CERVICAL CYTOLOGICAL PATTERNS AMONG HIV INFECTED WOMEN ON ANTIRETROVIRAL THERAPY AT KENYATTA NATIONAL HOSPITAL

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DECLARATION

I hereby declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been submitted to any higher learning institution.

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DEDICATION

I dedicate this dissertation to my parents for their support and encouragement.

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I thank God for this far He has brought me.

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ABBREVIATIONS

AGC- Atypical Glandular Cells

AIDS- Acquired Immuno Deficiency Syndrome

AIS- Adenocarcinoma in Situ

AOR- Adjusted Odds Ratio

CART- Combined Antiretroviral Therapy

ASC- H – Atypical Squamous Cell cannot exclude HSIL

ASCCP- American Society for colposcopy and Cervical Pathology

ASCUS- Atypical Squamous Cell of Undetermined Significance

CCC- Comprehensive Care Clinic

CI- Confidence Intervals

CIN- Cervical Intraepithelial Neoplasia

CP- Conventional Pap

DNA- Deoxyribonucleic Acid

DPX- Distyrene Plasticizer Xylene

EA- Eosin Azure stain

ECC- Endocervical curettage

FNA- Fine Needle Aspiration

HIV- Human Immunodeficiency Virus

HPV- Human Papilloma Virus

HR-HPV- High Risk Human Papilloma Virus

HSIL- High Grade Squamous Intraepithelial Lesion

ID- Identification

KNH- Kenyatta National Hospital

LAB- Laboratory

LR-HPV- Low Risk Human Papilloma virus

LSIL- Low Grade Squamous Intraepithelial Lesion

NILM- Negative for Intraepithelial Lesion or Malignancy

OG- Orange Green

OR- Odds Ratio

PAP- Papanicolaou Smear

SCC /SQC- Squamous Cell Carcinoma

SIL- Squamous Intraepithelial Lesion

SOP- Standard Operating Procedure

STI- Sexually Transmitted Infections

SPSS- Statistical Packages for Social Sciences

SPSS- Statistical Packages for Social Sciences

TBS- The Bethesda System

UK- United Kingdom

UON- University of Nairobi

WHO- World Health Organization

ABSTRACT

Introduction: HIV infection and HPV Persistence in immunosuppressed individuals is associated with development of cervical dysplasia and invasive cervical carcinoma. Combined Antiretroviral therapy (cART) has been shown to cause either regression or progression of cervical cytological lesions.

Objective: The aim of the study was to determine the cervical cytological patterns in HIV infected women on long term use over 5 years of cART**.**

Methodology: A cross sectional study. Pap smears were collected from two hundred and ten (210) HIV infected women on cART during November 2015-March 2016. CD4 cell counts and HIV viral loads were obtained from patient records at Comprehensive Care Clinic. Bivariate analysis correlated the cervical cytological lesions with CD4 cell counts, HIV viral loads and long term use of cART.

Results: Out of 210 HIV infected women sampled; the mean age was 42 years (SD=8.3). Age range was 24-61 years. The prevalence of cervical cytological lesions was 9.9%.Commonest lesion reported was high grade squamous intraepithelial lesion (HSIL) with 6%, followed by atypical squamous cells of undetermined significance(ASCUS), low grade squamous intraepithelial lesions(LSIL) and squamous cell carcinoma(SCC) having 1%. The distribution of the cervical cytological lesions was 2 with LSIL, 16 with HSILs. Infections occurred as follows twenty three patients had candida, seventeen had bacterial vaginosis, ten had co-infection, eighteen had atrophic cervicitis, three had atrophy and sixteen which amounted to 41%.The average duration on cART was 5-9 years (38.9%), 16% over duration of 10 years. The mean distribution of CD4 cell counts in the positive cytological lesions was 492.9/mm^3, with 62.5% with HIV viral loads of less than 500. The women with positive cytological abnormalities with 200 cells/mm^3 and below, were 66.7%.

Conclusions: This study demonstrated a reduction of cervical cytological lesions in HIV infected women on cART. The high grade cervical cytological lesions were leading followed by LSIL in this setting. In the development of cervical cytological lesions there was no statistical difference in the women with a history of cervical screening and those with no history.

Recommendations: Periodic Pap smear screening for all HIV infected women because VIA/VILLI is not specific. A larger study should be conducted to determine the long term effect of cART on cervical cancer

1.0 INTRODUCTION

Cervical cancer is the second most common malignancy after breast cancer affecting women. In sub-Saharan Africa, cervical cancer is the leading cause of cancer mortality among women. The cervical cancer burden is highest in developing countries with 270,000 new cases of cervical cancer occurring each year and accounting for 86% of cervical cancer deaths (1, 2).Although HIV prevalence has been declining, Kenya ranks fourth with the largest HIV epidemic. Studies have shown that the female population accounts for 50.2% incident HIV infections, majority living in Africa (3). Despite having a comprehensive antiretroviral access program, cervical screening coverage in Kenya is currently at 3.2%, which is low compared to the uptake in developed countries. All HIV positive women who are sexually active, and are 18–65 years old should be screened for cervical cancer as part of comprehensive HIV care. The screening cycle for this category is at diagnosis, after every 6 months in year one and then yearly if normal (11, 23)

HIV leads to chronic immunosuppression that propagates the Human Papillomavirus (HPV). HPV is the etiologic agent for cervical cancer. There is a greater prevalence of HPV infection among HIV infected women, ranging between 30% - 56% (32). The high risk HPV types 16 and 18 account for 56.6% and 18% majority of invasive cervical cancers worldwide (4).Meta-analysis carried out on the correlation between HIV, HPV and Squamous Intraepithelial Lesion (SIL) have shown that HIV infected women have a higher prevalence of HPV infection and Cervical Intraepithelial neoplasia (CIN) that tend to increase in proportion with the degree of immunodeficiency (3, 32).

With the advent of antiretroviral treatment, there has been a marked reduction on morbidity and mortality among people living with HIV. HIV is being termed as a ‘’manageable disease” because of the strides taken to improve the quality of life of HIV infected patients. Many studies have shown a marked decrease in high grade lesions for HIV infected patients under combined antiretroviral therapy but the impact on the use of the combined antiretroviral drug (cART) remains unclear. The purpose of this study is to determine cervical cytological patterns in cervical cancer HIV infected women on cART (5).

2.0 LITERATURE REVIEW

2.1 Incidence and Prevalence

Globally, cervical cancer causes the highest morbidity and mortality of all cancers. Cervical cancer is the 2nd most common cause of cancer death leading to approximately 270,000 deaths in 2012 of women worldwide. Most of the burden falls in low resource settings contributing to 70-80% of global burden of cancer. Approximately thirty five in one hundred thousand new cases of cervical cancer are diagnosed in sub Saharan Africa. Twenty three in one hundred thousand women die from the disease (1, 10, and 11).

Ten million Kenyan women aged 15 years and above are at risk of developing cervical cancer (24). According to statistics from the Ministry of Health the annual number of diagnosed cervical cancer cases and annual mortality rates are 2,500 and1, 676 respectively. The uptake years of cervical cancer screening is 18-69 for HIV infected patients. Prevalence of abnormal cytological lesions in general population is 3.6% while that in HIV infected women with high grade squamous intraepithelial lesion is still unknown. Prevalence of HPV 16 and 18 in women with HSIL is approximately 60.9%.Average age of presentation for invasive cervical carcinoma is 42 years, which in most cases is diagnosed late with 90% of patients presenting in stage II or worse.

