PREVALENCE OF PRE-MALIGNANT CERVICAL LESIONS AMONG HIV-POSITIVE WOMEN ON HAART ATTENDING HOME CARE CLINIC AT ST. FRANCIS HOSPITAL NSAMBYA, UGANDA

BY

ORINGO JOHN INNOCENT

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MAY 2020

DECLARATION FORM

I, **John Innocent Oringo**, hereby declare that this dissertation is my original work. It has never been published and or submitted for any other degree award to this or any other University before.

Signature: _____ **Date_____**

ORINGO JOHN INNOCENT

MSc. CLINICAL CYTOLOGY

DEPARTMENT OF HUMAN PATHOLOGY

UNIVERSITY OF NAIROBI

SUPERVISORS

PROF. LUCY. W. MUCHIRI, MBCHB, MMED (Path), PG-BRM, Ph.D., FCPath (ECSA).

Associate Professor,

Anatomic Pathology Unit,

Department of Human Pathology,

School of Medicine,

University of Nairobi

Signature: _____ **Date: _____**

DR. WAIRIMU WAWERU, MBCHB, MMED (Path) FCPath (ECSA).

Senior Lecturer,

Anatomic Pathology Unit,

Department of Human Pathology,

School of Medicine,

University of Nairobi.

Signature: _____ **Date: _____**

DR. EMMANUEL OTHIENO MBChB, M.MED (Path)

Associate Professor of Pathology

Senior Consultant

Department of Pathology

Soroti University

Signature: _____ **Date: _____**

DR. PETER SEKWEYAMA, MBChB, M.MED, (FECSACOG)

Deputy Medical Director.

Head Clinical Services

Consultant Obstetrician/Gynecologist

St. Francis Hospital Nsambya, Kampala

Signature: _____ **Date: _____**

DEDICATION

This dissertation is dedicated to my dear parents, my beloved wife, Jacklyn, children -Alfred, Benjamin & Clara, and to my siblings for their spiritual and social support.

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LIST OF ABBREVIATIONS

AGC	:	Atypical glandular cells
AIDs	:	Acquired Immune Deficiency Syndrome
AIS	:	Adenocarcinoma in-situ
ARVs	:	Antiretroviral
ASCCP	:	American society of colposcopy and cervical pathology
ASCH	:	Atypical squamous cells cannot exclude high grade
ASCUS	:	Atypical squamous cells of undetermined significance
cART	:	Combined antiretroviral treatment
CD4	:	Cluster of differentiation
CIN	:	Cervical intraepithelial neoplasia
CP	:	Convectional preparation
DNA	:	Deoxyribose Nucleic Acid
DPX	:	Distyrene plasticizer xylene
DVD	:	Digital versatile disk
EA	:	Eosin Azure
ECC	:	Endocervical curettage
ERB	:	Ethical Review Board

ERC	:	Ethical review committee
HAART	:	Highly active antiretroviral therapy
HCC	:	Home Care Clinic
NHC	:	Nsambya Home care
HIV	:	Human Immunodeficiency
HOD	:	Head of Department
HPV	:	Human Papilloma Virus
HR-HPV	:	High-risk human papilloma virus
HSIL	:	High grade squamous intraepithelial lesion
ICC	:	Invasive cervical cancer
ID	:	Identification
KNH	:	Kenyatta National Hospital
LAB	:	Laboratory
LAP	:	Lower abdominal pain
LIC	:	Low- income countries
LMIC	:	Low- and Middle-income countries
LR-HPV	:	Low-risk human papilloma virus
LSIL	:	Low grade squamous intraepithelial lesion

NILM	:	Negative for intraepithelial lesion or malignancy
OG	:	Orange G
PAP	:	Papanicolaou stain
PCB	:	Post-coital bleeding
PI	:	Principal Investigator
PVD	:	Per Vaginal Discharge
RNA	:	Ribonucleic Acid
SCC	:	Squamous Cell Carcinoma
SDF	:	Socio-demographic features
SIL	:	Squamous intraepithelial lesions
SOP	:	Standard operating procedure
STI	:	Sexually transmitted infection
TBS	:	The Bethesda system
UNAIDS	:	Joint United Nations programme on HIV/AIDs
UON	:	University of Nairobi
VL	:	Viral Load
WHO	:	World Health Organization

ABSTRACT

Background

Cervical cancer is the most prevalent cancer among women and the leading cause of cancer-related mortality in Uganda. In sub-Saharan Africa, one in six women will develop cancer during their lifetime, and one in eleven will die from it.

Cervical precursor lesions are associated with human immunodeficiency virus (HIV) among women on highly active antiretroviral therapy due to persistent human papilloma virus (HPV) infection. Low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells of undetermined significance (ASCUS), and high-grade squamous intraepithelial lesion (HSIL) have been the most frequent precursor lesions. However, the prevalence and correlations with other variables have not been documented among women living with HIV on HAART in Uganda.

Study objectives

To determine the prevalence and distribution of pre-malignant cervical lesions on Pap smear among HIV-positive women on HAART for three (3) years or more attending Home Care Clinic at St. Francis Hospital, Nsambya, in Uganda.

Materials and Method

A cross-sectional descriptive study was carried on HIV-positive women on HAART aged 18 to 69 years attending the Home Care Clinic (HCC) of St. Francis Hospital Nsambya between December 2019 and February 2020. They were examined for precursor cervical lesions on Pap smear. Socio-demographic and clinical information was obtained through a questionnaire. The baseline CD4 count and viral load were abstracted from the daily activity register. The data were

entered using Microsoft Excel spreadsheets and analysis done using R-software version 3.6.2 (2019). Logistic regression was done, and results were presented in tables, pie charts, histograms, and photomicrographs.

Results

Two hundred and ten (210) women were enrolled in the study, and a Pap smear was carried out in all of the participants and samples sent to the laboratory. Abnormal smears were reported in 12 (5.7%) of the women. The most common finding was ASC-US (2.4%) followed by LSIL (1.9%), while the rest of the women had atypical squamous cells cannot exclude high-grade (ASC-H), squamous cell carcinoma (SCC), and Adenocarcinoma lesions with 0.5% each. Interestingly SCC and adenocarcinoma were observed in the lower age group of between 30 to 39 years. Bacterial vaginosis, found in 24.8% of the study participants, was the most common infection, followed by Candidiasis which was observed in 8.1% of the subjetcs.

Conclusion

There was a low prevalence of pre-malignant cervical lesions among women living with HIV attending HCC of St. Francis Hospital, Nsambya, in Uganda. The study did not find a statistically significant relationship between the premalignant cervical lesions with the duration on HAART and viral loads.

INTRODUCTION

1.1 Background

Invasive cervical cancer is ranked the fourth most prevalent cancer and among the leading cause of mortality due to cancer in women worldwide. In 2018, approximately 570,000 new cases of the disease occurred, with about 311,000 related mortalities globally [1].

In sub-Saharan Africa, it is the second leading cause of cancer-related deaths among women after breast cancer. In 2018, about 28% of the new cases of cervical cancer occurred among women living with HIV accounting for approximately 42% of deaths among immunosuppressed patients. It was suggested that one in six women would develop cancer during a lifetime, and one in eleven will die from the disease [1,2]. Although cervical cancer is a preventable disease, most women in low-income countries have limited access to effective screening, timely diagnosis, and treatment. There is a need to have organized systematic screening to cover more vulnerable women [1,3,4].

The incidence risk of developing invasive cervical cancer is approximately ten times greater in low-income countries compared to high-income countries. This was as a result of effective routine screening, improved socioeconomic status, improved genital hygiene, reduced parity and diminished persistent high-risk HPV infection [1,2].

In Kenya, cervical cancer is the second most frequent cancer among women, and approximately 18.3% of the new cases were diagnosed in 2018 with an estimated 12% mortalities from cervical cancer; this is lower than in other countries in sub-Saharan Africa combined (28%) [5,6].

In comparison, in Uganda, cancer of the uterine cervix is frequently diagnosed among women and the leading cause of cancer-related mortality. Approximately 6,413 new cases occurred in

2018, resulting in 35.5% of the number of new cases of cervical cancer with about 4,301 mortalities [1,3–6]. Persistent high-risk HPV infection is the most critical risk factor worldwide. Other risk factors; lack of cervical cancer screening of HIV positive women, high parity, early sexual debut, oral contraceptive use, smoking may have contributed to the progressively rising incidence and mortalities due to cervical cancer [7].

East and Southern Africa were the most affected regions by the HIV epidemic, and the majority of the people lived with the disease between 2012-2017. Uganda, Kenya, and Mozambique were jointly ranked fourth-largest epidemics worldwide in 2017 [8]. It was estimated that 19.6 million people lived with HIV and about 6.8% prevalence among adults between 15-49 years. Approximately 800,000 new cases of HIV with 380,000 related mortality were reported in the region in 2017 and only about 66% of HIV infected adults were on HAART in the same year [8,9].

In Uganda, the rising number of new cases of AIDs-defining malignancies and non-AIDs defining malignancies were attributed to the HIV epidemic as a result of increased mortality and incidence of HIV infection. In 2017, it was estimated that 1.3 million people lived with HIV, and about 50,000 new infections occurred, contributing to 26,000 AIDs-related deaths. Women were disproportionately more affected by HIV than men with rates of 8.8% and 4.3%, respectively. It was noted that adults between 15-49 years had an HIV infection rate of 5.9%, and only 73 % were on HAART due to stigmatization and discrimination. Despite efforts to improve treatment initiatives, many people living with HIV are not on treatment [8,10].

In Kenya, however, 1.5 million people lived with HIV in 2017, 75% of adults were on HAART. Notably, there were 53,000 new infections with 28,000 AIDs-related deaths and a prevalence

rate of 4.8% among adults between 15-49 years in 2017, although the access to HAART has significantly reduced the mortality [7,9,11].

Therefore, the lowered incident rate of opportunistic infection and reduced mortality among HIV individuals are a consequence of easy accessibility to HAART [6-8]. Persons with HIV/AIDs now live longer while on highly active antiretroviral therapy, but cancer is still essentially a leading cause of morbidity and mortality [10,12,13]. Women living with HIV while not on HAART have an increased likelihood of developing precursor cervical lesions than those on HAART [14]. Long term positive benefits of HAART have been reported among seropositive women, resulting in a significant (67%) reduction in the rapid progression of precursor cervical lesions [15,16]. There is a need to expand the screening of women living with HIV in low-income countries to counter the rapid progress of precursor-cervical lesion.

The increased risk of HPV related malignancies has been significantly associated with HIV-immunosuppression [17]. Precursor lesions which pre-date invasive cervical cancer have frequently been found in over 77% of women living with HIV/AIDs and HPV positive [18]. Younger age and menopause may increase the likelihood of progression of cervical lesions [15]. While low-grade squamous lesions were the commonest lesion in West African women (6.3%), high-grade squamous lesions (17.8%) were found to be the most frequent among women between 19-62 years living with HIV/AIDs [19,20].

Oncogenic HPV subtypes 16,18,45 were noted to be prevalent among sexually active individuals and are the principal causative agent for invasive cervical cancer [14,21]. Over 37% of the HR-HPV was found to be more frequent among women living with HIV immunosuppression in a semi-urban population with HPV16 predominant in high-grade lesions [17]. It is known that HPV alone is insufficient for the development of cervical cancer as most women with the

infection never develop the disease. However, it is thought that other factors such as sexual practices that include multiple sexual partners, early age on sexual debut, and high parity increased the risk of HPV acquisition with persistent infection increasing the risk of cervical cancer [7,16].

Therefore, expanded easily accessible screening for precursor-cervical lesions and high-risk HPV genotypes among HIV infected women may be crucial for a progressive reduction in cervical cancer-related death rates as seen in high-income countries [22].

LITERATURE REVIEW

2.1 Cervical Cancer

Approximately 18.1 million new cases of all cancer occurred annually, with 9.6 million deaths globally. Among females, About 8.6 million new cases occurred among females with approximately 4.2 million deaths worldwide in 2018. An estimated 84.2% of total new cancer cases were reported in developing countries and an estimated total of 50% cervical cancer-related deaths. There was an estimated higher proportion of cancer-related deaths in developing countries compared to developed countries with an incidence of 7.3% and 5.8%, respectively. In sub-Saharan Africa, cervical cancer was the second most commonly diagnosed cancer after breast among women, significantly contributing to cancer-related deaths [1,3,4].

The increased burden of cancer of the uterine cervix has been contributed by a high number of cases of HIV/AIDs. About 60% of HIV infected women were at increased risk of having rapid progression to invasive cervical cancer in developing countries [9,23]. It has been estimated that 19.6 million people living with HIV and 6.8% were adults between 15-49 years. Approximately 800,000 new infections occurred in East and Southern Africa, with 380,000 AIDs-related deaths in 2017. Worldwide, this region had the majority of people living with HIV. As the number continues to rise, improved access to community-based HIV testing services increased awareness about own HIV status. In Kenya, it was estimated that about 53,000 new infections were among adults between 15-49 years, with 28,000 AIDs-related deaths [8,10].

