Volume per master mix = (N + 2) × 10 µL

Primer Mix = (N + 2) × 10 µL

For N = 300, N + 2 = 302, thus: Reaction Mix = 302 × 10 µL = 3,020 µL (3.02 mL)

Primer Mix = 302 × 10 µL = 3,020 µL (3.02 mL).

This resulted in 6.04 mL per master mix. Each mix was vortexed and briefly centrifuged before dispensing into PCR wells.

The calculated reagent volumes for the entire process are summarized in Table 1.

Table 1: Reagent Preparation and Volumes for PCR Analysis

Reagent	Formula	Calculation (N = 300)	Total Volume	Incubation Time
1X Lysis Buffer	**$C_1V_1 = C_2V_2$** **1 mL per sample**	**V_2 =** 300 × 1.0 mL = 300 Ml **V_1 =** 300 / 40 = 7.5 mL (40X stock) **Water =** 300 − 7.5 = 292.5 mL	300 mL= (7.5 mL stock + 292.5 mL water)	95 °C for 20 min
Master Mix (per PM-1 to PM-4)	**Reaction Mix** = (N+2) × 10 µL **Primer Mix** = (N+2) × 10 µL	**Reaction Mix** =(300+2) × 10 µL = 3,020 µL (3.02 mL) **Primer Mix** =(300+2) × 10 µL= 3,020 µL(3.02 mL)	6.04 mL per mix × 4 = 24.16 mL total	N/A
PCR Reaction (per well)	20 µL Master Mix + 5 µL Sample	—	25 µL per well	N/A

3.10.5 PCR PLATE SETUP

After preparing the master mixes, each PCR well received 20 µL of the appropriate master mix (PM-1 to PM-4) and 5 µL of the prepared sample. Positive and negative controls were added to designated wells in 5 µL aliquots. The plate was sealed with an optical adhesive film, vortexed gently to ensure mixing, and centrifuged briefly before amplification.

3.10.6 HPV GENOTYPING VIA REAL-TIME ISOTHERMAL AMPLIFICATION

The Atila AmpFire HPV High-Risk Genotyping Kit was used to detect 15 high-risk HPV types. The isothermal amplification process eliminates the need for thermal cycling, using a DNA polymerase that amplifies DNA at a constant temperature. Amplification was monitored in real time by detecting fluorescence signals, PCR plate was placed into a Bio-Rad CFX96 Real-Time System and run at 60°C for 60 minutes. Fluorescence data was collected every 60 seconds on multiple channels FAM, HEX/VIC, Cy5 to enable multiplexed detection.

3.10.7. QUALITY CONTROL

Each amplification run included the following controls, as specified by the manufacturer:

Positive Control (HPV Positive Template): A valid result is indicated by exponential amplification curves in all relevant fluorescence channels, Confirms amplification efficiency and verifies reagent performance.

Negative Control (No Template Control, NTC): A valid negative control displays no exponential amplification in any channel, Detects potential contamination in reagents or the testing environment.

Internal Control (IC): Monitors sample quality by confirming the presence of human DNA and the absence of PCR inhibitors. Successful amplification of the IC ensures that lysis and amplification steps were functional, even if HPV targets are absent.

3.11. FINAL ANALYTICAL SAMPLE SIZE

Following completion of laboratory processing for the 300 selected specimens, quality control checks identified six samples that could not be included in the analysis. Five samples yielded invalid outputs due to assay failure, and one sample lacked a corresponding participant identifier. These specimens were excluded from the dataset, resulting in a final analytical cohort of 294 samples. Restricting the analysis to complete and valid results preserved the methodological rigor of the study and ensured the reliability of the statistical inferences drawn.

3.12. STATISTICAL ANALYSIS

Data were analyzed using SPSS version 25 to calculate the prevalence of each HPV genotype. Descriptive statistics summarized participant characteristics, while HR-HPV prevalence was estimated with 95% confidence intervals. Chi-square tests were employed to evaluate associations between HPV positivity and other categorical variables, while logistic regression models were used to determine factors influencing HPV infection risk. Results were presented as adjusted odds ratios with 95% confidence intervals.