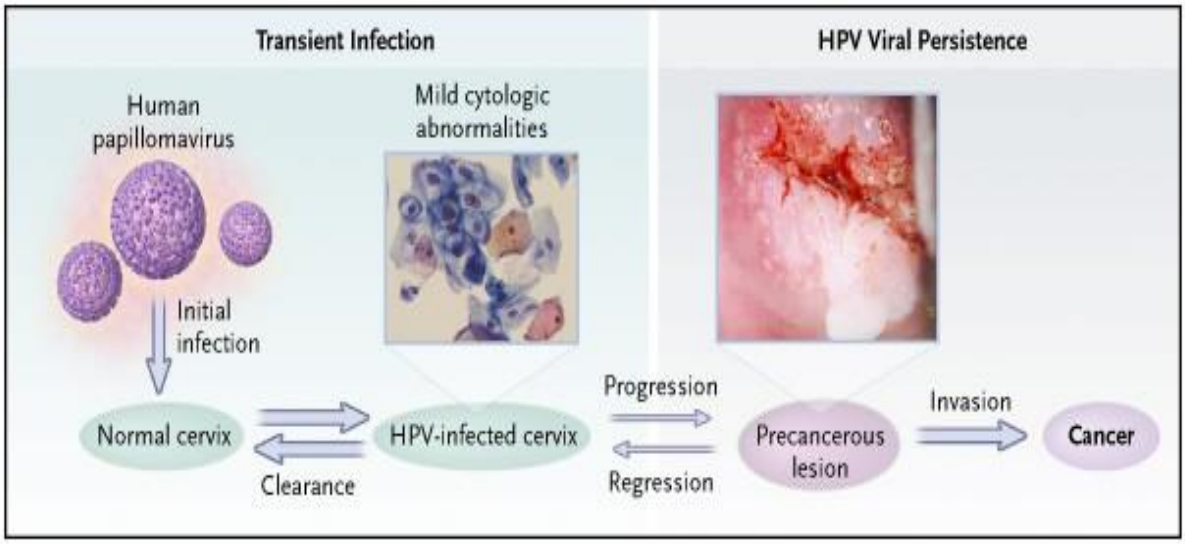
2.2 Cervical Cancer and HPV types

Cervical cancer is caused by the Human Papillomavirus(HPV).There are over 100 types of HPV, which are divided into three sub groups.-Low Risk, Intermediate Risk and High Risk HPV. Low-risk HPV types are mainly found in genital warts and other benign lesions and include HPV types 6, 11, 40, 42, 44, 54, 61, 70, 72 and 81. The high risk HPV types are oncogenic and are found in precancerous lesions and cancer. The high risk HPV types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 ,68, 73, and 82. Most oncogenic types of HPV include 16 and 18.HPV 16 seen in invasive cervical carcinoma and HPV 18 affects mainly the endocervical epithelium and leads to adenocarcinoma.

2.3 HPV Carcinogenesis

2.3.1 Natural History of Cervical Cancer

Most women are infected with at least one or several types of HPV during their sexual life. HPV is highly infectious with incubation periods of 3-4 weeks to months to several years. The majority of the high risk HPV positive infections clear and most of the women with infections do not develop cancer. Transmission of HPV occurs through skin to skin or mucosa to mucosa contact during sex. If there are tears in the mucosa the HPV infecting viral particles penetrate the epithelium and reach the germinal cells in the basal layer. The HPV infecting viral particles integrate into the host genome. Integration induces an over expression of viral E6 and E7 on coproteins. HPV DNA breakage occurs in the E2 gene region that leads to up regulation of E6 and E7. E6 interferes with host p53 gene function; E7 interferes with host tumor suppressor genes. Interference with p53 gene means there is no inhibition of apoptosis. E7 causes cell cycle inhibition. Without cell cycle regulation, tumor cells keep replicating and proliferating (30, 31).



Stages of cancer progression borrowed from *Wright & Schumann NEJM 2003*

2.3.2Persistence and Clearance

Infection with high risk HPV lasts 12-18 months and is cleared by the immune system. Approximately 10% fail to clear HPV infections, resulting in persistent infection. Persistent infection of High risk-HPV with time can lead to cytological abnormalities. The longer the persistence of High risk-HPV, the longer it takes for the clearance of HPV. Studies have shown that older women are more predisposed to persistent latent HPV than younger women probably due to latent infections over duration of time. Clearance of HPV is estimated to take approximately 10 years. In HIV-infected women latency of HPV can lead to cytological abnormalities as opposed to HIV non-infected patients. HIV negative patients may have HPV persistence, but may not necessarily have infections progress to squamous intraepithelial lesions (30, 31).

2.3.3 Progression to pre cancer and cancer

Pre cancer in cytology refers to the morphological diagnosis of HSIL or moderate and severe dysplasia. In pre cancer the undifferentiated cells with fixed genetic abnormalities have replaced normal cells in almost the entire thickness of the cervical epithelium. Average age of diagnosis of pre cancer varies from 25 to 35 years and depends on the onset of sexual exposure.

HPV type is the strongest factor that affects the absolute risks of viral persistence and of progression to pre cancer given viral persistence. HPV16 is the most carcinogenic, with an absolute risk of a pre cancer diagnosis approaching 40% after 3–5 years of persistent infection.
The total risk of pre cancer for a woman carrying several HPV types is increased compared with women infected with any one of the HPV types she carries, but it is not clear whether her risk is greater than the sum of the risks posed by individual HPV types. HPV and lack of cytology screening programs increase the risk of developing cervical cancer. Other external factors like multi parity, smoking, use of oral contraceptives and high number of sexual partners, contribute mildly to development of cervical cancer.

HIV-infected individuals are often infected with more HPV types than non-infected individuals, suggesting type differences in the success of immune suppression. The probability of invasion is not strongly affected by HIV. Combined antiretroviral therapy does not seem to affect HPV natural history or to reduce the risk of cervical pre cancer and cancer; however, the relation

between such therapy and risk is perhaps confounded because HIV-infected women on cART are living longer and allowing the cervix to have prolonged exposure to oncogenic HPV in the context of relative immunosuppression (30, 31, 32).

2.4 Squamous Intraepithelial Lesions

Squamous intraepithelial lesions encompass a spectrum of precursors to invasive squamous cervical cancer. Women with squamous intraepithelial lesions are at least 10 years younger on average than those with invasive cancer.

2.4.1 Low Grade Squamous intraepithelial lesion

LSIL comprises intraepithelial lesions showing a preservation of differentiation, maturation and organization of squamous epithelium with abnormal mitoses confined to basal and parabasal epithelial layers, koilocytosis, dyskeratosis, multinucleation, and enlarged hyper chromatic nuclei. Cytological abnormalities include superficial/ intermediate metaplastic cells with well preserved & well differentiated cytoplasm, slightly enlarged nuclei (3Xnormal intermediate cell nucleus), slightly altered N:C ratio, mild hyperchromasia, smooth nuclear membrane, finely granular & evenly distributed chromatin, presence of koilocytosis and prominent nucleoli(12, 13).In a critical literature review of CIN lesions, Östor has found that the rates of regression, persistence and progression to CIN 3 and to SCC for CIN 1 lesions were about 60%, 30%, 10% and 1%, respectively.

2.4.1.1Management of LSIL

The ASCUS/LSIL triage study found that high-risk HPV types were detected in 85% of
LSIL cases and that HPV DNA testing was not a useful triage strategy. Colposcopy is generally recommended for initial management of LSIL patients. Repeat Pap smear test should be done at 6 months and 12 months.

2.4.2 High Grade Squamous Intraepithelial Lesion

HSIL is characterized by a lack of squamous differentiation, epithelial disorganization, and severe cellular dysplasia with the presence of mitoses throughout the entire or lower two thirds of the epithelium. Cytological abnormalities manifest as mainly parabasal-like cells with nuclear atypia, singly occurring cells, sheets or syncytial-like aggregates, hyper chromatic clusters N: C

ratio is markedly increased over 50%, marked hyperchromasia, salt & pepper chromatin, many nuclei with irregular nuclear membranes

HSIL accounts for 0.5% of all Pap smears and 97% of women with HSIL Pap result are positive for high-risk HPV. If left untreated about 14% of them will develop cervical invasive squamous cell carcinoma (12, 13).

For CIN 2 lesions the corresponding approximations were 40%, 20% and 5%, respectively for rates of regression, persistence and progression .The likelihood of CIN 3 regression, persistence and progression to SCC were about 33%, 56% and over 12%, respectively(12, 13).

2.4.2.1Management of HSIL

Women with cytological result of HSIL will have biopsy-confirmed CIN 2 or CIN 3 identified at the time of colposcopy. The 2001 American Society for Colposcopy and Cervical Pathology consensus guidelines recommend that if biopsy confirmed CIN is not identified at colposcopy in a woman with a cytological interpretation of HSIL, all cytological results and histological material should be reviewed. If the cytological interpretation of HSIL is upheld on review, a diagnostic excisional procedure should be performed.