Young women, adolescent girls, and female sex workers were disproportionately affected by HIV infection in Uganda and with a high risk of progression of precursor cervical lesions, with about 5.9% between 15-49 years. About 8.8% of adult women live with HIV compared to 4.3%

of men. About 72% of all people were on treatment in 2017, and 56% were virally suppressed, this was attributed to increased access to treatment. Although women with HIV while on HAART can now live longer, they have significantly reduced the incidence of HPV16/18 infection compared to those not on treatment. Chronic immunosuppression may predispose them to continuous HR-HPV infection, persistence, and progression raising the risk of developing invasive cervical cancer [8,9,11,15,24].

Cervical cancer has been associated with the sexually transmitted oncogenic strains of HPV. There is an increased risk of developing cervical lesions with persistent HR-HPV infection [9,12]. A survey of 300 women in Kenya on the association of cancer with infectious agents, invasive cervical cancer was the commonest among females reported at approximately 20.7% followed by breast cancers at 19.7% [25].

Comparing the pattern of low-risk and high-HPV sub-types among seropositive women in South Africa, approximately 70.5% were high-risk HPV, while about 29.5% were low risk [26]. In Uganda, there was an increased prevalence of squamous-type cervical cancer and adenocarcinoma with a single train infection by HR-HPV [27]. A study showed that abnormal cervical cytology results were more frequent among HIV immunosuppressed women [28].

2.2 Epidemiology with HIV

Among women living with HIV in developing countries, cancer of the uterine cervix was still a significant burden to public health as a relatively large number of women develop aggressive precursor lesions and, eventually, the invasive disease [29]. Chronic immunosuppression is known to enhance the risk of developing HPV associated precursor lesions among HIV positive women [30]. Early HAART initiation was identified to significantly lower the risk of progression

of aggressive lesions to invasive cancer of the uterine cervix due to more rapid HR-HPV type clearance and regression of the precursor lesions [15,16,23,31].

A study suggested that HIV-positive women with a competent immunological status on HAART had a similar incidence of cervical lesions to those HIV-positive but not enrolled on HAART [32]. However, various studies have supported the relationship between cervical lesions and HIV infection [33,34]. In South Africa, there was a significantly large proportion of cervical lesions and invasive cervical cancer among women living with HIV than HIV-negative [35]. There was a marked association between HR-HPV infection with incident HIV infection acquisition; there was moderate LR-HPV infection among HIV-positive women suggesting a vital role in the initiation and progression of precursor lesions into invasive cervical cancer [36]. In Tanzania, the proportion of cervical lesions and invasive cervical cancer was 6.1% and 7.3%, respectively, among HIV-positive women, which were significantly high [37]. In another study, many precursor lesions and invasive cervical cancer were noted to be 38.3% to 5.8%, respectively, among HIV-positive women. High-grade squamous lesions were the most prevalent among HIV seropositive women [33].

There were significant reductions of cervical lesions among HIV seropositive women on combined ART in Kenya. In Uganda, HIV infection and low CD4 counts were proposed to be responsible for the progression of cervical lesions to invasive cervical cancer among HIV seropositive women. With a negative cytology outcome and known HR-HPV positive, a study showed an increased risk of developing precursor lesions among HIV- positive women [10,32,38].

There was a higher risk of developing aggressive cervical lesions among HIV-positive women with low-grade cervical lesions than the HIV-negative [39]. In another study of all women who had CIN III, over 77% had HR-HPV infection predominated by HPV16 [17].

The prevalence of precursor lesions was reduced among HIV immunosuppressed women on HAART, despite the persistence of HPV infection [40]. Long HAART duration and high CD4-count positively influenced the reduction of the prevalence of HR-HPV, which was essential in the progression of cervical lesions into invasive cervical carcinogenesis without complete regression of lesions to normal [17,18,22]. A study showed 92.9% of HR-HPV among women with high-grade squamous lesions, HPV subtype 16 was predominant [41].

2.3 Squamous Lesions

2.3.1 Typical Squamous Cells

Atypia affects both the mature and immature squamous and metaplastic cells and presents less than 5% of pap smears. It includes changes suggestive of squamous intraepithelial lesions. However, they are qualitatively or quantitatively insufficient for a definitive interpretation as low or high grade squamous intraepithelial lesions.

It is categorized as atypical squamous cells of undermined significance and atypical squamous cells, cannot exclude high-grade lesions. The diagnosis is made on suspicion of squamous intraepithelial lesions.

2.3.1.1 Atypical Squamous Cells of Undetermined Significance

It involves atypical superficial and intermediates cells. It represents about 90% of atypical squamous cells. These cells are more abnormal than the reactive cells but insufficient for a

definitive diagnosis of the low-grade squamous intraepithelial lesion [36,42]. It was shown to be among the most frequent precursor lesions among HIV- positive women on HAART [28,43].

2.3.1.2 Atypical Squamous Cells, cannot exclude high-grade lesion

It involves atypical metaplastic or parabasal cells (immature cells) and represents between 5-10% of ASC lesions. Cells are cytomorphological, more abnormal than reactive cells. However, features are insufficient for a definitive diagnosis of a high-grade squamous lesion [43,44].

Management

According to the American Society of Colposcopy and Cervical Pathology (ASCCP), women over 30 years with ASCUS should have HR-HPV DNA testing due to mixed HPV infection with both low and high-risk types. A Follow-up Pap test should be done every six months, followed by colposcopy in case of persistent infection [43].

For the management of ASC-H, colposcopy is recommended due to the high positive predictive value for CIN2/3 on histology. If HR-HPV DNA is positive, then immediate colposcopy is recommended [43].

2.3.1.3 Low-Grade Squamous Intraepithelial Lesion

This lesion exfoliates mature squamous cells, superficial and intermediate cell types. They are in sheets or as singly dispersed cells. It includes flat condylomata, a plaque of ectocervix identified with koilocytosis. About 60% of LSIL will regress due to LR-HPV sub-types. Approximately 10% are due to HR-HPV sub-types and are likely to subsequently progress to the invasive disease through high-grade lesions if untreated meanwhile 30% may persist [43].

LSIL comprises mild dysplasia, CIN I, or HPV cytopathic effects on the superficial layer of the squamous epithelium. Koilocytes are characterized by a perinuclear clearing of three times the nuclear size, peripheral condensed cytoplasm with or without keratinization, and nuclear atypia [44]. A study showed 6.3% making it the commonest precursor lesion among HIV-positive women [18].

Management

According to ASCCP, as HPV-DNA testing does not help in the triage study of about 85% of LSIL with detectable HR-HPV, colposcopy is recommended as initial management and deferred six weeks postpartum for pregnant women [45].

2.3.1.4 High-Grade Squamous Intraepithelial Lesion

This lesion exfoliates metaplastic and parabasal squamous cells. It consists of moderate, severe dysplasia, CIN II, and CIN III cells in sheets or as singly dispersed. About 0.5% of all pap smears are HSIL, and 97% are positive for high-risk HPV genotypes resulting in about 14% progression rate to invasive cancer. Cellular and nuclear pleomorphism, chromatin clumping, and hyperchromasia are features of the high-grade squamous lesions; irregular nuclear outline and nuclear enlargement are other criteria [44,45].

Management

ASCCP recommends mandatory colposcopy and biopsy. Deferred six weeks' postpartum colposcopy should be considered for pregnant women [36,42].

2.3.1.5 Squamous Cell Carcinoma

The majority of 80% of all malignant cervical lesions are invasive squamous cell carcinomas and is the most frequently diagnosed type in women. About 14% of high-grade lesions progress to invasive cervical cancer. The peak incidence of the oncogenic HR-HPV infection among older women was between 50-60 years, and this implies that the more they age, the higher the likelihood of having persistent HPV infection.

Post-coital bleeding, as well as sudden abnormal bleeds, are the principal clinical manifestation of invasive cervical cancer. The predominant cells have an orangeophilic cytoplasm in small aggregates or clusters, prominent nucleoli, hyperchromasia, pleomorphism, and tumor diathesis in the background are present. Invasive cervical cancer has been found to have glandular involvement and may coexist with adenocarcinoma in-situ [36,42].

Management

According to ASCCP, colposcopy and biopsy have been recommended for invasive cervical cancer. However, total hysterectomy has been the recommended treatment of choice [36,42].

2.4 Glandular Lesions

2.4.1 Atypical Glandular Cells

The changes are more than a reactive process but are cytomorphologically insufficient for the interpretation of invasive adenocarcinoma.

2.4.2 Adenocarcinoma In-Situ

May coexist with high-grade precursor lesions or severe dysplasia. It is considered to be a glandular counterpart of HSIL. It forms the non-invasive endocervical epithelial. The cells are in sheets or loose clusters with feathering, pseudo-stratification, abnormal mitotic figures, hyperchromasia, increased nuclear to cytoplasmic ratio, and abnormal chromatin distribution [45].

2.4.3 Invasive Adenocarcinoma

It has been frequently reported among older women of over 50 years and the second common cancer of the uterine cervix after squamous cell carcinoma. Adenocarcinoma contributes to about 25% of all cancers of uterine cervix. The abnormal cells spontaneously exfoliate in loose groups [45]

About 37% of high-risk HPV-18 is predominantly associated with cervical adenocarcinoma than HPV-16. Endocervical malignancy, mucinous-type, is the most frequent with about 70-90% of the cases than endometrial adenocarcinoma. It may be well-differentiated, poorly differentiated, or undifferentiated adenocarcinoma. Cytomorphologically, cells are in sheets or loose clusters with pleomorphism, pseudo-stratification, abnormal mitotic figures, hyperchromasia, increased nuclear to cytoplasmic ratio, abnormal chromatin distribution [46].

Management

ASCCP recommends colposcopy with endocervical or endometrial sampling, followed by total hysterectomy as a choice of treatment.

2.5 Human Papilloma Virus

Oncogenic HPV is the principal causative agent of invasive cervical cancer in the majority of women. Most infections suddenly clear without clinical presentations, as small proportions will persist and progress to precursor lesions and invasive disease. The HPV infection may take weeks, months to several years, while the rate of infection clearance lies between 1-3 years. The immune response will play a pivotal role in ensuring a small proportion of the viral infection persists and progresses to the invasive stage of the disease [7,29,30].

In sub-Saharan Africa, there was a significantly high incidence rate of cervical cancer with an age-standardized rate of 31.0 per every 100,000 women. Oncogenic HPV subtypes 16/18, which are vaccine-preventable, contribute to the majority of invasive cervical cancer [47]. A study in Italy showed a significantly high association of HPV infection with invasive cervical lesions of over 70% compared to low-grade lesions and normal cervix. HR-HPV subtypes 16/18 were predominantly common in high grade and invasive lesions [32,38].

In Zambia, HPV 16/18 strains were predominant at about 21%, and this was two times higher than in the USA. HPV-18 was nine folds in HIV infected women, which implies that those living with HIV have an increased likelihood of HR-HPV infection and, therefore, high cervical cancer to dysplasia among this group [25,35].

Persistent HPV infection has consistently been associated with an early sexual debut, multiple sexual partners, and alcohol consumption; however, there was no association with smoking [38]. However, a study among young students showed a high rate of contraction of both high-risk and low-risk HPV infection with subsequent high clearance rate; smokers showed a high acquisition rate than nonsmokers suggesting an association [48]. A significantly high ratio of invasive

cervical cancer to dysplasia has been commonly associated with more aggressive HR-HPV 16 than 18 subtypes. However, LR-HPV 6,11 subtypes were related to genital warts instead of precursor high grade and invasive cervical lesions [36,42,46].

In West Africa (Mali and Senegal), two-thirds of HPV were sub-types HPV-16/18/45. A follow-up study on female sex workers in South Africa showed a significant number of anogenital HR-HPV among HIV-positive women. This study suggested that HIV may be responsible for the occurrence of invasive cervical cancer. There was a strong correlation between HIV prevalence and high-risk HPV-18 in invasive disease in sub-Saharan Africa [7,14,35,37,39,49].

2.5.1 Classification of HPV

Papillomavirus belongs to the Papillomaviridae family of non-enveloped DNA viruses comprising a circular double-stranded DNA molecule [50]. Two (2) main categories have been identified to play a role in various squamous lesions. Mucosal low-risk subtypes 6 and 11 are commonly associated with anal-genital benign lesions and cervical condylomata [51]. Studies have shown that over ten various high-risk types have been associated with over 50% of high grade squamous intraepithelial lesions and over 90% of invasive cervical cancer. These include 16,18,45,31,33,35,39,51,56,58,59,66,68,73 and 82. HR-HPV types 16/18 are the oncogenic types accountable for about 50% and 20% invasive cancer, respectively. HPV18 predominates in invasive adenocarcinoma and less frequently invasive squamous lesions [16,44,46,48,52].

2.5.2 Carcinogenesis

2.5.2.1 Role of Human Papilloma Virus

During reproductive life, the majority of sexually active individuals will contract one or several HPV subtypes. The mode of acquisition of HPV is through mucosa-mucosa, skin contact during sex. Injury to the basal cells of the squamous epithelia exposes the cells to the HPV virion through abrasions [49].