3.13. ETHICAL CONSIDERATIONS

The Rwanda National Ethics Committee provided ethical approval for this laboratory-based, cross-sectional study, which utilized clinical and demographic data along with archived anal swab samples (Reference Number: RNEC 520/2024). Informed consent was obtained in writing from all participants during the initial sample and data collection, which also covered consent for the use of anonymized data in future research. Participant confidentiality was strictly maintained throughout the study, with data access restricted to the authorized research team members only.

CHAPTER 4. RESULT ANALYSIS AND DISCUSSION

4.0 Results

This section details the findings of the study, beginning with the demographic characteristics of the cohort, followed by the prevalence and distribution of anal high-risk HPV (hr-HPV), and concluding with the statistical analysis of factors associated with hr-HPV infection.

4.1.QPCR(QUANTITATIVEPOLYMERASECHAINREACTION)AMPLIFICATION PLOT RESULTS

The qPCR amplification plot shown in Figure 5 illustrates the real-time amplification of target DNA sequences detected in this study. This method, widely used in molecular biology, enables quantification of specific nucleic acid sequences by monitoring fluorescence signals generated during amplification cycles.

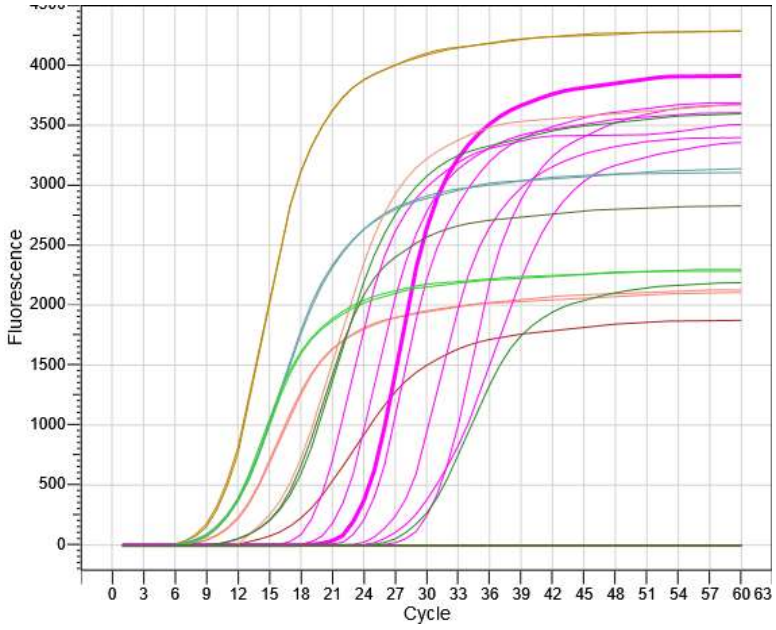

Figure 5: Real-time PCR amplification plot, Generated in this study.

This graph shows the real-time amplification of a target DNA sequence from multiple samples. The key metric is the Cycle Threshold (Ct), which is the cycle number where the fluorescence signal rises significantly. A lower Ct value means a higher starting amount of DNA.

Key Observations:

Wide Dynamic Range: The experiment successfully detected samples with a broad range of DNA concentrations, indicated by the curves spanning from early to late cycles.

Highest Concentration Sample: The gold curve on the far left has the lowest Ct value (~cycle 11), indicating it contained the highest initial amount of target DNA.

Lowest Concentration Samples: The curves on the far right have the highest Ct values (~cycles 27-33), indicating they contained the lowest amounts of target DNA.

Valid Negative Control: The flat line at the bottom is a negative control (NTC), showing no amplification. This confirms the absence of contamination and validates the experiment's results.

The experiment was successful, providing reliable quantification of the target DNA across various concentrations.

4.2. DESCRIPTIVE STATISTICS

Descriptive statistics were used to summarize the central tendency and variability of the age distribution within the study sample (N = 294).

Table 2: Descriptive Statistics of Age Distribution in HIV-positive Women

Variable	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	294	22	33	30.44	2.076

The mean age of participants was 30.44 years (SD = 2.076). The small standard deviation, combined with a narrow age range of 22 to 33 years, indicates a relatively young and chronologically homogeneous sample with low variability in age

The study population represents a relatively young and chronologically homogeneous cohort of women. The narrow age range 11 years and small standard deviation indicate low variability, which is advantageous for controlling age as a potential confounding variable in the analysis of HPV prevalence. This age group 22-33 years is of particular clinical interest as it falls within the peak years of sexual activity and HPV acquisition. In HIV-

positive individuals, infections acquired during this period are more likely to persist due to immune dysregulation, thereby increasing the long-term risk of developing HPV-associated malignancies (Clifford *et al.*, 2005). The homogeneity of the sample, while beneficial for internal validity, may limit the generalizability of the findings to older or younger populations of Rwandan women living with HIV.