2.3.3 Atypical Squamous cells

Atypical squamous cells (ASC) are less than 5% of all Pap smears. Patients with ASC are found to have a CIN lesion in 10% to 20% of cases. In The Bethesda System- 2014, ASCs are divided in 2 categories: ASC of undetermined significance (ASC-US) and cannot exclude a high-grade squamous intraepithelial lesion (ASC-H). An ASC diagnosis is made when an SIL is suspected cytologically. Cytologic criteria for identification of ASC-US and ASC-H cells are somewhat subjective, and the diagnoses suffer high interobserver and intra-observer variation rates. ASC-US represents about 90% of all ASC cases.

2.3.3.1 Atypical Squamous cells of undetermined significance

ASC-US cells show cellular features that are more severe than those of squamous cells with reactive changes but less than those of a SIL. The diagnosis of ASCUS is made by exclusion of cells with known features. The ASCUS cells are usually the size of intermediate or superficial

squamous cells and have nuclear changes that are suggestive but not diagnostic of LSIL or SIL not otherwise specified (12, 13).

2.3.3.1.1Management of ASCUS

Oncogenic high-risk HPV DNA testing is the preferred management for patients with ASCUS especially when it can be performed concurrently. The test should not be performed in women younger than 30 years of age because the HPV infection in these patients is often caused by a mixture of low- and high-risk virus types, making the interpretation of the test results difficult, if not impossible. Follow-up with repeat Pap tests at 6-month intervals or immediate colposcopy is also acceptable. If cellular atypia persists over 2 years refer for colposcopy for further evaluation (13)

2.3.3.1.2 Atypical squamous cells, cannot exclude HSIL. (ASC-H)

ASC-H represents 5% to 10% of all ASC cases. ASC-H cells are usually the size of metaplastic cells and maybe seen singly or in clusters; they are suggestive but not diagnostic of HSIL (12, 13).

2.3.3.1.2.1 Management of ASCH

Patient with ASC-H diagnosis should be referred to colposcopy as ASC-H has a positive predictive value for histologic CIN 2 or 3 much higher than that of ASC-US (50% versus17%). If a CIN 2 or 3 is not found a repeat Pap test in 6 months or a HPV DNA test is done. If repeat Pap test result is ASC-US or worse, or if HPV DNA is positive for high-risk viruses, should be referred for a second colposcopy (12).

2.3.4 Squamous cell carcinoma

Invasive SCCs are the most common type of cervical cancers, accounting for 60% to 80% of all malignant tumors of the cervix. HSIL is an immediate precursor of cervical squamous cell carcinoma. They occur mainly in adults with a peak incidence in the 5th and 6th decades of life. Their common clinical manifestation is abnormal vaginal bleeding that may occur spontaneously or following sexual intercourse (post-coital bleeding). (12, 13).

Cellular cytological features include cells with bizarre configurations (spindle snakes, caudate tadpoles. Marked pleomorphism, orangeophilia associated with dense keratinization, eosinophilia may be more prominent. Cells occur singly, although occasional syncytial-like aggregates can be seen. Nuclei are 1.5 to 3 times the size of an intermediate cell nucleus. The chromatin is typically coarse, with pyknotic nuclei. Macronucleoli may be present and seen mostly in non-keratinizing squamous cell carcinoma. Keratinizing squamous cell carcinoma is usually accompanied by evidence of hyperkeratosis, parakeratosis, atypical parakeratosis, and keratinizing dysplasia. A tumor diathesis consisting of old blood and necrotic debri is often observed (37)

2.3.4.1 Management of Squamous cell Carcinoma

Colposcopy and biopsy for histological evaluation is recommended.

2.3.5Glandular Abnormalities

2.3.5.1 Atypical Glandular cells

Adenocarcinomas are common invasive malignancies of the female genital tract. Unfortunately, the Pap smear is not nearly as good a screening test for glandular lesions as it is for squamous lesions (12). AGC cytology is most commonly associated with squamous lesions including CIN 1. However, glandular and squamous lesions often coexist, with CIN found in approximately half of women with Adenocarcinoma in situ, so identification of CIN does not preclude AIS or adenocarcinoma. HPV types 16 and 18 have been identified in cervical adenocarcinoma tissues suggesting a common etiology with squamous cell cancer. Although cervical adenocarcinoma is HPV associated and can be detected with HPV testing, endometrial cancer is not, therefore HPV DNA testing is not a useful test.

2.3.5.2 Endocervical Adenocarcinoma of the Uterine Cervix

It accounts for a third of all invasive carcinomas of the uterine cervix and occurs more frequently in the fifth decade.Most in situ and invasive endocervical adenocarcinomas occur in women over 30 years of age, a slightly older age group than patients with comparable squamous lesions. AIS are found in about 5% of cervical HSILs. Endocervical adenocarcinoma in situ is considered to

be the glandular counterpart of cervical intraepithelial neoplasia (CIN3) and the precursor to invasive endocervical adenocarcinomas and adenocarcinoma in situ (13). HPV 18 is predominantly associated with adenocarcinoma of the uterine cervix. It may be well- to poorly differentiated, and a mixed pattern consisting of areas of well differentiated and poor differentiated in the same patient is not uncommon. Endocervical mucinous carcinomas are the most common type, accounting for 70% to 90% of cases followed by carcinoma of endometrioid type.

Criteria for Endocervical AIS include abnormal architecture: pseudostratified strips, rosettes, feathering, increased nuclear-cytoplasmic ratio, nuclear enlargement (75μm mean area) with pleomorphism of size and shape, coarsely granular evenly distributed chromatin, nucleoli typically not prominent. Mitoses and apoptotic bodies may be present. Tumour diathesis typically not present, but inflammatory background may be present. (12, 13)Cytological manifestations of endocervical adenocarcinoma are similar as AIS.

2.4.4Effects of cART on SILs

Combined Antiretroviral Therapy (cART)is known to improve the quality of life of HIV infected patients by restoring or preserving the immunologic function, suppressing viral replication and in reduction of HIV related morbidities and mortalities. HIV associated malignancies brought about by the immunosuppression of HIV, are greatly reduced because of the effects of cART.

A study done by Adler et al discovered that women on cART with normal smears initially were 38% less likely to develop cytological abnormalities in follow up pap smears(20). Another study by Firnhaber et al, in South Africa associated cART use with a reduction of the incidence and progression of cervical lesions among HIV positive women (16). Konopnicki et al in Belgium followed a large cohort of women on cART duration of 3 years, with a sustained CD4 cell count of 500 cells/ mm^3 for over one and a half years, and found the outcome was decreased risk of persistent cervical high risk HPV infection (34).

A recent study in Canada by Sandra Blitz et al showed that cART assists in the clearance of HPV in HIV infected patients who present with LSILs at initial screening of cervical cancer as

opposed to patients presenting with HSILs due to the presence of HR-HPV types 16.The likelihood of cART users with normal cervical cytology results is significantly less likely to have cytological abnormalities in subsequent cervical smears. Some of these studies support the benefit of cART because of the immune reconstitution and the adherence to cART the patients are following, depending on the levels of CD4 cell counts at baseline during initiation of antiretroviral therapy Studies have shown there is a decrease in prevalence of SIL among HIV infected women on use of cART for over five months on treatment (16, 18, 20, 36).

A few studies show there is no impact on the natural history of HPV hence less reduction in the incidence and regression of cervical lesions. In India, Saharsrabuddhe et al found that HIV-infected women currently receiving cART were at higher risk of presenting a CIN compared to untreated women (19).In Kenya, DeVuyst et al found no significant association between cART use and the presence of high grade CIN. Another study done in India by Bansal B et al showed that cART has no relationship on the development of cervical lesions in HIV positive women (19, 38).

2.5 JUSTIFICATION

Invasive cervical cancer is a HIV defining malignancy lesion with high prevalence in sub Saharan Africa. Kenya has the highest burden of HIV, HPV co-infection second to South Africa.

With the introduction of cART, there has been a marked reduction in HIV associated morbidities and mortalities. In Kenya, many of the HIV infected women are on cART but it is unknown what the cervical cytological patterns are. HIV infected women on cART are expected to have less cytological lesions. This is due to better clearance of HPV, opportunistic and AIDS associated infections and a gradual decrease in the incidence and progression of cervical lesions.