The Early region of the genome expresses regulatory proteins E6 and E7. They are oncogenes that inactivate tumor suppressor genes P53 and Retinoblastoma, respectively. They are responsible for the propagation of the carcinogenesis of HR-HPV sub-types. E6 oncogene interferes with associated protein to form E3 ubiquitin complex, which degrades P53 meanwhile, E7 oncogene degrades Retinoblastoma by modulating ubiquitin ligase activity.

These will interrupt regulatory protein activities resulting in suppression of apoptosis and cell cycle regulation, disrupted cell adhesion, epithelial differentiation, and reduced immune recognition resulting in uncontrolled and unregulated abnormal cell growth [44,45,48].

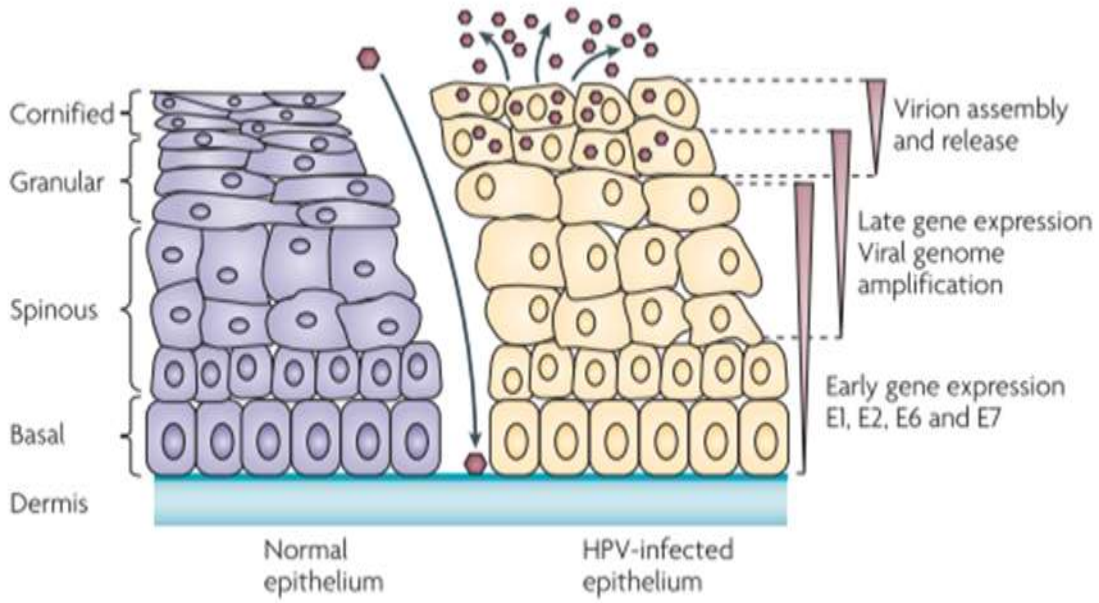


Figure 0.1: Pathways to the transformation of HPV (Moody et al. 2010)

2.5.3 Persistence and Clearance of Infection

The risk of developing HPV associated abnormal cervical lesions dramatically depends on the rate of persistent HR-HPV infections as the major risk factor. A study showed that infection might last about two years, followed by subsequent clearance, out of which about 10% will persist within ten years and progress to invasive cervical cancer [53].

The rate of regression and persistence of HPV induced squamous lesions are about 30% to 60% in low-grade lesions and 33% to 56% for high-grade lesions, respectively, implying that there was a high probability of progression of aggressive precursor lesions to invasive cervical cancer [54].

However, a meta-analysis study by Melnikow et al. has shown a slight improvement to 35% regression. High-risk HPV infection alone has been crucial but insufficient for the growth of invasive cervical cancer. However, co-factors like cell gene mutations, genomic instabilities,

induced chromosome rearrangements as well as old age, multiple HPV types infection, multiple sexual partners, and early sexual debut may play a role in the progression [55].

HIV induced immunosuppression has an increased risk of chronic HPV infection leading to genetic damage resulting in cytological abnormalities, therefore resulting in a subsequent progression of cervical dysplasia [31,45,48].

2.5.4 Progression of Precursor Lesions

Persistent oncogenic HPV sub-types infection substantially possesses a higher likelihood of progression of the precursor lesions to invasive cervical cancer among women. HPV-16/18 was predominantly common in aggressive cervical precursor lesions [56].

HPV-18 was known to be the second commonest oncogene sub-type responsible for the majority of adenocarcinoma and a few squamous cell carcinomas worldwide. However, a highly significant prevalence in more aggressive squamous lesions and invasive cancer were associated with the most common HPV-16 genotype with an absolute risk of about 40% within five years of persistent infection [34,42].

Studies have shown an increased prevalence rate and persistent HPV infection among women living with HIV. The use of HAART has extended the shelf of life as people with HIV can now stay longer while on treatment. The risk of multiple and persistent HPV infection was high as the lack of sufficient data exists about the natural history of the virus; this suggests that persistent HPV infection may result in rapid progression to invasive cervical cancer.

Therefore, inadequate systematic cytology screening and HPV DNA testing have resulted in the progression to invasive cervical lesions among women living with HIV [4,15,57].

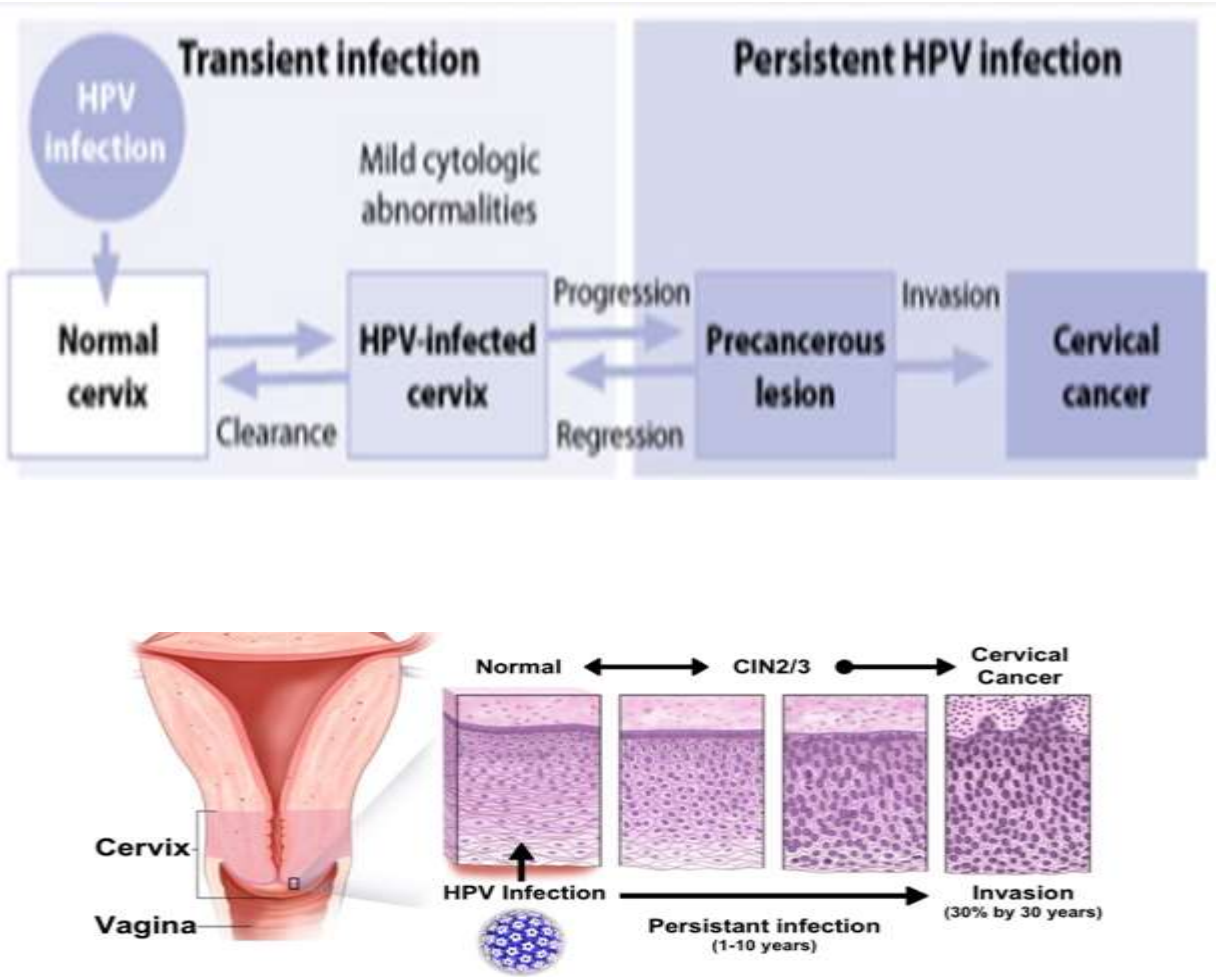


Figure 0.2: Adopted from Wright, Schiffman et al. 2003 on HPV DNA to cervical cancer screening; New England Journal of Medicine

2.5.5 Effects of Combined Antiretroviral Therapy on Squamous Intraepithelial Lesion

As women with HIV/AIDs on combined antiretroviral therapy can now live longer, the incidence of cervical cancer is still on the rise among this group of people. Studies have reported a significant reduction in cervical cancer among women living with HIV while on HAART. Still, a

few available data exist to explain the effects of HAART on the development of cervical lesions [51,58].

HIV-immunosuppressed women have an increased risk of developing cervical lesions than immunocompetent partly due to low CD4 count. As the prolonged use of HAART has significantly been associated with elevated CD4 count, there was a significant reduction of HR-HPV genotypes among HIV-immunosuppressed women on HAART compared to those not on treatment [56].

In Nigeria, a study showed that low-grade lesions were more frequent in low CD4 count than high CD4 counts. Another study showed that older women with low CD4 count have a 70% likelihood of progression of precursor lesions to more aggressive lesions than younger women [59]. Although increased CD4 counts have been associated with a remarkable reduction in the risk of development of precursor lesions, there was no significant relationship between CD4 count, viral load, and precursor squamous lesions among women living with HIV in Uganda [50,60].

2.6 Problem Statement

As HIV is a worldwide epidemic causing high morbidity and mortality among adults, the highest percentage of HIV infection is through sexual transmission. In Uganda, the total Population affected was increasing by the day to 1.3 million currently [9]. The introduction of HAART has prolonged life expectancy of HIV-infected individuals increase in the number of individuals living with HIV [24].

The most typical route of HIV transmission is similar to HPV transmission [7,12]. HIV immunosuppression and HPV infection increase the likelihood of developing cervical pre-

malignant lesions, which may progress to invasive cancer in-case of delayed diagnosis and treatment [60,61].

As the proportion of women infected with HIV continues to rise in Uganda, there was need to establish the prevalence and distribution of cervical lesions and to determine the association between immune-suppression among HIV-positive women on HAART and the prevalence of cervical lesions [62].

2.7 Justification for the study

Invasive cervical cancer is the commonest and the leading cause of cancer-related deaths among women of reproductive age in Uganda [6].

In Uganda, approximately 73% of HIV infected adults are on HAART. Over 50,000 new cases are added annually. Most of the affected Population is between 15-49 years, predominantly women. Most HIV-positive women on HAART are living longer, enabling the oncogenic effects of chronic HPV infection to increase the risk of malignancies [24]. An estimated 40% of HIV infected persons will get malignancy in their lifetime [55,60].

HIV positive women in Uganda are inadequately screened and, therefore, at high risk of cervical cancer. It was noted that expanded routine screening detects early pre-cervical lesions; however, suboptimal screening rates among HIV-positive women have increased the likelihood of precursor lesions to progress to invasive cervical cancer [63,64].

2.7 Research Questions

1. What is the prevalence of pre-malignant cervical lesions among HIV-positive women on HAART attending HCC at St Francis Hospital, Nsambya, in Uganda?
2. What is the distribution of pre-malignant cervical lesions among HIV-positive women on HAART?

2.8 Research Objectives

2.8.1 General Objective

To determine the prevalence and distribution of pre-malignant cervical lesions on Pap smear among HIV-positive women on HAART attending HCC at St Francis Hospital, Nsambya, in Uganda.

2.8.2 Specific Objectives

1. To determine the prevalence of pre-malignant cervical lesions on Pap smear among HIV-positive women on HAART attending HCC.
2. To determine the distribution of pre-malignant cervical lesions among HIV-positive women on HAART.
3. To correlate the duration on HAART and viral loads with pre-malignant cervical lesion grades among HIV-positive women.

METHODOLOGY

3.1 Study Design

A descriptive cross-sectional prospective study was carried out among HIV-positive women attending HCC of St. Francis Hospital Nsambya in Uganda.

3.2 Study Site

The study was carried out at Home Care Clinic (HCC) of St. Francis Hospital Nsambya in Uganda, where all persons living with HIV go for treatment, follow-up, and management of other opportunistic infections. The clinic is located on Nsambya Hill in Makindye Division approximately 5 kilometers southeast of the central business district of Kampala, and it operates from 08:00 am to 05:00 pm serving about 100 women living with HIV daily from Monday to Friday. Over 14,600 patients and their families are served annually at HCC, out of which 6,931 patients are on HAART, and 4,000 are females.