4.3 ANAL HPV GENOTYPE PREVALENCE

The following table presents the frequency distribution for high-risk hr-HPV genotyping results within the sample (N = 294).

Table 3: Prevalence of anal hr-HPV among HIV-positive women.

Result	Frequency (n)	Percent (%)
Negative	178	60.5
Positive	116	39.5
Total	294	100

This finding demonstrates that nearly 4 in 10 HIV-positive women carried anal hr-HPV, reinforcing the heavy burden in this population and the urgent need for targeted screening interventions.

The observed prevalence of 39.5% high-risk anal HPV infection among HIV-positive women in this study highlights a significant burden in this population. HIV-related immunosuppression has been well documented to reduce the body's ability to clear HPV infections, leading to greater risk of persistence and progression to anal intraepithelial neoplasia and, eventually, anal cancer. Our results align closely with findings from similar populations in sub-Saharan Africa conducted reported a 43% prevalence of anal high-risk HPV among HIV-positive women in Johannesburg, South Africa (Mbulawa *et al*., 2017). Their study also noted a high proportion of abnormal cytology and anal lesions, suggesting that persistent HPV infection in HIV-positive women carries substantial clinical consequences.

Further regional comparison study conducted a systematic review and meta-analysis of high-risk HPV among Nigerian women(Ezechi *et al*., 2023) , reported a pooled prevalence of 38.6% high-risk HPV among HIV-infected women, with predominant genotypes including HPV16, HPV35, HPV52, and HPV58. This prevalence is remarkably consistent with our findings and reinforces the conclusion that high-risk HPV is widespread among HIV-positive women in Africa, regardless of country-specific variations.

4.4 SINGLE ANAL HPV GENOTYPE DISTRIBUTION

Table below shows the frequency and percentage of the 15 different single anal HPV genotypes. The predominance of HPV56, together with the unexpectedly low prevalence of HPV16, highlights a distinct regional genotype pattern, with direct implications for vaccine and screening strategies in Rwanda.

Table 4: Distribution of single anal HPV genotypes in HIV-positive women.

HPV Genotypes	Frequency (N)	Percent (%)
HPV16	1	0.9
HPV18	5	4.3
HPV31	1	0.9
HPV33	3	2.6
HPV35	6	5.2
HPV39	4	3.4
HPV45	2	1.7
HPV51	5	4.3
HPV52	3	2.6
HPV53	6	5.2
HPV56	10	8.6
HPV58	3	2.6
HPV59	4	3.4
HPV66	5	4.3
HPV68	4	3.4
Total	**56**	**48.3**

In this table, HPV56 was the most frequently detected high-risk genotype among HIV-positive women, accounting for 8.6% of all single-type infections, followed by HPV35 at 5.2% and HPV18 at 4.3%. HPV16, which is globally recognized as the most oncogenic and widely distributed genotype, was found in only 0.9% of the samples. This is a surprisingly low rate considering its strong association with anal and cervical cancers, as described by Clifford et al.., 2017 and differs from global patterns where HPV16 usually dominates.

The low prevalence of HPV16 is consistent with findings from other sub-Saharan African countries. According to Akarolo-Anthony et al .., 2019 HPV56, HPV35, and HPV58 were more common than HPV16 among HIV-positive women in Nigeria .Also a Similar patterns were also reported in Uganda and Kenya, where non-16 and non-18 types such as HPV52, 58, and 35 were frequently detected in HIV-infected populations (Banura *et al*., 2008).

The predominance of HPV56 in this study may reflect persistence patterns linked to immunosuppression. As noted by DeVuyst *et al*.,2015 HIV-related immune dysfunction may limit viral clearance, allowing genotypes like HPV56 and HPV35 to persist more readily compared to HPV16 or 18 .

These observations highlight the need for region-specific HPV surveillance and prevention strategies. While current vaccines are highly effective against HPV16 and 18, they offer limited or no coverage for other high-risk types such as HPV56 and 35. Relying on global genotype trends alone to guide national vaccination or screening programs may leave key populations under protected. Incorporating broader-spectrum vaccines and genotype-inclusive screening tools would be more appropriate in settings where these other types are prevalent.