This study will determine the cervical cytological changes seen in long term use of cART in HIV infected women. The study findings may provide new or improved strategies for prevention of cervical cancers among immuno-compromised HIV infected women

2.6The Research Question

Does long term use (more than 5 years) of cART affect the patterns of cervical cytological lesions in HIV infected women attending the CCC in KNH?

2.6.1 Broad Objectives

To determine the prevalence and patterns of cervical cytological lesions in HIV infected women on long term use of cART attending the CCC in KNH?

2.6.2 Specific objectives

1. To determine the prevalence and patterns of the cervical cytological lesions in HIV infected women.
2. To determine current CD4 cell counts and HIV viral loads among women on cART.
3. To correlate Pap smear results with CD4 cell counts and HIV viral loads.
4. To correlate the grade of cervical neoplasia with the length of combined antiretroviral therapy.

3.0 METHODOLOGY

3.1 Study design

Cross sectional descriptive study.

3. 2 Eligibility Criteria

3. 2.1 Inclusion criteria:

1. HIV infected women in the reproductive age who were on cART more than 5 years eligible for a pap smear.
2. HIV infected women are also eligible 6 weeks post-delivery.
3. HIV infected women who have consented to participate in the study.

3. 2.3 Exclusion criteria

1. HIV infected women who have undergone hysterectomy/cervical ablation.
2. Pregnant HIV positive women
3. Those on therapy for high grade squamous intraepithelial lesions.
4. Women on menstrual cycle

3.3 Sample size determination

The number of samples for the study population was calculated using prevalence of a study done to assess the prevalence and identified associated risk factors for precancerous cervical cancer lesions among HIV-infected women in resource-limited settings in Kenya. HIV- infected women attending the ART clinic at the Nazareth Hospital between June 2009 and September 2010(17). The prevalence of HIV infected women taking cART was 15.7%using the Fishers formulae

The sample size will be calculated using the following formula:

$$n = \frac{z^2 \times p(1-p)}{d^2} = \frac{1.96^2 \times 0.157(1-0.157)}{0.05^2} = 210$$

Sample size n= 210

n is the minimum sample size

P is the known prevalence

Z is the normal standard deviate that correspond to 95% confidence interval

d is margin of error degree of precision set at +/- 5%

3. 4 Study site

The study was conducted in Kenyatta National Hospital-Comprehensive Care Clinic and cytology laboratory.

3. 5 Target Population

The target population was HIV infected women on long term use of cART more than 5 years attending the Comprehensive Care Clinic in Kenyatta National Hospital.

3. 6 Recruitment and data collection

This study was carried out at Comprehensive Care Clinic (CCC) in Kenyatta National Hospital. The PI contacted the manager of CCC once ethical approval of the study was obtained. The manager with the matron introduced the PI to a trained nurse who assisted in the study.

The PI recruited two hundred and ten (210) study participants who met the inclusion criteria and were given a written informed consent to participate in the study. Every day, recruited study participants were grouped and a short education session was given by the PI on the risk of cervical cancer, benefit of early detection, research study aims and procedure of the Pap smear in both English and Kiswahili.

3.6.1 Data Collection

The PI administered the questionnaire (Appendix III) to each study participant which includes demographic characteristics, lifestyle factors such as history of tobacco smoking, number of sexual partners and last menstrual period. The CD4 cell counts, HIV viral loads, information associated with cervical abnormalities such as coitarche, previous STI's and ART history (treatment regimen and duration) were obtained and verified from client's records.

3.6.2 Pap Smear Collection and processing

1. All pelvic examinations and Pap smear collections were performed by nurse trained in obtaining pap smears.
2. Patients assumed lithotomy position and plastic disposable speculums were inserted in the vagina to examine the cervix.
3. The cervix was examined for gross lesions and abnormal discharge.
4. A Cervex brush (appendix X) was used to take specimens from the squamo-columnar junction. The Cervex brush was rotated 360º to achieve an adequate sample.
5. Smears were made on the frosted end of the labeled glass slide provided in the Pap kit (appendix X) and were fixed using the fixative provided in the pap kit.
6. The fixed pap smears were put in a zip lock polythene bag and sealed. These smears were transported at room temperature to the cytology laboratory by the principal investigator. All the pap smears were processed and stained using the Papanicolau staining technique (appendix III).

3.6.3 Screening and reporting of the smears

All the Pap smears were screened by the principal investigator and signed out together with a consultant pathologist (supervisor). The Bethesda system 2014 for reporting cervical cytology was used for reporting all the cytological abnormalities observed during examination and reporting.

All abnormal and 10% of “normal” smears were reviewed by a second pathologist. Any discrepancies in cytological result were referred to a third pathologist who acted as the tie-breaker.

The results were filed into the respective patients file. For the patients who had suspected lesions, the PI called the study participant to come to CCC and linked the patient to the Reproductive Health Clinic for further management of the lesion.

3.7 Quality Assurance

Specimen collection was carried out by experienced health professionals who perform Pap smears regularly. Pap smears were stained in line with the KNH cytology laboratory standard operating procedures (SOPs) used for staining gynecological specimens and approved by the Pathologist-in-charge. The stains and reagents were prepared using relevant standard operating procedures (SOPs) and filtered before each use to ensure good quality staining. Deteriorated stains were discarded and new stains prepared.

The PI examined all the smears followed by a review with the pathologist (supervisor). All abnormal and 10% of "normal" smears were reviewed by a second pathologist. Any discrepancies in cytological result were referred to a third pathologist who will act as the tie-breaker.

3.8 Ethical Considerations

Ethical clearance was obtained from KNH/UON ethics review committee to conduct the study at Comprehensive Care Clinic. Informed consent was sought from the prospective study participants using ethical guidelines. No woman was coerced into taking the cervical smear test without their permission. Assurances were offered to calm the women who were unsettled when performing the procedure. Pap smear sampling had minimal risks. Patient privacy and confidentiality was strictly observed. All Pap smear results were communicated to the attending physician in a timely manner for further management.

3.9 **Data Collection, Management and Statistical Analysis Plan**

Data was collected using predesigned questionnaires, report forms and stored in hard cover register, Microsoft excel as well as SPSS software. Data was collected from the questionnaires and in hard cover register and kept in lockable cabinets where only the researcher had access thus confidentiality was maintained. Information stored in soft copies was protected from access

from unauthorized persons by a password. All records will be identified by study identification numbers. All data was analyzed using SPSS version 21. All statistical tests were performed at 5% level of significance (95% confidence levels). The descriptive statistics were presented as proportions and percentages in form of tabulation charts and graphs.

4.0 RESULTS

4.1 SOCIO DEMOGRAPHICS

Out of the 210 HIV infected women sampled, the mean age of the women was 42 years (SD=8.3). Those married were 95(42.8%). Only 1 had a history of tobacco/ bhang smoking. The women with secondary education were 96 (43.6%). The number of children living was 2 and the average range of miscarriages was 1-4. Over half of the women, 124 (56.6%), had a pap smear taken (Table 1).