3.3 Study Population

The study participants were HIV-positive women between 18-69 years who were attending daily routine cART clinic at Home Care Clinic and on any regimen of treatment between December 2019 to February 2020.

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

HIV-positive women between 18-69 years on HAART for three years or more.

HIV-positive women who were six weeks-postpartum on HAART for three years or more.

Eligible HIV-positive women who consented.

3.4.2 Exclusion Criteria

All HIV-positive women who were pregnant on HAART on disclosure or based on the last menstrual period

HIV-positive women who were on HAART with a history of total hysterectomy.

All HIV- positive women who were on HAART and previously treated for CIN or on treatment

HIV-positive women who were on HAART and confirmed to have had invasive cervical cancer

Those who declined to participate were excluded to take part in the study

3.5 Sample Size

The sample size was calculated using Fisher's Formulae. The prevalence (15.7%) of women living with HIV on HAART in Kenya at Nazareth Hospital between June 2009 and September 2010 (17, 77), was used to obtain the sample size.

$$n = z^2 * \frac{p(1-p)}{e^2}$$

n= (1.96x1.96) x0.157 (1-0.157)/ 0.05x0.05

n= 210

Where:

n- Sample size

p- Prevalence

z- Normal standard deviation with 95% confidence interval

e -Margin error.

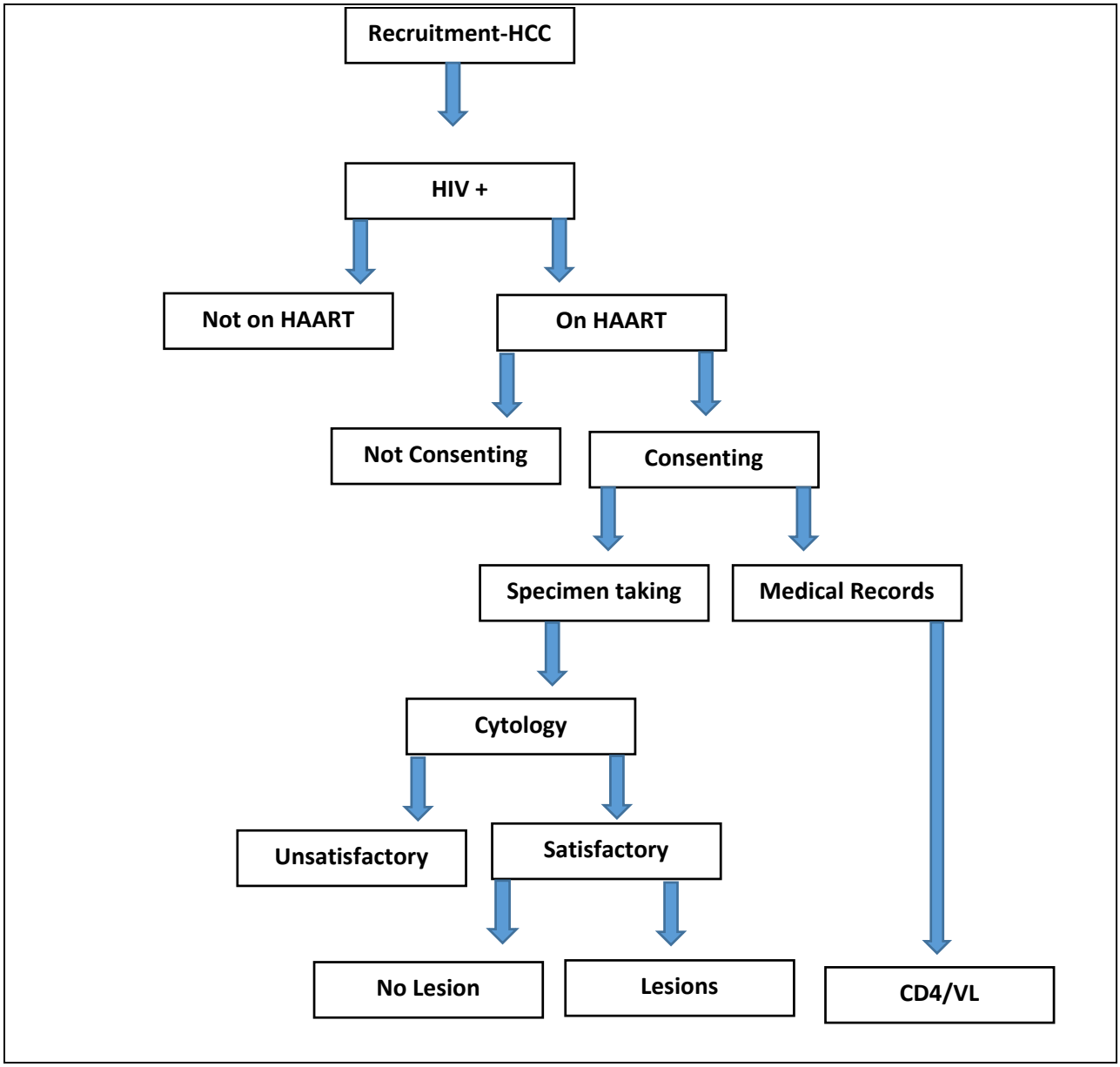


Figure 0.1: A flow chart below illustrates the steps of specimen processing and reporting

3.6 Recruitment and Consenting

The potential study participants were identified through daily activity clinic registers and randomly sampled by the research assistant who was an enrolled nurse.

The purpose, procedure, benefits, and risks of the study were explained to the participants, those who consented were taken aside for further explanation about the research and recruitment (Appendix I). The consented participants filled the questionnaire, which was also translated into the local language (Luganda), then they were sampled. Medical records access code was granted to the investigator, and CD4 count/Viral load results were abstracted as illustrated by the flow chart above.

3.7 Study Instrument

A pre-coded questionnaire for the interviews was issued by the research assistant (Appendix II). The patient's medical records were checked for viral load and CD4 count results.

3.8 Data Collection

3.8.1 Research Assistants

An enrolled nurse was trained and recruited as a research assistant. The research assistant obtained informed consent from the identified potential participants strictly following the exclusion and inclusion criteria. The participants were then recruited into the study; the test procedures were explained and then filled the questionnaire.

3.8.2 Training of Research Assistants

The training was carried out by the principal investigator before data collection to ensure the required standard. The areas included knowledge of clinical research terminologies, research site management, research protocols, professional ethics, data, and specimen collection.

3.8.3 Specimen Collection and Processing

The sample collection was carried out in a suitable gynecological consultation room at HCC. Clean frosted-end glass slides and patient forms were assigned and labeled with a unique laboratory identification number (e.g., N001/19). A trained research assistant collected the samples and prepared the smears following the standard protocol (Appendix III). The principle investigator assessed the slides for any pre-analytical errors.

3.8.4 Staining Procedure

The slides were stained using the Papanicolaou staining procedure in the Cytopathology laboratory by the investigator.

Hematoxylin stained the nucleus due since it's a basic stain, while Orange G-6 stained the cytoplasm of the mature keratinized squamous cells. In contrast, Eosin-Azure stained the cytoplasm of the immature squamous cells. The standard operating procedure was followed, as shown in (Appendix III).

3.8.5 Screening and Interpretation of Smears

The stained slides were screened by the investigator and then reported with the Pathologists (supervisors) following The Bethesda System 2014 for reporting cervical cytology (Appendix

IV). A review of abnormal Pap smear was done by the second and third Pathologists who were the tiebreakers. All the results were filed into the participant's file, and those with the abnormal results were informed. The patient referred to a gynecologist for further management as per protocol.

3.9 Quality Assurance

The study was conducted by trained and competent personnel in specimen collection, processing, and analysis of results at St. Francis Hospital Nsambya. The standard operating procedures for specimen collection, processing, and reporting Pap smears were followed. Daily quality control of the reagents was done to ensure good quality smears. Manufacturers' instruction for commercially purchased stains were followed to ensure certified standards. Smear processing, examination, and reporting were carried out by the principal investigator and the Pathologist before a tiebreaker.

3.10 Ethical Consideration

Ethical approval was obtained from Kenyatta National Hospital and the University of Nairobi ethical review committee to authorize the study. Approval was also obtained from the ethical review board of St. Francis Hospital Nsambya and permission from the Department of HCC. A non-institutional ethical approval was also obtained from the Uganda National council of science, technology, and innovation. The participants filled their informed consent to participate in the study issued by the research assistant. There were maximum confidentiality and voluntary participation. No names of participants were used in any way; only study identification numbers were assigned. The naive and anxious participants were counseled about procedure and outcome by the research assistants. Patient results were submitted to their files, and their physicians

informed those with abnormal results for further management. Questionnaires in hardcover registers were kept in a lockable cabinet accessible only to the researcher while the soft copy access was only authorized through a password. Slides were archived in a lockable cabinet where it will stay for up to 5 years before disposal or during which time any other study may be done on after ethical approval.

The researcher and the clinician contacted participants with abnormal results, were explained and counseled about her findings, and after that referred for further management and treatment at the Oncology Department of St. Francis Hospital Nsambya and at the Uganda Cancer Institute.

3.11 Participant's Benefits

This was a free screening service, and participants had to access their cervical cancer screening results at the end of the study.

3.12 Data Management and Statistical Analysis

A standard questionnaire and a unique identification number were used for data collection. Access was granted by the Department records for the CD4 counts and viral load. Microsoft excel data entry was carried out by the investigator, and then statistical analysis using R-Software ver. 3.6.2 (2019) was performed with the biostatistician. Logistic regression (Fisher's exact test) was used for the analysis of the data. The results were displayed in tables, charts, and photomicrographs.

3.13 Data Dissemination and Utilization

The data was presented to the Department of Human Pathology at the University of Nairobi and St. Francis Hospital Nsambya, Uganda. Publications in peer review journals, poster presentations in scientific conferences and seminars will also be considered in the future.

RESULTS

4.1 Socio-demographic characteristics

A total of 210 women aged between 18-69 years were screened for pre-malignant cervical lesions at Home Care Clinic of St. Francis Hospital Nsambya in Kampala between December 2019 and February 2020. All the women were HIV-positive and on HAART for at least three years. About 82(39%) were aged between 40-49 years, as shown in Figure 4.1. More than half of the women, 114(54%), had attained a minimum of secondary level education. Majority, 130(62%) were self-employed. About 18(9%) reportedly had unprotected sex with multiple sexual partners in the past six (6) months, with the median number of 4 sexual partners. Only 3 of the wome (1%) were cigarette smokers. The mean number of children was 3.06 (SD 2.4), and the mean age at first sexual intercourse was 18 years (SD 3.11). Family planning methods such as pills, injectable-plan, and implants were used by 47 of the women (22.4%).

Table 0.1: Social demographic characteristics

VARIABLE	**FREQUENCY (%)**
EDUCATION LEVEL	
NONE	16 (8%)
PRIMARY	80 (38%)
SECONDARY	85 (40%)
TERTIARY	29 (14%)
TYPE OF EMPLOYMENT	
EMPLOYED	43 (20%)
SELF-EMPLOYED	130 (62%)

UNEMPLOYED	37 (18%)
MULTIPLE SEXUAL PARTNERS	
NO	192 (91%)
YES	18 (9%)
SMOKING	
NO	207 (99%)
YES	3 (1%)
PARITY MEAN (SD)	3.06 (2.04)
SEXUAL DEBUT MEAN (SD)	18.0 (3.11)
NUMBER OF SEXUAL PARTNERS MEDIAN (IQR)	4 (3,5).
FAMILY PLANNING	
NO	163 (77.6%)
YES	47 (22.4%)

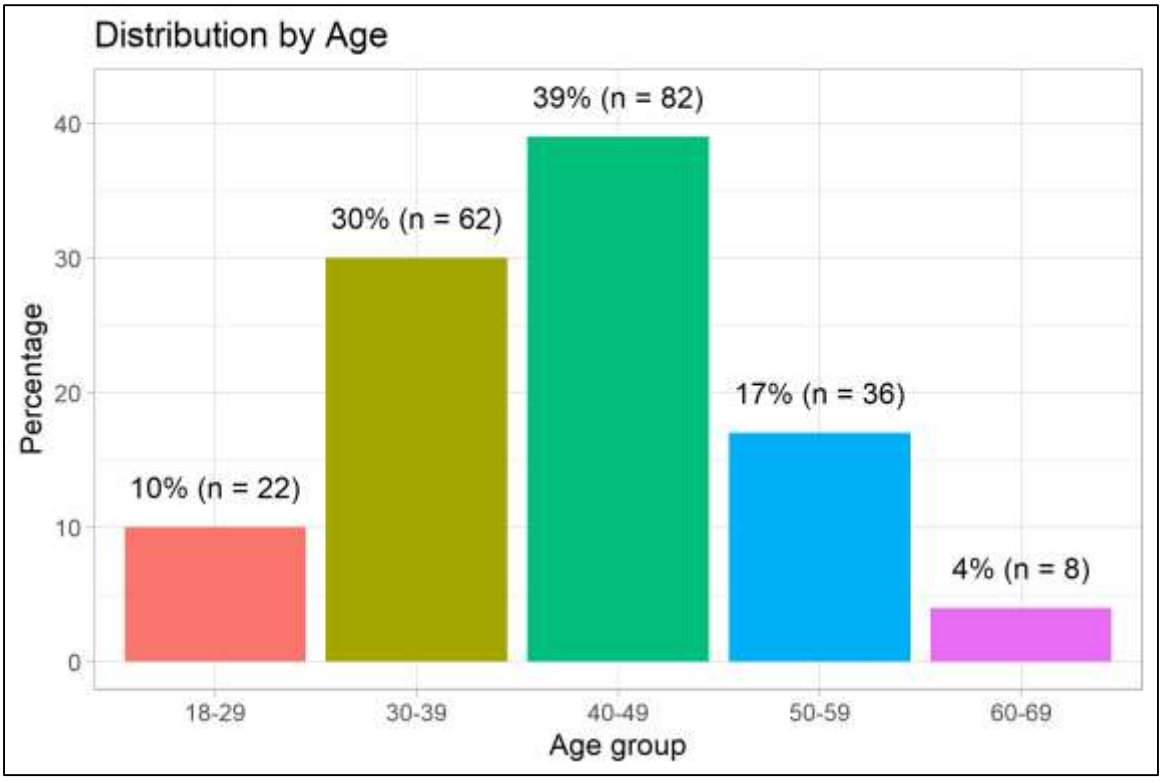


Figure 0.1: The distribution of the age at the screening of the study participants

4.2 Clinical Symptoms

Out of the 210 women, about 42(20%) had experienced lower abdominal pain. Only 49(23.3%) of the women had previously been screened for cervical cancer and were all negative. A small number, 11(5.2%), reported post-coital bleeding, while 14(6.7%) of the women had abnormal vaginal bleeding.