4.5 MULTIPLE HIGH RISK HPV GENOTYPES DISTRIBUTION

The wide range of multi-genotype infections, particularly the frequent combinations of HPV39 with HPV51 and HPV66, reflects viral diversity facilitated by HIV-related immunosuppression, complicating clinical management.

The following table details the distribution of specific high-risk human papillomavirus (hr-HPV) co-infections identified within the study cohort. The data illustrates the frequency and percentage of multi-genotype infections.

Table 5: Distribution of multiple high-risk HPV co-infections among HIV-positive women (N=294).

HPV Genotype	Frequency	Percent
39/51	2	3.7
39/66	2	3.7
52/66/68	1	1.9
16/59	1	1.9
39/52	1	1.9
52/53	1	1.9
35/68	1	1.9
51/53/68	1	1.9
16/39/56	1	1.9
39/68	1	1.9
16/51	1	1.9
52/68	1	1.9
31/66/68	1	1.9
39/56/68	1	1.9
53/68	1	1.9
51/52	1	1.9
33/39/51/56	1	1.9
18/39/51/53/56/66	1	1.9
56/68	1	1.9
35/56	1	1.9
18/51	1	1.9
16/18/51/68	1	1.9
16/53	1	1.9
18/51/58	1	1.9
18/39/56	1	1.9
16/35/52	1	1.9
33/66	1	1.9
39/53	1	1.9
51/59	1	1.9
35/52/53/66	1	1.9
31/39/52/68	1	1.9
18/35/51/53	1	1.9
53/56	1	1.9

35/51/56	1	1.9
51/68	1	1.9
16/18/66/68	1	1.9
33/35/58	1	1.9
31/56	1	1.9
53/56/59	1	1.9
35/39/51/58/66	1	1.9
16/18/45	1	1.9
58/66	1	1.9
51/52/56	1	1.9
39/58	1	1.9
51/56	1	1.9
45/52/68	1	1.9
35/52	1	1.9
31/45/56	1	1.9
31/53/66	1	1.9
39/45/58/68	1	1.9
16/68	1	1.9
33/56	1	1.9

Above table reveals a complex results and diverse pattern of hr-HPV co-infections, with a wide variety of unique genotype combinations. Most of these combinations appeared only once 1.85% of cases, indicating significant genetic diversity in the infections. HPV39 with HPV51 and HPV39 with HPV66 were the most frequent co-infections, each accounting for 3.7% of the cohort.

The findings from our study are consistent with Swase et al..,2025 they emphasized that HPV31, HPV33, HPV35, and HPV58 are emerging as key contributors to cancer progression, particularly in immunocompromised populations, such as those with HIV co-infection (Swase *et al*., 2025). These genotypes are increasingly implicated in anal cancers, especially in individuals living with HIV, due to the prolonged viral persistence enabled by immunosuppression.

From Swase *et al*.., 2025 highlighted that HPV16 and HPV18 are frequently co-infected with other high-risk genotypes in HIV-positive individuals, leading to a higher viral load and prolonged infections. This is particularly concerning in the context of anal cancers, as individuals with HIV are at an increased risk of persistent HPV infections, which can lead to neoplastic transformations. Similarly, in our study, HPV16 and HPV18 co-infections with HPV31, HPV33, HPV35, and HPV58 were prevalent in the cohort. These raise the risk of anal dysplasia, which can progress to anal cancer.

The observed high prevalence of HPV31, HPV33, HPV35, and HPV58 in our study is of particular significance in regions with a high burden of HIV, such as sub-Saharan Africa. Studies, including those reviewed by Swase *et al*.., 2025 indicate that in such regions, HPV genotypes outside of HPV16 and HPV18 are increasingly contributing to anal cancers. This underscores the need for targeted screening and vaccination programs that include these emerging genotypes, particularly in immunocompromised populations.

Within our study cohort, co-infections of HPV39 with HPV51 and HPV39 with HPV66 were the most frequently observed, representing 3.7% of all cases. This finding, combined with the detection of other high-risk genotypes including HPV31, HPV33, HPV35, and HPV58, highlights the substantial genetic diversity inherent in anal HPV infections. Such heterogeneity could complicate diagnostic and therapeutic efforts, a concern that is especially pronounced in immunocompromised populations, such as individuals living with HIV.