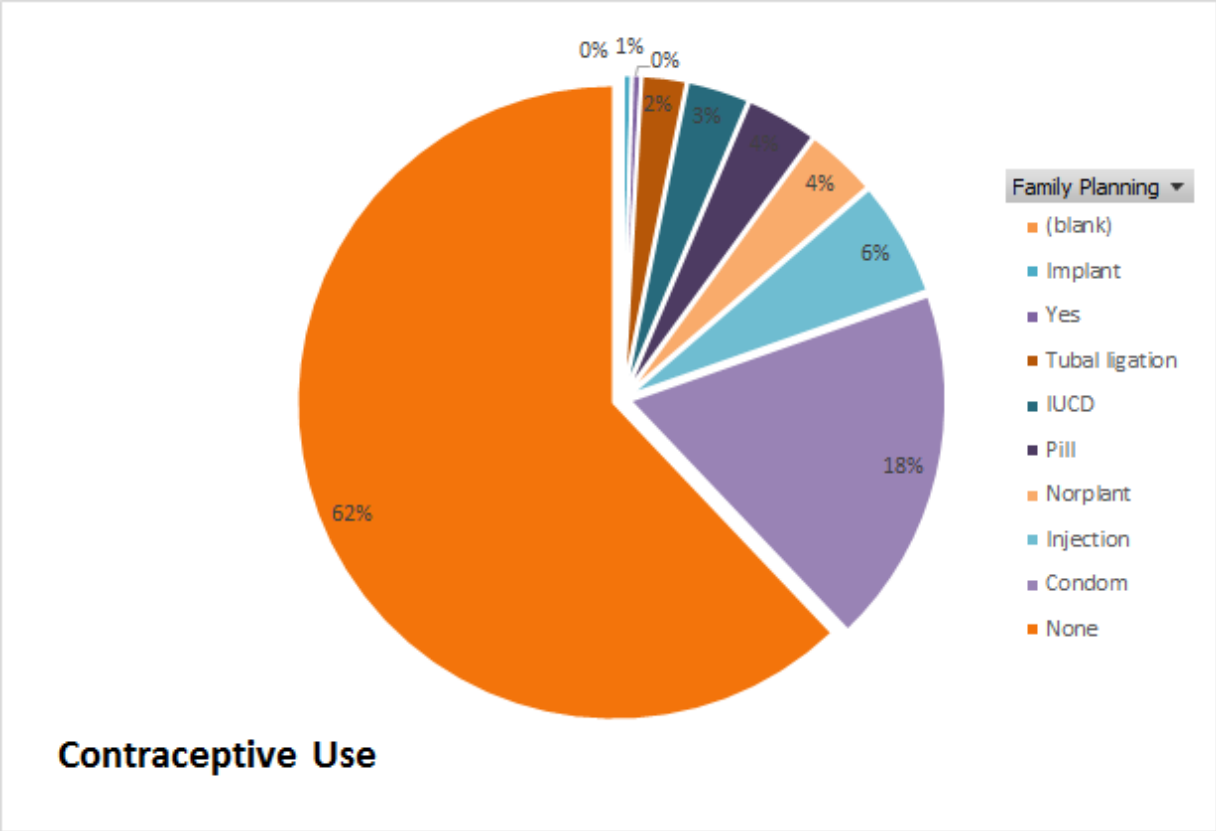
The most used family planning methods were Condoms and Injection with 40 (18.3%) and 13 (5.9%) respectively (Figure 1). The number of sexual partners was 1 and median age/year of first sexual intercourse was 18 years with a range of 16 to 20 years old.

Table 1: Socio-demographics

SOCIO DEMOGRAPHICS	n (%)
Age (Years) Mean (SD)	41.9 (8.3)
Marital Status	
Single	90 (40.5)
Married	95 (42.8)
Divorced	7 (3.2)
Widowed	30 (13.5)
History of Tobacco/ Bhang smoking	
No	220 (99.1)
Yes	2 (0.9)
Education	
Primary	46 (20.9)
Secondary	96 (43.6)
College	77 (35.0)
Not gone to school	1 (0.5)
No of children	
How many are living, Median (IQR)	2 (1-3)
Miscarriages, Median (IQR)	0 (0-1)
Ever had a pap smear	
Yes	124 (56.6)
No	95 (43.4)
Family Planning	
No FP	154(68.9)

Condom	40 (18.3)
Injection	13 (5.9)
Pill	8 (3.7)
IUCD	7 (3.2)
No of sexual partners, Median (IQR)	1 (0-1)
Age/Year of first intercourse, Median (IQR)	18(16-20)

Figure 1: A pie chart showing contraceptive use in the HIV infected women



4.2 Clinical History

Cervix appearance was normal in 204 (92.3%) and with erosions 16 (7.2%) of the participants. Those with a history of cART for 5-9 years were 86 (38.9%) followed by 16% on cART for 10 years.

Half the women, 159 (73.6%) had CD4 cell counts of <200 cells/mm3. HIV viral loads show 58(77.3%) with <500 copies/ml, 16 with >1000 copies/ml and 1(1.3%) with 500-100 copies/ml (Table 2)

Table 2: Clinical History

CLINICAL HISTORY	n(%)
Appearance of the cervix	
Normal	204 (92.3)
Eroded	16 (7.2)
Inflamed	1 (0.5)
Suspicious	0 (0)
Others specify	0 (0)
History on ART	
Less than 6 months	21 (9.5)
6-12 months	14 (6.3)
1-4 years	64 (29.0)
5-9 years	86 (38.9)
>10 years	36 (16.3)
Specimen Adequacy	
Satisfactory	220 (99.1)
Unsatisfactory	2 (0.9)
Epithelial cell features	
Negative	204(91.89)
ASCUS	2 (0.5)
LSIL	2 (1.8)
Inflammatory	0 (0)
Reactive	0 (0)
ASCH	2 (0.9)
HSIL	12(3.6)
SCC	2 (0.9)

AGC	0 (0)
AIS	0 (0)
Adenocarcinoma	0 (0)
HIV Viral Loads, copies/ml	
>1000	16 (21.3)
500-1000	1 (1.3)
<500	58 (77.3)
CD4 Cell counts, cells/mm3 Mean (SD)	556.04 (243.2)
CD4 Cell counts Category, cells/mm3	
>500	31 (14.4)
200-500	26 (12.0)
<200	159 (73.6)

Figure 2.1 Graph with distribution of CD4 cell counts

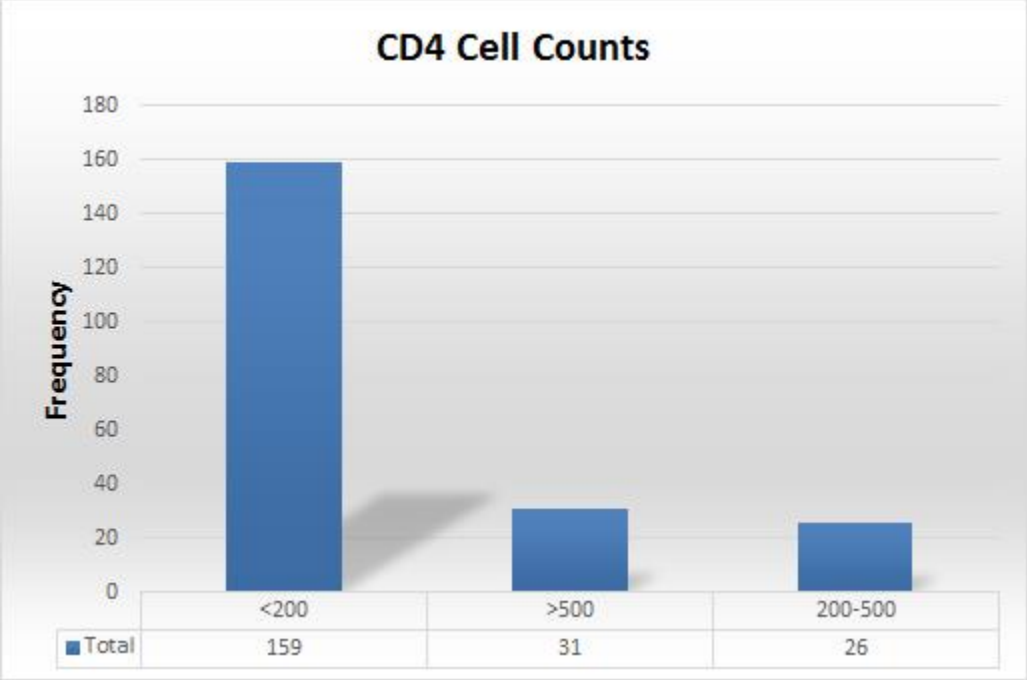
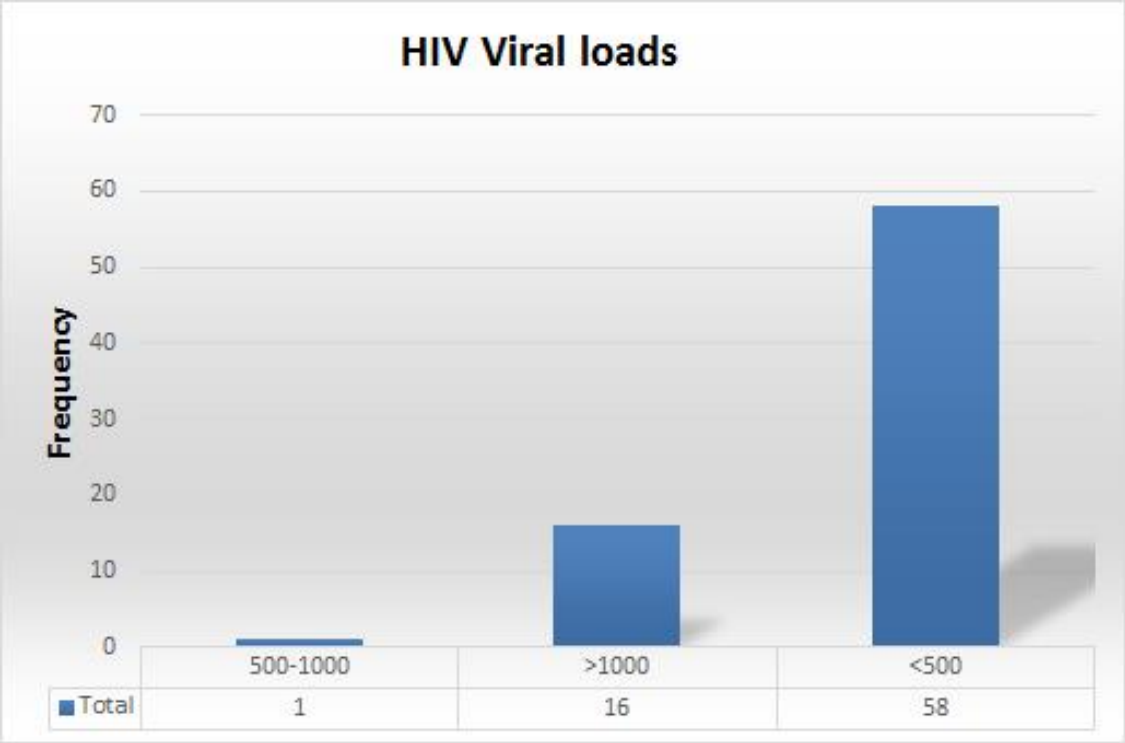