4.3 Pap smear results

The results in Table 4.2 indicate that for most women, 183(87.1%) transformation zone components (endocervical and squamous metaplastic cells) were sampled. On cytology,

198(94.3%) of the women had negative pap smear results (NILM), 5(2.4%) had ASC-US, while 4(1.9%) of the women had LSIL.

ASC-H, Adenocarcinoma, and SCC lesions each accounted for 1(0.5%) of the women, while the data did not show any prevalence of HSIL, AIS, AGC lesions. About 52(24.8%) of the women had Bacterial vaginosis, followed by candidiasis, which was prevalent in 17(8.1%) of the women.

Table 0.2: Pap smear results

VARIABLE	**FREQUENCY (PERCENTAGE)**
ENDOCERVICAL / SQUAMOUS METAPLASTIC CELLS	
NO	27 (12.9%)
YES	183 (87.1%)
PAP SMEAR RESULTS	
NILM	198 (94.3%)
ASCUS	5 (2.4%)
ASC-H	1 (0.5%)
LSIL	4 (1.9%)
HSIL	0 (0.0%)
SCC	1 (0.5%)
AGC	0 (0.0%)
AIS	0 (0.0%)
ADENOCARCINOMA	1 (0.5%)
VAGINAL INFECTION	

BACTERIAL VAGINOSIS	52 (24.8%)
BACTERIAL VAGINOSIS AND CANDIDA	1 (0.5%)
CANDIDIASIS	17 (8.1%)
NONE	136 (64.8%)
TRICHOMONAS VAGINALIS	4 (1.9%)

4.4 Clinical appearance of the cervix

The results in Figure 4.5 show that the cervix appeared normal in the majority of women, 167(80%), while 22(10%) had an inflamed cervix.

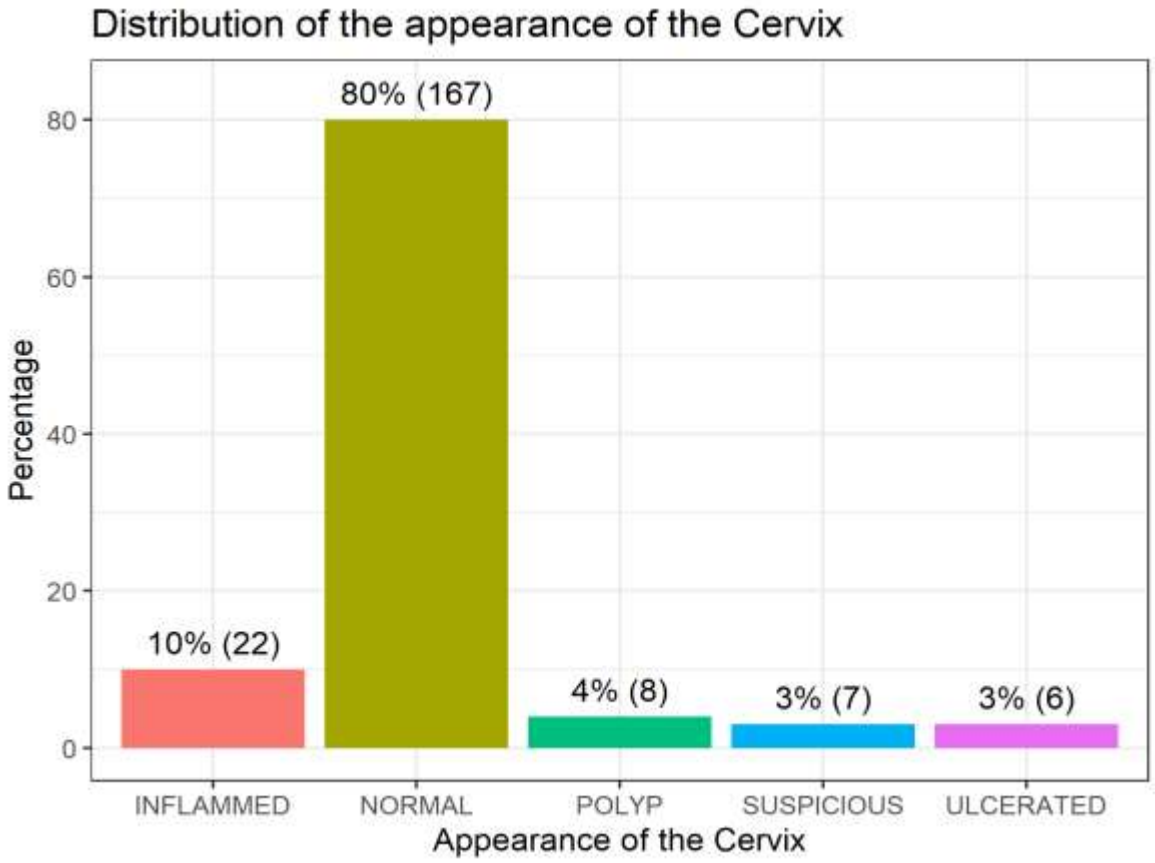


Figure 0.2: The clinical appearance of the cervix

4.5 Distribution of viral loads

The majority of the women, 208(99%), had low current viral load copies (<1000 copies/ml) during the study 208(99%). Only 2(1%) had detectable copies (>1000 copies/ml).

4.6 Distribution of baseline CD4 count

The results represented in Figure 4.3 indicate that more than half of the women, 112(53%), had a baseline CD4 counts result of < 250 cells/mm^3, followed by 70(33%) with CD4 counts between 250-500 cells/mm^3. Only 28(13%) of the women had CD4 counts > 500 cells/mm^3.

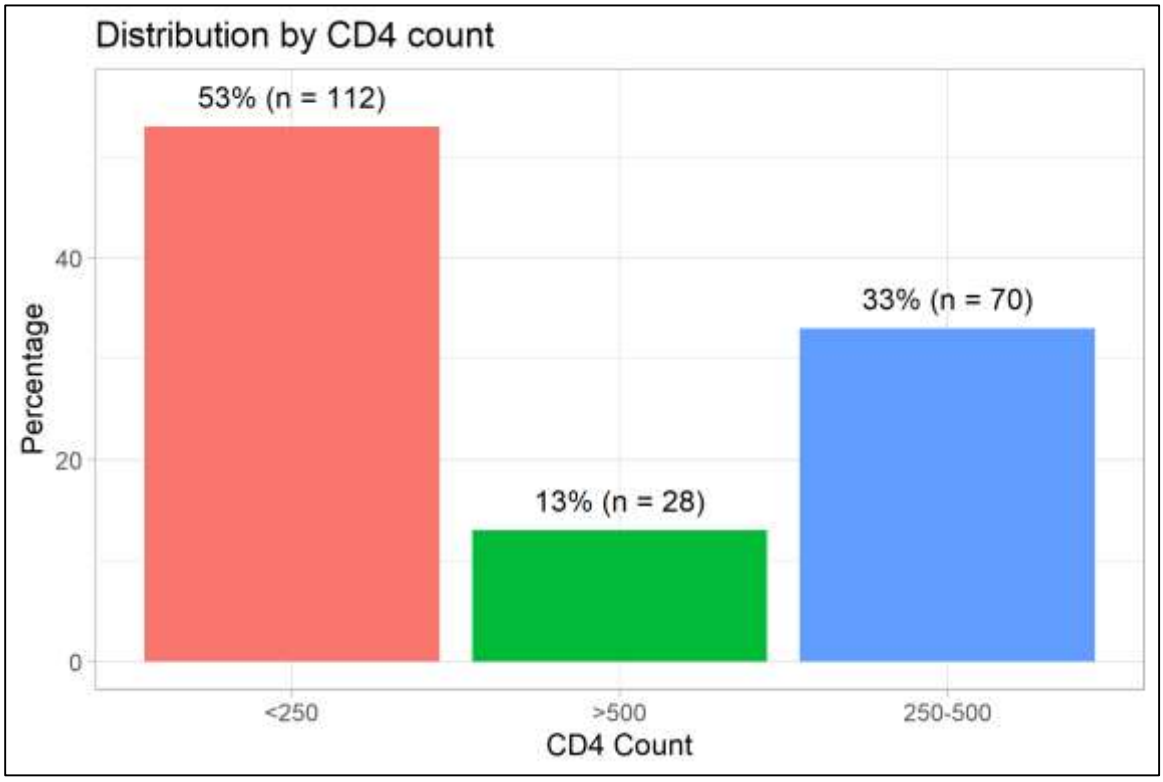


Figure 0.3: The distribution of a baseline CD4 counts

4.7 Duration on HAART

Majority of the women, 178(85%), had been on combined ART for more than five years, 17(8%) below four years, while 15(7%) of the participants had been on HAART for between 4-5 years at the time of the study (Fig 4.3)

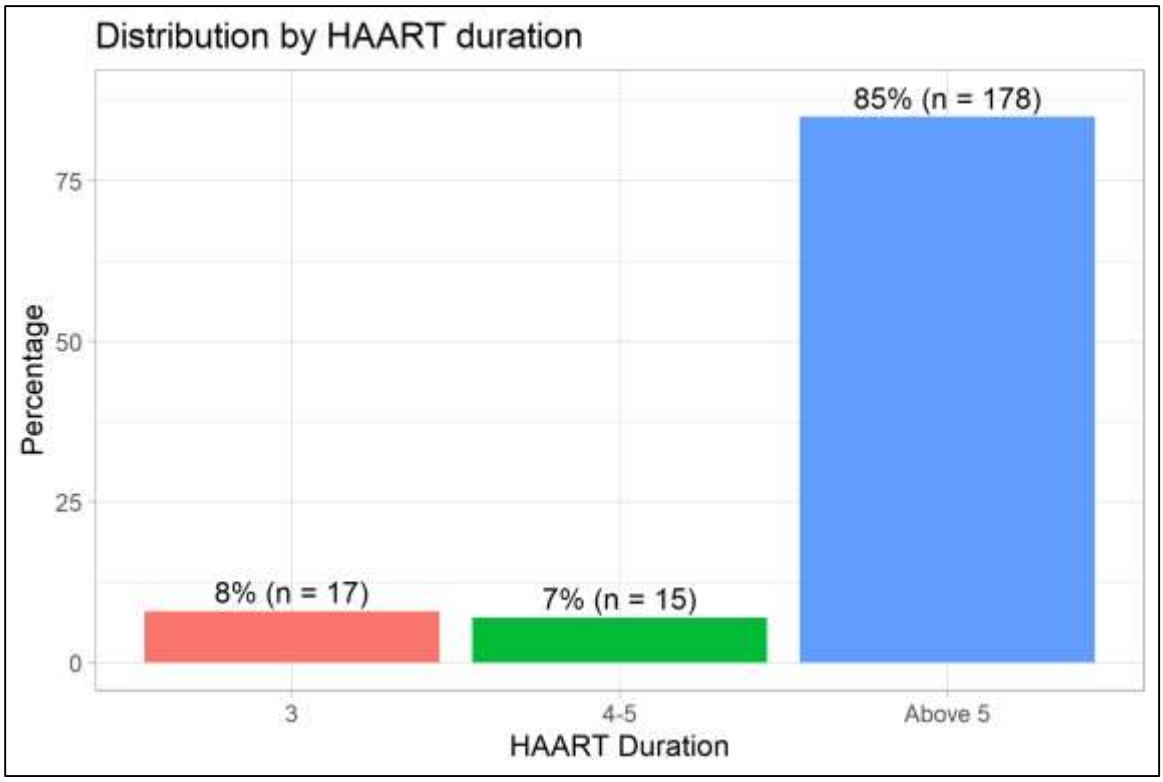


Figure 0.4: The variation of duration on HAART

4.8 Distribution of Pap smear results.

4.8.1 Pap smear results versus age distribution

The data in Table 4.3 indicates that the majority of participants who had ASC-US and LSIL were in the age group of 40 to 49 years. ASC-H was present primarily in the age group of 60 to 69 years. The participants who had squamous cell carcinoma and adenocarcinoma were in the age group of 30 to 39 years while, HSIL, AGC, and AIS were not reported among women in any of the participants. Of the women with a negative pap smear, 60(30.3%) were in the age group 30 to 39, and 75(37.9%) were in the age group 40 to 49 years.