Based on these findings, we recommend the incorporation of HPV genotyping into routine screening protocols for anal HPV, with specific surveillance for HPV31, HPV33, HPV35, and HPV58. Given that these genotypes play a critical role in the progression toward anal HPV-related malignancies, their early and accurate detection is crucial for timely intervention. This necessitates the development of comprehensive screening programs and targeted vaccination strategies to mitigate the risks associated with these emerging genotypes, particularly among women living with HIV.

4.6 CHI-SQUARE FOR Age Group, Vaccination Status, and hr-HPV Genotypes

The Chi-Square tests assessed the associations between age group, HPV vaccination status, and anal high-risk HPV (hr-HPV) genotyping results in the study population (N = 294). Observed and expected frequencies were compared using Pearson's Chi-Square, Fisher's Exact Test, and likelihood ratio tests.

Table 4: Chi-Square test results for age group, HPV vaccination status, and hr-HPV infection

Comparison	Test	Df	p-value
Age group vs hr-HPV	Pearson χ^2	1	0.466
Age group vs hr-HPV	Fisher (2-sided)	—	0.476
Vaccination vs hr-HPV	Pearson χ^2	2	0.784
Vaccination vs hr-HPV	Likelihood ratio	2	0.782

The present study examined the associations between age group, HPV vaccination status, and the prevalence of anal high-risk HPV (hr-HPV) infection. The analysis revealed no statistically significant associations for either variable in this cohort (Table 1).

Association between Age Group and hr-HPV Infection

For the relationship between age group and hr-HPV, statistical testing indicated a non-significant result (Pearson's $\chi^2(1) = 0.530$, $p = 0.466$; Fisher's Exact Test, $p = 0.476$). This finding suggests that hr-HPV prevalence was consistent across the age categories studied, without the typical decline in older age groups often observed in the general population.

This result is consistent with existing literature on women living with HIV. For instance, Stelzle et al., 2021 observed a high prevalence of anal hr-HPV across all age groups in immunocompromised women, without a clear age-dependent trend. Similarly, a study in the Netherlands reported that hr-HPV prevalence remained relatively stable among HIV-positive women of different ages(Castle, 2021). This phenomenon is attributed to the reduced immune clearance of HPV in individuals with HIV.While immunocompetent individuals often clear the virus over time, HIV-mediated immunosuppression can lead to persistent infections that endure across the lifespan, masking the age-related trends seen in the general population.

Association between HPV Vaccination Status and hr-HPV Infection

Similarly, HPV vaccination status was not found to be significantly associated with the prevalence of anal hr-HPV infection (Pearson's χ^2 (2) = 0.487, p = 0.784; Likelihood Ratio χ^2 (2) = 0.491, p = 0.782). While this may seem counterintuitive given the known efficacy of HPV vaccines, several factors in a high-risk cohort like this could explain this non-significant finding

Collectively, these findings suggest that in this specific cohort of women living with HIV, the dominant role of immunosuppression in driving persistent HPV infection may override the influence of demographic factors like age. This aligns with broader observations in high-risk populations, where traditional predictors of HPV infection are often less reliable. For example, Chinyowa *et al*., 2019 found a high prevalence of anal HPV across age categories in a Zimbabwean cohort without strong links to demographic variables, and Winer *et al*.,2011 showed that in sexually active young women, variables such as age did not reliably predict infection risk.

4.7 Logistic Regression Age Group, HPV vaccination status and Anal hrHPV Genotyping Results

This logistic regression model examines the relationship between Age Group, HPV vaccination status and Anal hrHPV Genotyping Results. The table provides the regression coefficients (B), standard errors (S.E.), Wald statistic, significance (Sig.), and odds ratios (Exp(B)) to understand the influence of age group on the outcome.

Table 5: Logistic Regression Age Group, HPV vaccination status and anal hrHPV Genotyping Results

Predictor	Odds Ratio	95% CI	p value
Age group	0.71	0.28–1.80	0.467
HPV Vaccination status	0.91	0.59–1.41	0.676

A binary logistic regression analysis was performed to identify significant predictors of anal high-risk HPV (hr-HPV) infection in the study population. Both age group and HPV vaccination status were examined as independent variables. As shown in Table 1, neither predictor was found to have a statistically significant association with the presence of anal hr-HPV.