Figure 2.2: Distribution of HIV viral loads



4.3 Bivariate Analysis of SIL and Age

The women who had ASCUS were in the age group of 40 to 50 years, those women with HSIL were in the age group of 40 to 50 years and those with SCC were in age group 40 to 50 years. The women with LSIL result were in age group 20 to 39 years. In the categorization of lesions, NILM 90 (44.3%) was in age group 40-49 years and 69 (34%) 30-39 age group. Three out of the eighteen HIV infected women who had lesions were on cART for longer than 10 years. Four out of the eighteen HIV infected women were on combined antiretroviral therapy for an average 5 to 7 years. Out of the remaining 210 on cART, two had LSIL, fourteen had HSILs.

However, the results of the bivariate analysis did not show any statistically significant differences in age group, p=0.276 as shown in table 3.

Table 3: Prevalence of SILs versus age selected categories in HIV infected-positive women attending CCC, in Kenyatta National Hospital

	NILM	ASCUS	ASCH	LSIL	HSIL	SCC	Test stat.	P
	n=203	n=2	n=0	n=2	n=12	n=2		
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)		
Age group							Fisher's test	0.276
20-29	11 (5.4)	0 (0)	0 (0)	2 (50)	0 (0)	0 (0)		
30-39	69 (34)	0 (0)	1 (50)	2 (50)	1 (12.5)	0 (0)		
40-49	90 (44.3)	1 (100)	0 (50)	0 (0)	5 (50)	2 (100)		
50-59	26 (12.8)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)		
60-69	7 (3.4)	0 (0)	0 (0)	0 (0)		0 (0)		

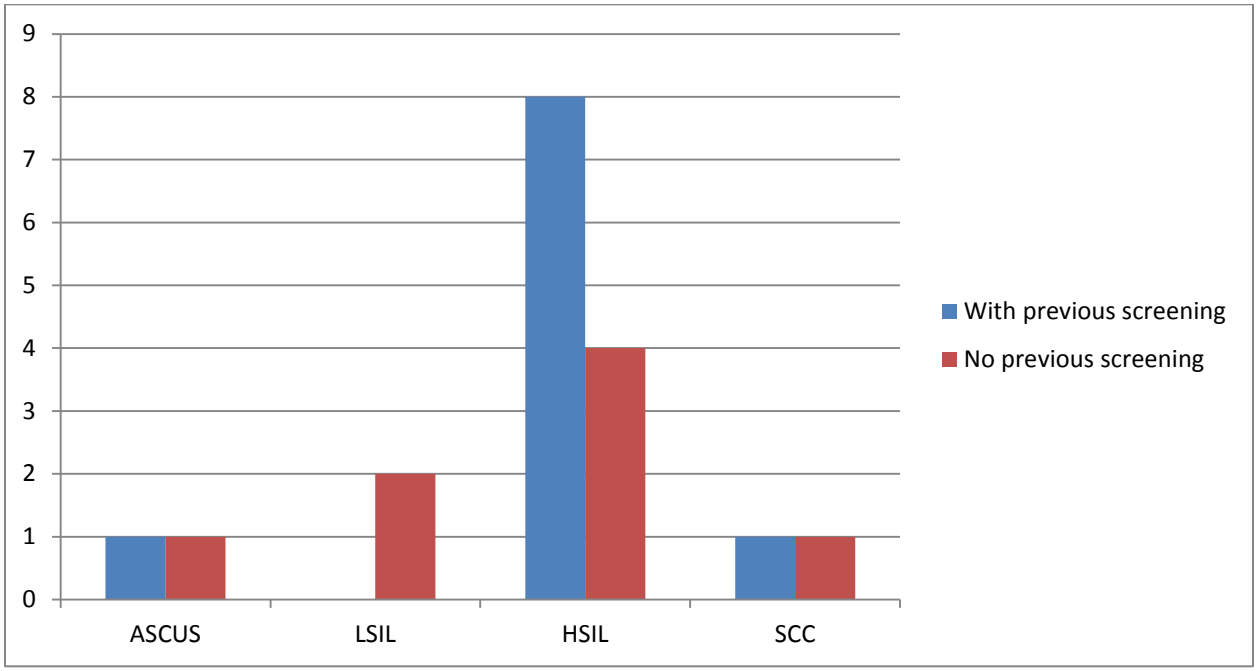
4.4 Bivariate Analysis of SILs in relation to CD4 cells counts, viral load (VL), and use of cART.

Eighteen women with positive cytological abnormalities were on cART. A hundred and ninety two (94%) had negative cytological abnormalities with no statistical significance, p =0.605. The mean CD4 count of those with a negative cytological result was slightly higher, 561.3 (SD= 244) than those who had positive cytological results 492.9 (SD= 231.1), without statistical significance, p = 0.297. A few women 147(74%) with a negative cytological abnormalities had CD4 less than 200 cells/mm^3. The women with positive cytological abnormalities with CD4 cell counts less than 200 cells/mm^3 were 12 (66.7%), without statistical significance p= 0.555. Similarly for HIV viral loads, a slightly higher proportion of women with a negative cytological abnormalities had viral loads less than 5000 copies compared to those with a positive cytological abnormalities, 53(79.1%) vs. 5 (62.5%), without statistical significance p= 0.427(Table 4).

Table 4: Distribution of SILs in relation to CD4 cells counts, viral load (VL), and use of cART among HIV positive women.