Table 0.3: Pap smear results versus age distribution.

	AGE GROUP (YEARS)					
	18-29 n = 22	30-39 n = 62	40-49 n = 82	50-59 n = 36	60-69 n = 8	
PAP SMEAR RESULT	n (%)	n (%)	n (%)	n (%)	n (%)	TOTAL
NILM	21 (10.6)	60 (30.3)	75 (37.9)	35 (17.7)	7 (3.5)	198
ASCUS	1 (20)	0 (0)	3 (60)	1 (20)	0 (0)	5
ASC-H	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)	1
LSIL	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	4
SCC, ADENOCARCINOMA	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2

4.8.2 Pap smear results versus duration on HAART

The data in Table 4.4 indicate that all women 12(6.7%) who had a cervical lesion had been on combined ART for five years and above. The majority of these women had ASC-US (n=5), followed by LSIL (n=4). The rest of the women had ASC-H, SCC, and Adenocarcinoma lesions.

Table 0.4: Pap smear results versus duration on HAART

	HAART DURATION		
	3 Years n = 17	4-5 Years n = 15	Over 5 Years n = 178
PAP SMEAR RESULT	n (%)	n (%)	n (%)
NILM	17 (100)	15 (100)	166 (93.3)
ASCUS	0 (0)	0 (0)	5 (2.8)
ASC-H	0 (0)	0 (0)	1 (0.6)
LSIL	0 (0)	0 (0)	4 (2.2)
SCC	0 (0)	0 (0)	1 (0.6)
ADENOCARCINOMA	0 (0)	0 (0)	1 (0.6)

4.8.3 Prevalence of pre-malignant cervical lesions on Pap smear among HIV-positive women on HAART attending HCC

The prevalence of pre-malignant cervical lesions on Pap smear among HIV-positive women on HAART attending HCC was found to be 5.71 per 100.

4.8.4 Pap smear results versus baseline CD4 counts

As shown in Table 4.5, more than half 104(52.5%) of the women with negative results had CD4 counts of less than 250 cells/mm^3. The cervical lesions are distributed between women with a single baseline CD4 count of 500 cells/mm^3 and below. None of the women with a CD4 count above 500 cells/mm^3 had an abnormal pap smear. About two thirds, 8(66.7%) of the positive cervical lesions were seen in women with a CD4 count below 250 cells/mm^3. Less than a half, 33.3% of these positive lesions were seen in women with a CD4 count between 250 – 500 cells/mm^3.

Table 0.5: Pap smear results versus baseline CD4 counts

	BASELINE CD4 COUNTS (CELLS/MM^3)		
	<250 n = 112	250-500 n = 70	>500 n = 28
PAP SMEAR RESULT	n (%)	n (%)	n (%)
NILM	104 (92.9)	66 (94.3)	28 (100)
ASCUS	2 (1.)	3 (4.3)	0 (0)
ASC-H	1 (0.9)	0 (0)	0 (0)
LSIL	4 (3.6)	0 (0)	0 (0)
SCC	0 (0)	1 (1.4)	0 (0)
ADENOCARCINOMA	1 (0.9)	0 (0)	0 (0)

4.8.5 Pap smear results versus viral loads

The Pap smear results indicate that only women with viral load copies below 1000 had cervical lesions (Table 4.6)

Table 0.6: Pap smear results versus viral loads

	VIRAL LOAD COPIES	
	< 1000 Copies n = 208	1000+ Copies n = 2
PAP SMEAR RESULT	n (%)	n (%)
NILM	196 (94.2)	2 (100)
ASCUS	5 (2.4)	0 (0)
ASC-H	1 (0.5)	0 (0)
LSIL	4 (1.9)	0 (0)
SCC	1 (0.5)	0 (0)
ADENOCARCINOMA	1 (0.5)	0 (0)

4.9 Correlation of viral loads and duration on HAART with pap smear results among HIV-positive women

The data in table 4.9 indicate that the majority 198(94.3%) of the cervical lesions were negative for the pre-malignant cervical lesion.

All the women (n=12) with pre-malignant cervical lesions had viral loads of fewer than 1000 copies. There was no statistically significant relationship between pre-malignant cervical lesions grade and viral loads of the women (P-Value = 1.0000).

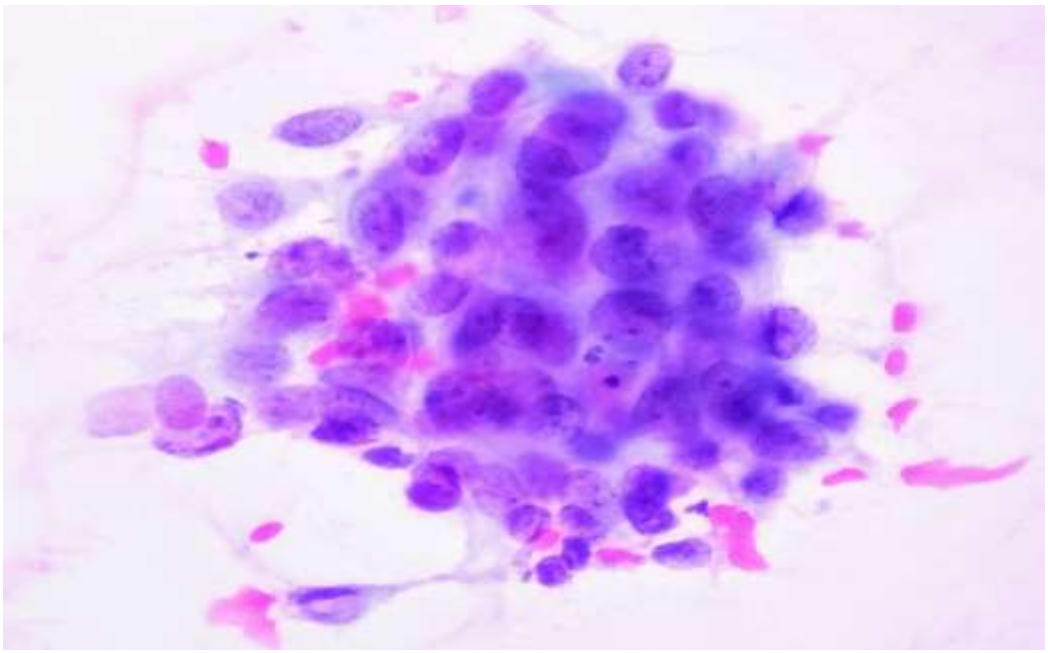
All the women (n=12) with cervical lesions had more than five years of duration on HAART; there was no statistically significant relationship between pre-malignant cervical lesion grade with the duration on HAART (P-Value = 0.5463).

Table 0.7: Fisher's Exact Test for correlation between pap smear results, viral loads & duration on HAART.

VARIABLES	**PAP SMEAR RESULT**		
	Negative n=198	Positive n=12	
	n (%)	n (%)	**P-value**
VIRAL LOADS			1.0000
< 1000 COPIES	196 (99%)	12 (100%)	
1000+ COPIES	2 (1%)	0 (0%)	
DURATION ON HAART			0.5463
3 YEARS	17 (8.6%)	0 (0%)	
4-5 YEARS	15 (7.6%)	0 (0%)	
ABOVE 5 YEARS	166 (83.8%)	12 (100%)	

4.10 List of the photomicrographs

A



B

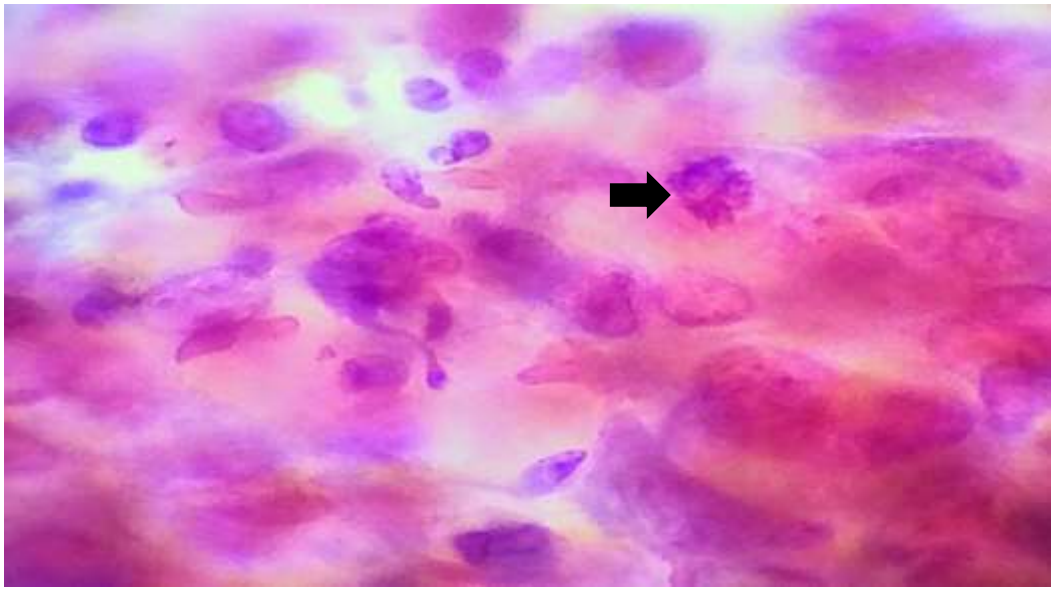


Image 1: Photomicrographs A&B above show Endocervical Adenocarcinoma, X40

The above photomicrographs show a crowded dyshesive group of endocervical cells with loss of honey-comb or palisading architecture, moderate nuclear atypia is seen. Abnormal mitotic figure in photomicrograph **B (arrow).**

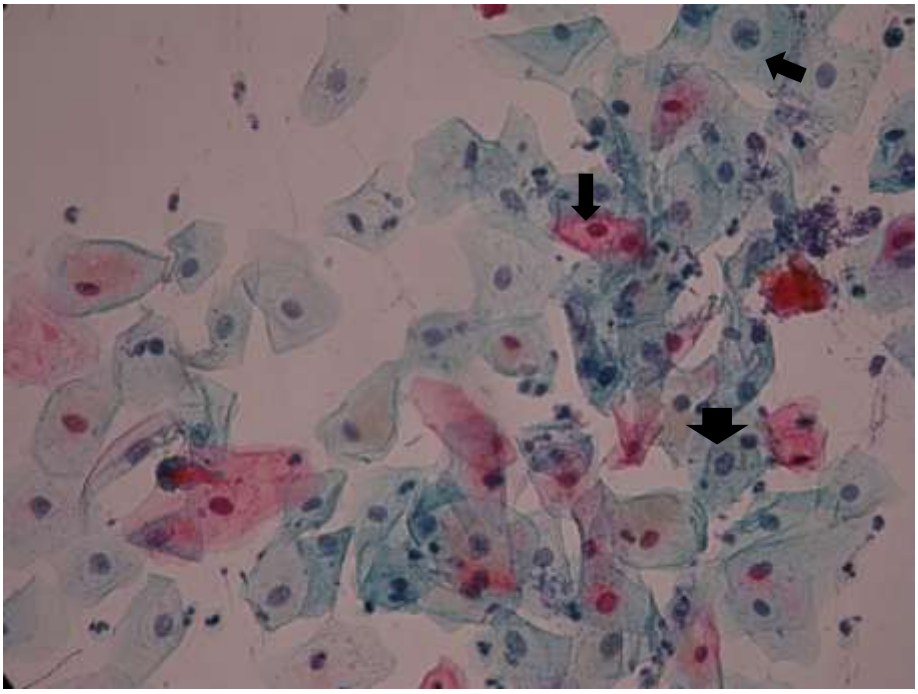
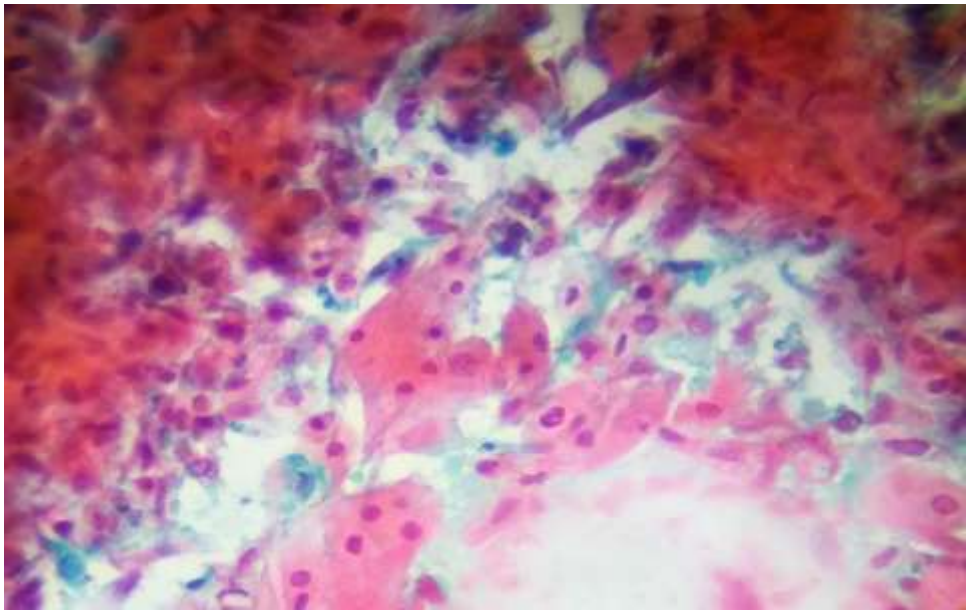
C 

Image 2: Photomicrograph C shows Atypical squamous cells of undetermined significance (ASCUS), X10. It shows parakeratosis in the mature squamous cells with koilocytic changes and mild nuclear atypia (arrows).