Age Group as a Predictor of hr-HPV Infection

Age group was not a significant predictor of anal hr-HPV infection. The model yielded an odds ratio (OR) of 0.71 with a 95% confidence interval (CI) of 0.28 to 1.80, which was not statistically significant ($p = 0.467$).

The odds ratio of 0.71 suggests a slight, non-significant trend toward lower odds of hr-HPV infection with increasing age, but the wide confidence interval, which crosses 1.0, confirms the lack of a reliable association. This absence of a significant age effect is likely due to the relatively narrow age range of the study participants (22–33 years). Within this window, most individuals are in a period of high HPV acquisition and persistence. As noted by Moscicki *et al* ., 2012 HPV prevalence tends to remain stable in early adulthood, with significant age-related declines in prevalence typically emerging after the age of 30 .

This biological pattern, combined with the limited age variability in our sample, makes it difficult to detect a statistically significant trend. Our finding is also consistent with epidemiological analyses showing a weak age effect on anal hr-HPV prevalence, especially in high-risk populations such as women living with HIV (Fracella *et al.,* 2024).

HPV Vaccination Status as a Predictor of hr-HPV Infection

Similarly, HPV vaccination status was not found to be a significant predictor of anal hr-HPV infection in this cohort (Odd ratio = 0.91, 95% CI [0.59–1.41], $p = 0.676$). The odds ratio is very close to 1.0, indicating almost no difference in the odds of infection between vaccinated and unvaccinated individuals in this sample.

This non-significant finding does not necessarily contradict the known efficacy of HPV vaccines. Instead, it likely reflects the context of vaccination in this high-risk population. Studies shown that while the HPV vaccine is safe and immunogenic in individuals with HIV, its clinical effectiveness is greatest when administered prophylactically, before HPV exposure. In populations where many individuals are vaccinated after becoming sexually active, the vaccine may not clear pre-existing infections (Mavundza *et al* ., 2020). Therefore, the lack of a protective association in our cross-sectional analysis could be explained by vaccination occurring after initial HPV exposure, a factor that cannot be discounted without detailed behavioral and clinical histories.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study provides compelling evidence of a high anal hr-HPV prevalence at 39.5% among Rwandan women living with HIV, confirming their status as a significant high-risk group whose vulnerability is amplified by immune dysregulation. Critically, the research uncovers a unique virological landscape that deviates from global trends, with HPV56 emerging as the predominant genotype instead of the more common HPV16. Furthermore, while the classic oncogenic types HPV16 and HPV18 were present, they occurred overwhelmingly within complex, multi-genotype co-infections. These findings demonstrate that public health strategies must be tailored to local data, as models based on global patterns are insufficient to address the specific and complex risks faced by this population.

5.2. LIMITATIONS OF THE STUDY

This study provides important insight into the prevalence and genotype distribution of anal high-risk HPV among Rwandan women living with HIV. However, a few limitations should be acknowledged. Study population was restricted to a narrow age range 22 to 33 years, reduces the generalizability of the findings to older or younger HIV-positive women who may exhibit different immune responses or risk patterns. The study lacked access to detailed sociodemographic, clinical, and behavioral data. Important variables such as CD4 cell counts, HIV viral load, duration on antiretroviral therapy, and sexual behavior were not available. These factors are known to influence HPV infection dynamics, including acquisition, persistence, and clearance. Their absence limited the ability to conduct more nuanced analyses that could have revealed associations between host characteristics and HPV outcomes.

5.3. RECOMMENDATIONS

Based on the findings of this study, it is strongly recommended that routine, comprehensive anal high-risk HPV screening be integrated into the clinical management protocols for HIV-positive women. Public health strategies should prioritize the inclusion of the nonavalent HPV vaccine, which covers a broader spectrum of oncogenic HPV types, in vaccination programs targeting high-risk populations. Additionally, regional HPV surveillance should be strengthened to monitor the prevalence, genotype distribution, and potential shifts in the viral landscape. Health education initiatives should also be enhanced to increase awareness of HPV-associated risks and prevention strategies. Furthermore, longitudinal cohort studies are essential to delineate the long-term trajectory of HPV infections, identify key host and behavioral risk factors, and assess the sustained effectiveness of vaccination interventions in this immunocompromised group.

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