Variable	Negative n= 204	Positive n=18	Test stat.	P value
	n(%)	n(%)		
ART use			Fisher's exact test	0.605
No	12 (5.9)	0 (0)		
Yes	192 (94.1)	18 (100)		
CD4 CELL COUNTS			t-test (194 df) = 1.05	0.297
mean(SD)	561.3 (244)	492.9 (231.1)		
CD4 Cell Counts Categories			Fisher's exact test	0.555
<200	147 (74.2)	12 (66.7)		
>500	27 (13.6)	4 (22.2)		
200-500	24 (12.2)	2 (11.1)		
HIV Viral loads Categories			Fisher's exact test	0.427
<500	53 (79.1)	5 (62.5)		
>1000	13 (6.4)	3 (37.5)		
500-1000	1 (1.5)	0 (0)		

Figure 2.3 Proportion of women with positive cytological lesions versus screening history



Photomicrographs of Squamous Intraepithelial Lesions and Infections (Leica) Pap Stain

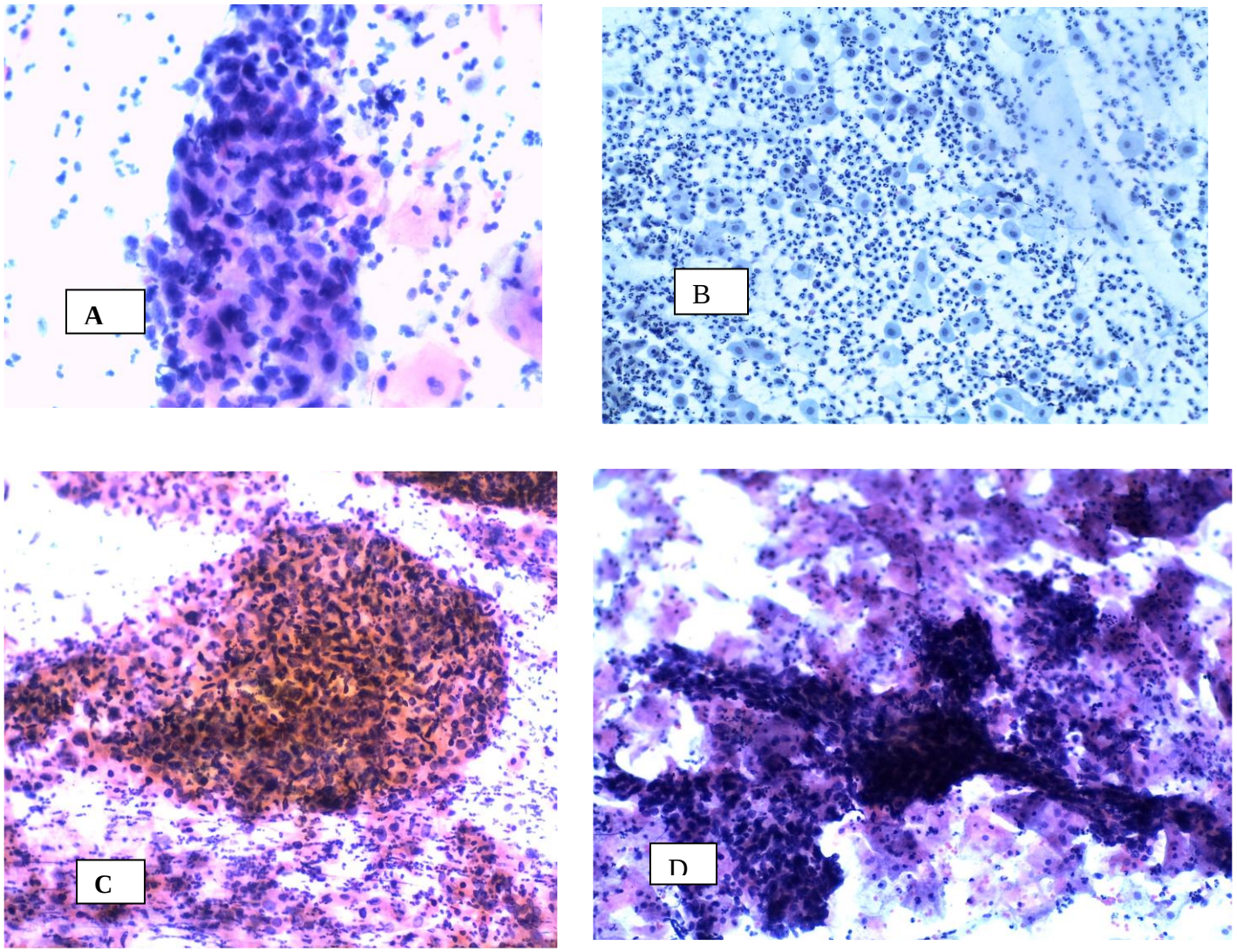


Figure 1: Photomicrographs of **(a)** Low grade squamous intraepithelial lesion (LSIL) showing intermediate squamous cells exhibiting slight nuclear enlargement, hyperchromasia, coarse chromatin and abundant cytoplasm (**b**) Atrophic cervicitis showing parabasal sized cells with dense cytoplasm, regular round nuclear outlines (**c**) Keratinizing squamous cell carcinoma (KSCC) with small cells showing hyperchromasia, irregular nuclear membranes (**d**) HSIL cannot exclude micro invasion showing three dimensional hyperchromatic crowded groups with marked hyperchromasia, pleomorphism, coarse chromatin and irregular nuclear membranes

5.0 DISCUSSION

Combined Antiretroviral Therapy (**cART**)is known to improve the quality of life of HIV infected patients by restoring or preserving the immunologic function, suppressing viral replication and in reduction of HIV related morbidities and mortalities.

In this study, the prevalence of cervical cytological lesions is 9.9% which is lower as compared to other studies done in Kenya and Africa. In a study done in Kenya by Memiah *et al* among HIV infected women the prevalence rates was 26.7% (17)Another unpublished study done in the Comprehensive Care clinic in 2014 was 36.7%. Studies conducted in South Africa, Uganda and Zambia reported a prevalence of cervical pre-cancer and cancer among HIV-positive women of 66.3%, 73.0% and 76%, respectively (38-40). The high prevalence of cytology lesion in these studies can be explained by a high prevalence of High risk HPV types. Several studies in West Nigeria found the prevalence rates to be between 10.9-17.8% (5). The prevalence rate in this study was lower probably due to early initiation and duration of combined antiretroviral therapy as opposed to other studies who had unknown status of antiretroviral therapy.

Frequent cervical cancer screening may have contributed to less prevalence rates. The national cervical cancer screening guidelines recommends that HIV infected women should be screened every 1 year because of their high risk to development of cervical cytological lesions. At the comprehensive care center, VIA/VILLI are done annually and positive cases referred for further management in national hospitals. Most of the women in this study assented to having the VIA/VILLI done in 2014 at the Comprehensive Care Center. Studies done by Adler *et al* and Minkoff *et al* (20) discovered that women on cART with normal smears initially were 38% less likely to develop cytological abnormalities in follow up pap smears. They also demonstrated a regression of SILs and prevalence rates for women on antiretroviral therapy for over a median of over 5 months Of the HIV infected women in this study, half of the women with previous cervical cancer screening did not develop any lesions.

Studies done by Memiah *et al* (17) and Bansal B *et al* showed that women with CD4 cell counts of 200 cells and below are at 1.6 times risk of developing cervical cytological abnormalities compared to those with CD4 cells 200 cells/mm³and above. In this study the bivariate results showed that women with CD4 cells counts of 500 cells and less were associated with having

cervical dysplasia. This explains the role immunosuppression has on progression of cervical dysplasia in HIV infected women.

Although cervical cancer screening has taken awhile to be integrated in our health facilities, the beneficial effect of cART on the incidence and prevalence of cervical cytological abnormalities can be used as a tool for advocating service integration especially in settings like ours where pap smears are costly. The see and treat interventions currently existing are effective even as the women are integrated in on antiretroviral therapy.

The women for this study were recruited from Comprehensive Care Centre; which was a true representative of the entire population. The women were drawn from all over the country, from different socioeconomic backgrounds and lifestyles. We were also able to capture an entire average of duration on antiretroviral therapy that was useful in analyzing some of our results. Other studies should be carried out over a longer period of time and a larger cohort that could look at the effectiveness and the adherence to antiretroviral therapy over duration of time.

5.1 Limitations of the study

Cytology results were not correlated with histology which acts as the gold standard.

The clinical information given was not accurate in all instances.

Conclusions

This study demonstrated a reduction of cervical cytological lesions in HIV infected women on cART. The high grade cervical cytological lesions were leading followed by LSIL in this setting. In the development of cervical cytological lesions there was no statistical difference in the women with a history of cervical screening and those with no history.

Recommendations: Periodic Pap smear screening for all HIV infected women because VIA/VILLI is not specific. A larger study should be conducted to determine the long term effect of cART on cervical cancer

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APPENDIX 1: CONSENT FORM

My name is Monica Akinyi Odhiambo, a postgraduate student at the University of Nairobi (M.Sc. Clinical Cytology).I kindly request you to participate in the above study of your free will. The purpose of this consent form is to give you information about my study that will help you in making an informed decision whether to participate in the study or not. I humbly ask you to feel free to ask any questions about the purpose of the research, your role in the study, the possible risk and benefits you will encounter, your rights as a volunteer and anything else about the research for clarification. You are at liberty to be included in the study or not without any coercion. You are entitled to have a copy of this consent for your records.