D 

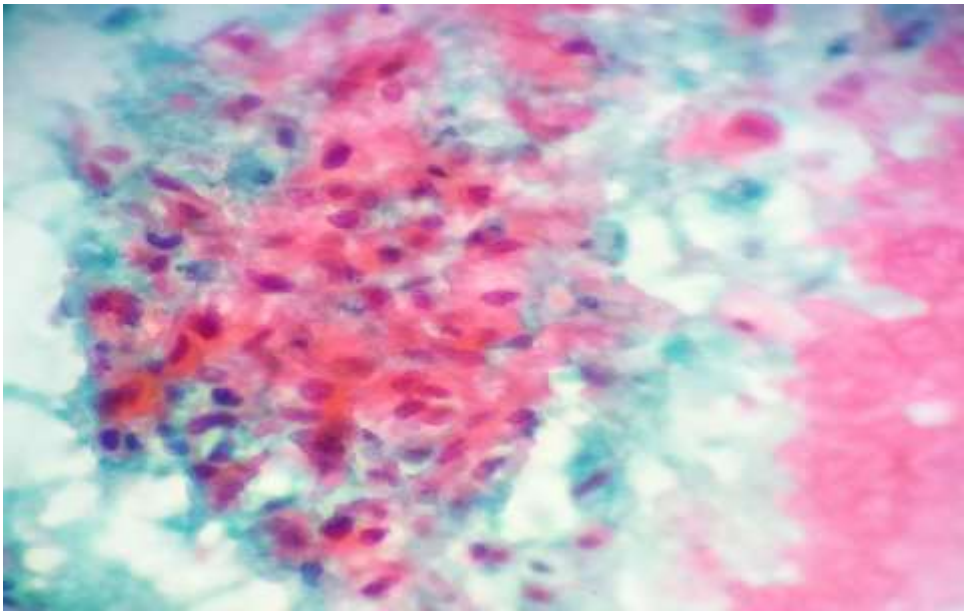
E 

Image 3: Photomicrographs D&E show Squamous cell carcinoma (SCC), X40. The photomicrographs show atypical spindling squamous cells exhibiting marked nuclear pleomorphism and hyperchromasia, irregular nuclear outline, and dense keratinized cytoplasm.

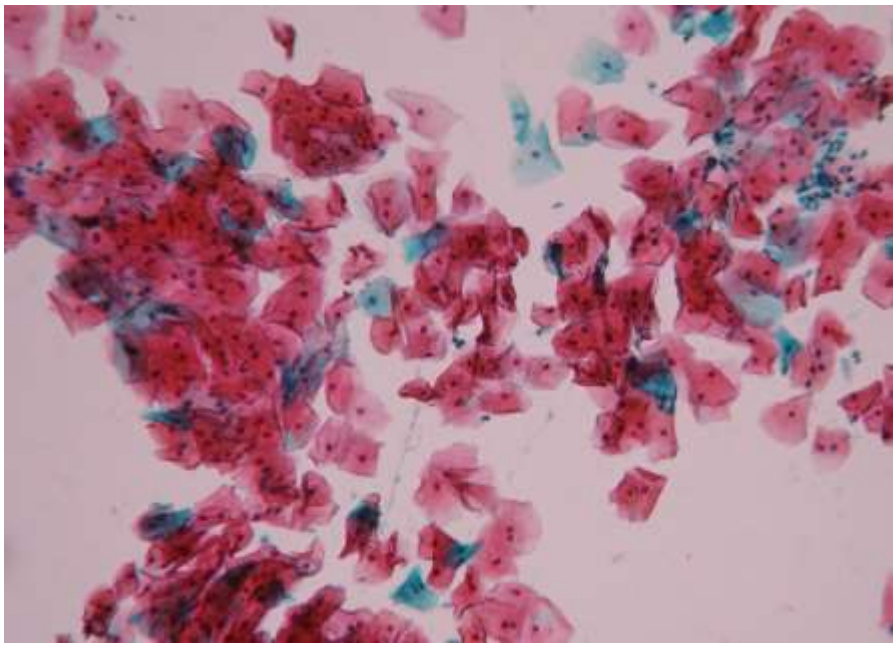
F 

Image 4: Photomicrograph F shows Negative for intraepithelial lesion or malignancy (NILM), X10. The photomicrograph shows intermediate and superficial squamous cells, no cellular and nuclear atypia.

DISCUSSION

5.1 Socio-demographics and Clinical findings

In our study, most of the women were between 30-49 years of age. The mean age at sexual intercourse was 18 years and about 9% of the women had an active multiple sexual partners in the last six (6) months with the median number of four (4). In this study, a total of 25(11.9%) women reported post-coital bleeding and abnormal vaginal bleeding, only a few (n=7) had been previously screened and were negative for cervical lesion. More than a half of our study population had attained post-primary education and the majority (94.3%) of the women screened had normal cytology. A study by Kayumba et al. (2010) in Rwanda, the majority of participants were between 30-49 years, a significant proportion (87.7%) had early sexual debut at sixteen (16) years or more. In the same study, most of the women (52.9%) had more than four (4) lifetime sexual partners and almost all (99.3%) had never been screened for cervical cancer [65]. Another similar study by Odhiambo et al. (2016) in Kenya, most of the participants were between 30-49 years and a significant number (43.5%) of the women had never had pap smear. The same study observed that a few (20%) of the women had first sexual intercourse at 18 years while most (92.3%) had normal cervical smear [66]. As the mean age at sexual debut of the women lowers, the risk of multiple active sexual partners, and high parity may increase the likelihood of developing cervical lesions due to exposure to more HPV infection [17].

In this study, out of 167(80%) women with normal cervical appearance, about 48(29%) had vaginal infection. Although vaginal and cervical examination may look normal, this can not exclude other co-infections and precancer lesions. According to Zheng et al. (2019), increased pathogenicity of HPV infection and occurance of cervical lesions may be potentially associated

with the imbalance in floral ratio, reduction in lactobacilli, and pathogen infections [67]. However, Muwonge et al. (2017) observed that co-infections with candida spp and TV does not increase the risk of cervical neoplasia in HPV positive women. Therefore, as normal cervix is subjective to the experience of the examining gynecologist, Pap smear test should be used to give a decisive information on the clinical appearance of the cervix.

5.2 Prevalence of pre-malignant lesions, viral loads, and duration on HAART.

In this study, a prevalence (5.7%) of cervical lesion was observed among HIV-positive women living longer while on HAART. Un-published study by Mbulwa H.C et al. (2016) that used visual inspection with acetic acid (VIA) and biopsy at the Infectious Disease Institute (IDI) in Uganda observed a prevalence of 4.4%. Another study by Kapiga et al. (1999) observed a prevalence of 2.9% of cervical lesions among HIV-positive women in Dar es Salaam-Tanzania. Other studies among HIV-positive women on HAART in Kenya, Nigeria, and Rwanda observed higher prevalence of 9.9%, 14.3%, and 20% respectively [34,65,66]. These finding were comparable to our study however, the use of a larger sample size, and a larger study area at a comprehensive care center (CCC) represented an ideal study population resulting into the variations of the prevalence. Furthermore, as majority of the women were not on combined ART and had CD4 count below 200 cells/mm3 due to delayed HAART initiation, this may have potentially lead to early HIV-related deaths before the advancement of precancer cervical lesions or increased the number of women living with HIV to support the oncogenic effects of HPV infection [68].

In our study, atypical squamous cells of undetermined significance (2.4%) was the most frequent lesion, followed by the low-grade squamous intraepithelial lesion (1.9%). Mbulwa et al. (2016)

in Mulago observed low-grade squamous intraepithelial lesion at 2.7% while high-grade squamous intraepithelial lesion was at 1.6%. In Rwanda, low-grade squamous intraepithelial lesions (11.3%) was the most frequent followed by atypical squamous cells of undetermined significance at 5.1% [65]. Other similar studies, Enebe et al. (2015) in West Africa observed 6.3% of LSIL and 2.3% HSIL as the commonest lesions, Kiplangat et al.(2008) in Kenya reported LSIL at 17% while ASCUS was at 9%, and Odhiambo et al. (2016) observed HSIL as the commonest cervical lesion at 6% at CCC in Kenya [18,66,69]. The use of both VIA and biopsy for histological conformation at IDI in Mulago-Uganda may have increased the diagnostic accuracy of the lesions compared to the findings in our study which used a conventional pap smear. While at Comprehensive Care Center (CCC) in Kenya, early screening of women on prolonged HAART use may have resulted into higher number of women with high-grade squamous lesions compared to our findings. Incident HIV infection and subsequent immunosuppression has been significantly associated with HPV infection [34,36]. Increased prevalence (32.8%) of high-risk HPV infection (subtypes 35,18,52) were observed commonly among young women between 25-34 years with normal cytology and at risk of cervical squamous intraepithelial lesions [70]. In our study, the majority of particpants with cervical lesions were between 18-49 years probably due to a potential failure of compliancy, inaffordability and inaccessibility to free condoms which may have exposed them to more HPV infection suggesting the argue for screening and prevention [71].

According to the World Health Organisation (WHO), all HIV-positive women should undergo annual cervical cytology screening following two (2) initial normal pap smear at six (6) months interval. Furthermore, all HIV-positive women with atypical squamous cells of undetermined significance should have colposcopy [72]. The Bethesda System (TBS) for reporting cervical

cytology recommends all women below thirty (30) years with ASCUS to have a follow-up pap smear between six to twelve (6-12) months, while those above thirty (30) years with the same lesion should undergo HPV-DNA testing [44].

In our study, all women (n=12) with cervical lesion had a baseline CD4 counts below 500 cells/mm^3 and suppressed viral loads below 1000 copies while on HAART for more than 5 years. The availability of baseline CD4 counts were not significant enough to monitor the disease progression in this group of women and determine the association of the cervical lesion with CD4 counts. However, a study by Sonia et al. (2017) observed a reduction in the risk of cervical lesions among women with CD4 counts above 500 cells/mm^3 and suppressed viral loads below 1000 copies [73]. In Kenya and Nigeria, majority of the women with the lesion had suppressed load copies and were on longer HAART use for more than three (3) years with CD4 counts above 200 cells/mm^3 [18,66]. A study by Makurdi et al. (2012) observed that CD4 counts do not reconstitute the immune system to halt disease progression [34]. All HIV-positive women with low CD4 counts were observed to have an increased likelihood of having consecutive HPV-positive test results more than 6 years than HIV-negative women and therefore at increased risk of developing cervical lesion [68,74]. However, these studies above did not show any statistically significant relationship between cervical lesions with CD4 count, Viral loads and duration on HAART observed which was comparable to our findings. In other studies, Memiah et al. (2012), Minkoff et al. (2010), Ezechi et al. (2014) observed an increased likelihood of developing cervical lesion among women with low CD4 counts, and high viral load copies and there was a statistically significant association shown in their study [73,75].

5.3 Conclusion

This study observed

1. A relatively low prevalence of pre-malignant cervical lesions (5.7%) among HIV-positive women on HAART for three (3) years or more.
2. Atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) were the most common lesions among women.
3. In the progression of pre-malignant cervical lesions, there was no statistically significant association between cervical lesions with viral load and duration on HAART.

5.4 Limitations of the study

1. Lack of adequate power to influence the national policy since this was a hospital-based study in an already selected community.
2. Constraints due to limited time and resources during the study period to perform current CD4 count tests for all the women screened.

5.5 Recommendation

1. A larger study to obtain more data to interrogate the current status, or confirm the low prevalence should be conducted among women living with HIV.

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APPENDICES

7.1 Appendix I: Client Information and Consent Form

7.1.1 Title:

Prevalence of pre-malignant cervical lesions among HIV-positive women on HAART attending Home Care Clinic at St. Francis Hospital Nsambya, Uganda.

7.1.2 Study Objective

To determine the prevalence and distribution of pre-malignant cervical lesions on Pap smear among HIV-positive women on HAART attending HCC at St Francis Hospital Nsambya, Uganda.

7.1.3 Consent explanation:

My name is John Innocent Oringo, a post-graduate student at the University of Nairobi, School of Medicine, in the Department of Human Pathology. I request you to participate in this study voluntarily. The purpose of this consent form is to give you information about the study to enable you to decide whether you want to be in the research or not. Please feel free to ask any questions about the survey. You are entitled to have a copy of this consent for your records.

After obtaining adequate clinical information, you will lie in the dorsal lithotomy position. A sterile vaginal speculum will gently be inserted into the vaginal canal to expose the cervix. Endocervical brush passed through the cervical opening into the endocervix and turned 180° clockwise. Then quickly, a wooden spatula placed against the ectocervix and turned 360° in a

clockwise direction. The smear is immediately made on a labeled, clean frosted end glass slide and fixed with alcohol.

7.1.4 Risks and benefits

Pap smear procedure is a rapid, non-invasive, relatively safe. There is usually no bleeding experienced during and after the procedure; however, any minimal bleeding will be managed by the research assistants collecting the sample.

There will be no payments offered to the participants, and you will not be denied the services to which you are entitled in this institution in case you decide not to participate or to withdraw from the study at any point in time. The information provided by the results may be used for better management of your condition by your health care provider. Early diagnosis of cervical lesions and other infections will guide the gynecologist to give comprehensive management.

Personal information will not be disclosed to anyone. I kindly request you to participate in the study.

7.1.5 Compensation

There will be no incentives offered to the study participants before, during, or after the study.

7.1.6 Alternative treatment

There will be no special treatment accorded to the participant other than the usual channels already in place for the management of patients.

7.1.7 Confidentiality

All records will be identified by serial numbers only to maintain your confidentiality, and we will not record your names.

7.1.8 Storage of specimen

In case of further analysis with permission from the KNH/UoN ERC, all specimens and data will be kept safely for five years in the department of human Pathology, the University of Nairobi, at the end of the study after which it will be destroyed. In-case of further research to be done on the sample, ethical approval will be obtained. Abnormal results will be put in the participant's file, and her physician will contact her.

7.1.9 Contact information

If you have any questions regarding the study, please contact me, Oringo J. Innocent, University of Nairobi P.O. Box 19676-00202 Nairobi. Mobile +256-778811-228/+254 700781658 or my supervisors, Prof. Lucy Muchiri, Dr. Waweru Wairimu, at the University of Nairobi, Dr. Othieno Emmanuel and Dr. Peter Sekwayama at St. Francis Hospital, Nsambya.

You may contact KNH-UoN ERC through Email: uonknh-erc@uonbi.ac.ke.

7.1.10 CONSENT FORM

I.....have read and understood the purpose of the study, the procedure to be done on me, the benefits, risks, and inconveniences associated with participating in the study and have agreed to participate without force or coercion of any kind. I am aware that I can withdraw from the study at any time without loss of any benefit or quality of management to which I am entitled.

Participant's signature/Thumbprint.....Date.....

Doctor's Name.....Signature.....Date.....

Principal investigator's signature.....Date.....

7.1.11 Luganda Translated consent form

FOOMU EKKIRIZIBWAAKO OKWETABA MU KUNOONYEREZA.

Ennamba mu kunnonyereza:.....

OMUTWE

OKUKEBERA EBIZIMBA KU MUMWA GWA NABAANA MU BAKYALA ABALI KU DDAGALA LYA KAWUKA KAMUKENENYA NGA BALIFUNIRA MU DDWALIRO LYA ST.FRANCIS NSAMBYA (HOME CARE) MU UGANDA.

EBIGENDERERWA MU KUNONYEREZA KUNO

Okwekebejja enddwadde mu bakyala abali kuddagala lyamukenenya nga balifunira mu ddwaliro lya St. Francis Nsambya.

OKUNYONYOLA KUKUKIRIZA

Amannya gange nze John Innocent Oringo, ndi muyizi mu tendekero lyobusawo e Nairobi mutabbi erikebera obunyama bwabantu. Nkusaba wenyigire mu kunonyereza kuno mukusalawo kwo. Omugaso gwa kukuwa bikwaata kukusoma.

Kino kijja kuyamba mukusalawo oba oyagala okwetaba mu kusoma oba nedda. Totya kubuuza kibuuzo kyonna ku musomo guno. Kikukakatako okuba ne fomu yo oyokukiriza kubanga oyinza okubaako byojjuzaamu.

Oluvanyuma lwokubuzibwa abasawo byonna, ojja kwebakira ku mugongo, ekyuma ekiyonjo ennyo kijja kutekebwa mubukyaala bwo okwekebejja nabaana yo. Ka bulashi akatono kajja kwetololera ku mumwa gwa nabaana katono, nga kadda ku ddyo. Mangu ddala akabaawo

akatono enyo kajja kutekebwaayo era ketolole nga kadda kuddyo, okujayo otuzi tutono ddala tutekebwe ku ka gilaasi katwalibwe okukeberebwa.

OKWEKENGERA N'OKUGOBOLOLA

Enkebera eno eyamangu, terina buzibu bwonna era yesigika, tewali musaayi gwonna gukuvaamu nga bakebera, era singa akasayi akatono kajja, kakolebwako mangu nnyo.

Tewali nsasula yonna ewebwa oyoayetabyemu, era tewali kigana kukolako singa oba tewetabye mu musomo guno oba nga oguvuddemu.

Ebiva mukukebera biyinza okukozesebwa omusawo wo, era okumanya amangu ku bizimba byokunabaana oba enddwadde endala kiyamba okufuna obujjanjabi obwamangu.

Ebiva mukukebera kuno bya kyama. Nkusaba nekissa kingi wetabe mukunonyereza kuno.

ENGASSI

Tewali kiwebwa beetabye mukononyerea kuno

OBUJANJABI OBULALA

Tewali bujanjabi bulala okuleka ebyabulijjo ebiriwo.

OKWEKISIZA

Ebiwandiiko byonna binonyerezebwako na namba, era amannya gamwe tetugeetaaga

ENTEREKA YA KANYAMA AKOKEBERA

Bwewabaawo ekirala kyonna, nga olukusa luva KNH/UoN ERC, ebijiddwako byonna nebwino biterekebwa bulungi okumala emyaka etaano mutelekero lyobunyama, obukeberwa mu tendekero lya waggulu e Nairobi, era oluvanyuma lwokunonyereza, tujja kusanyaawo akanyama

ako. Bwewanaabawo okukebera okwetagisa okulala kukanyama, olukusa lujja kuwebwa. Singa ebikebereddwa binasangibwa nga sibirungi, bijja kuterekebwa, nanyini byo ategezebwe omusawo omukungu.

ABATUKIRIRWA

Bwoba olina ekibuuzo kyonna mukunonyereza kuno kubira nze Oringo John Innocent, mutendekero lya waggulu e Nairobi Kenya, kasanduke 19276-00202 Nairobi. Essimu +256-778811228/+254-700781658, oba bakalabalaba bange, Pulofesa Lucy Muchiri, Dr. W. Waweru, Dr. Othieno Emmanuel ne Dr. Sekweyama Peter ku ddwaliro lya St. Francis Nsambya.

ENZIKIRIZA

Nze.....nasomye era netegera omugaso gwokunonyereza kuno, nekigenda okunkolebwako, emigaso, okwekengera ne bitataganya ebirirmu era nsazeewo okwetaba mu nga sikakiddwa. Era nkimannyi nti nsobola okuvaamu nga sirina wensaliddwa oba awokusasulira wonna.

Omukono gwoyo ayetabyeemu.....Ennaku z'omwezi.....

Amannya gomusawo.....omukono..... Ennaku z'omwezi.....

Omukono gwomunonyereza omukulu..... Ennaku z'omwezi.....

7.2 Appendix II: Questionnaire

PREVALENCE OF PRE-MALIGNANT CERVICAL LESIONS AMONG HIV POSITIVE WOMEN ON HAART ATTENDING HOME CARE CLINIC AT ST. FRANCIS HOSPITAL, NSAMBYA IN UGANDA.

This form is to be filled by the participants before sample collection.

Social Demographic Features

Study ID……. Date……

Gender……Age…….

Residence…….

What is your level of education?

None ☐

Primary ☐

Secondary ☐

Tertiary ☐

What is your occupation?

Unemployed ☐

Self Employed ☐

Employed (salaried) ☐

Others (specify).....

How many children do you have? Parity…….

Are you currently pregnant? Yes ☐ No ☐

When was your last menstrual period? State.....

Are you on family planning? Yes ☐ No ☐

if yes, state type……...

When was your sexual debut?.....

Do you have multiple sexual partners? Yes ☐ No ☐

if yes, state how many.....

Do you smoke? Yes, ☐ No ☐ if yes, how many cigarettes per day? .....

Do you have lower abdominal pain? Yes ☐ No ☐ if yes, for how long?.....

Do you have any history of abnormal pelvic bleeding? Yes ☐ No ☐ if yes, for how long?.....

Do you have any history of post-coital bleeding? Yes ☐ No ☐

if yes, for how long?.....

Have you ever screened for cervical cancer? Yes ☐ No ☐

When were you diagnosed with HIV? Months……Years...…

How long have you been on ARVs? Months…..... Years…...

What is your viral load and CD4 cell count?

<250cells/mm^3 ☐ 250-500cells/mm^3 ☐ >500cells/mm^3 ☐

7.3 Appendix III: Pap smear specimen collection and preparation

7.3.1 Collection

A complete record of the participant was taken, including previous clinical history.

The participant was put to lie in a lithotomy position in a comfortable place. The sterile disposable vaginal speculum was used to expose the cervix.

Cyto-brush was gently inserted through the cervical canal until only the bottom-most bristles were exposed to the OS A slow 180° rotation in a clockwise direction was made, and the brush was removed immediately.

The contoured end of a wooden spatula was gently inserted and pressed against the ectocervix. Holding the stem firmly between thumb and forefinger, the spatula was rotated 360° in a clockwise direction, and the sample was obtained within a few seconds.

The smear was and fixed immediately on a labeled, clean frosted end glass slide.

7.3.2 Preparation Technique

The wooden spatula was placed against the upper third of the frosted end clean slide

Both sides of the spatula were swirled against the slide surface in a circular motion spreading it evenly.

The endocervical brush was rolled on the lower two-thirds of the slides in a longitudinal direction towards the tail of the slides.

Immediately fixation of the smear using spray fixative was made.

7.3.3 Pap staining protocol

Post fixation of the smear in 95% ethanol was done for 15 minutes

Then slides were transferred to Hematoxylin solution for 6 minutes

One quick dip in 1% acid alcohol was done, followed by bluing in gentle running tap water for 5 minutes

The slides were dehydrated in 70%, 85%, 90% ethanol for 1 minute in each, respectively.

Then slides were transferred into OG-6 staining solution for 2 minutes

Rinsing of the slides in two changes of 95% alcohol for 1 minute each was done followed by staining with EA-50 solution for 4 minutes

Dehydration of the slides was done by dipping in three changes of increasing concentration of 95% ethanol than in absolute ethanol for 2 minutes in each.

Clearing in two changes of xylene for 2 minutes in each was done, followed by mounting with DPX and then left to air dry.

7.3.4 Results

Nucleus stained Blue to Black

Cytoplasm stained Pale Blue, Green to Pink or Orange

7.4 Appendix IV: The Bethesda System for Reporting Cervical Cytology 2017

CLINICAL HISTORY:

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APPEARANCE OF THE CERVIX

Normal ☐ Polyp ☐ Inflamed ☐ Ulcerated ☐ Suspicious ☐

SPECIMEN ADEQUACY

☐ Satisfactory for Evaluation

☐ Transformation zone: Present ☐ Absent ☐

☐ Endocervical cells: Present ☐ Absent ☐

☐ Endometrial cells present ≥45 years

☐ Unsatisfactory for evaluation: >75% Blood ☐ >75% inflammation ☐ Acellular ☐

INTERPRETATION

☐ NILM ☐ SCC

☐ ASC-US ☐ AGC

☐ ASC-H ☐ AIS

☐ LSIL ☐ Endocervical Adenocarcinom

☐ HSIL ☐ Endometrial Adenocarcinoma

ORGANISM

☐ Trichomonas Vaginalis

☐ Fungal organisms consistent with candida species

☐ A shift in flora suggestive of Bacterial Vaginosis

☐ Bacteria morphologically consistent with Actinomyces

☐ Cellular changes consistent with Herpes Simplex Virus

☐ Cellular changes consistent with Cytomegalovirus

P.I.: .....Date.....

Pathologist: .....Date.....

Tie Breaker: .....Date.....