BENEFITS

This study will help in the early diagnosis of cervical pre-cancerous and cancerous lesions and other abnormalities that will guide the clinician to offer you comprehensive management. In cases where the patient is found to have cancer, I will contact the patients to come over to CCC whereby together with the clinician we will link them to the Reproductive Health Clinic for treatment. Even if there is no financial benefit directly arising from this study, the results obtained in this study will help policy makers in making informed decisions that are best suited in treatment and management of cervical cancer in high risk groups.

RISKS AND INCONVENIENCES

The questions that will be asked during the interview may be quite sensitive and personal, but we will do our best to ensure that you're comfortable and that all the information collected is confidential. If you're not comfortable with the questions, please feel free to skip them. In case you don't understand the language, we can switch to your language of choice. If you're not happy with the whole procedure you are free to withdraw from the study. Slight discomfort may be felt, but the procedure is not associated with any major complications. For confidentiality purposes, all records will be identified by serial numbers only. Completed study forms will be kept in locked cabinets, in an access limited room at the study site. The questionnaire data will be delinked and your name will not appear on any database. No added cost will be asked from you as a result of participating in the study.

Pap collection explanation for study participants

You will be required to lie on an examination couch.

A nurse or a doctor will insert a speculum in your vaginal canal and visualize the cervix.

Suspicious areas will be sampled using Pap smear sterile collection devices in the kit.

The microscope slide will be fixed by the fixative provided in the kit.

The principle investigator will take the sample to the laboratory for examination.

CONFIDENTIALITY

Participation in this study is voluntary and it is part of your routine evaluation. Declining to participate will by no means affect the services you are seeking. You are free to withdraw any time without losing the benefits to which you are entitled in this institution. Names will not be required in the study and you will be identified by study numbers. Questionnaires will be kept for one year then destroyed. Any information given to us will remain confidential and will be for your own benefit. You results will be sent to your file and be communicated to in the usual manner by the doctor or nurse counselor taking care of you during your next visit.

Who to Contact

If you have any questions regarding the participation in this study at any time, you may contact any of the following people:

1. Monica Akinyi Odhiambo (Principal investigator) on telephone number 0712138142 or my supervisors Prof Muchiri (telephone number 0722703364)and Dr Micah Oyaro(telephone number 0733638285), Dr Patel (telephone number 0724214350), Prof M. L. Chindia ERC Chairperson 02-2726300

I……..after reading and being explained the study purpose do hereby give informed consent to participate in the diagnostic study fully aware of the benefits and risks.

I am aware that I can withdraw from this study without loss of any benefit or quality of management to which I am entitled.

Participants Signature/Thumb print ……Date……..

Doctor/Nurse……Date……..

Principal investigator……Date…….

APPENDIX 1 B: FOMU YA IDHINI

KICHWA CHA UTAFITI: UTAFITI KUTUMIA‘‘PAP SMEAR’’ KWA WANAWAKE WALIOGUNDULIWA KUWA NA VIRUSI VYA UKIMWIAMBAO WANAOMEZA MADAWA ZA ANTIRETROVIRALS KWENYE HOSPITALI KUU YA KENYATTA.

Jina langu ni Monica Akinyi Odhiambo, mwanafunzi wa chuo kikuu cha Nairobi idara ya Human Pathology. Ningependa kuomba usaidizi wenu kufanya utafiti huu.Umuhimu wa hii fomu ya idhini nikukusaidia kuelewa na kufanya uaamuzi kama ungependa kuendelea na hii utafiti ama la. Tafadhali kuwa na uhuru wa kuuliza maswali yoyote juu ya umuhimu wa utafiti huu, jukumu lako, faida na madhara utakaopitia kama aliyejitolea na chochote kile ambao unataka maelezo zaidi.

MAELEZO KWA UFUPI NA NJIA YA UTAFITI HUU

Njia ya kizazi yaweza kuwa na dalili za ugojwa wa saratani ambao huletwa na virusi vya HPV kwa wanawake ambao wako na virusi vya ukimwi. Utatolewa kipimo mara moja na njia ya uchunguzi ni rahisi.

Hali yako ya kua na virusi vya HPV itajulikana na uchunguzi kwa njia ya Pap smear yatalinganishwa.

FAIDHA YA UCHUNGUZI NA MADHARA YA UTAFITI HUU KWAKO

Kipimo ya Pap smear ina uwezo wa kugundua dalili ya saratani. Dalili ya magojwa mengine yaweza kupatikana. Hivyo basi utafaidhika kutokana na uamuzi bora wa uchunguzi na uamuzi bora wa matibabu utakayopewa.Hakuna faidha yeyote ya kifedha utakayopata kutokana na utafiti huu.Utaratibu wa kupata kipimokwa njia ya Pap smear hauna madhara yeyote, muhudumiwa aweza kuhisi usumbufu kiwango kidogo ile anaweza vumilia. Matokeo yako itashugulikiwa kwa njia ya siri na hakuna yeyote anayeruhusiwa atakayesisoma. Hakuna malipo ya ziada utakayo hitajika kwa kuhusika katika uchunguzi huu. Majina halisi hayatatumiwa ila nambari ndizo zitakazotumiwa. Matokeo yatawekwa kwa njia ya siri na ni mchunguzi pekee yake ambaye atayapata. Watakao shiriki katika uchunguzi huu itakua kwa njia ya hiari bila kushuritishwa. Kutoshiriki haitapoteza kwa njia yeyote haki yako kuhudumiwa unavyostahili.Majibu ya uchunguzi huu uitapata kwa njia ya kawaida wakati wa kufuata kiliniki yako ya kawaida.

IDHINI YA MSHIRIKI

Kama utashiriki tafadhali tia sahihi yako kwenye pengo lilioachwa hapa chini

Mimi----------nimesoma na nimeelewa nia ya uchunguzi huu, utaratibu utaotumika kuchukua kipimo, faida na madhara yanayohusika na uchunguzi huu. Nimekubali kushiriki kwa hiari bila kushurutishwa.

Sahihi ya mushirika----------Tarehe ----------

Sahihi ya shahindi----------Tarehe

Unaweza wasiliana nasi wakati wowote kupitia nambari zifuatazo iwapo una swali lolote;

ANWANI MUCHUNGUZI,

MONICA AKINYI ODHIAMBO

Chuo Kikuu Cha Nairobi

SLP 19676-00202Nairobi

Numbari ya simu 0721319898

Chuo kikuu cha Nairobi, idara ya Human pathology

Maadali ya utafiti ya KNH/UONERC

SLP 20732, Nairobi Kenya.

Nambari ya simu 02-726300 Ext 44102

APPENDIX II: QUESTIONNAIRE

CERVICAL CYTOLOGICAL PATTERNS AMONG HIV INFECTED PATIENTS ON COMBINED ANTIRETROVIRAL THERAPY AT KENYATTA NATIONAL HOSPITAL.

All consenting participants will be required to fill the questionnaire before specimen collection. Kindly tick one of the choices given.

Section A: Socio demographic information (sdm)

Study number…….

Date ☐☐ ☐☐ ☐☐

DD/ MM/ YR

Residence……

1. Age…….

2. Marital Status

Single ☐

Married ☐

Divorced ☐

Widowed ☐

3. History of Tobacco/ Bhang smoking

NO ☐

YES ☐

If Yes, how long have you been smoking…….

How many cigarettes or packs per day…….

4. Education

Primary ☐

Secondary ☐

☐

College

Not gone to school ☐

5. No of children…….

How many are living……..

Miscarriages ……

6. Last menstrual period…….

7. Ever had a Pap smear

Yes ☐

No ☐

If yes, when…….

8. Family Planning

Natural ☐

Condom ☐

Injection ☐

Pill ☐

IUCD ☐

No of sexual partners……

Age/Year of first intercourse……..

Section B: Clinical history (CH)
Tick appropriately

Appearance of the cervix

1. Normal ☐
2. Eroded ☐
3. Inflamed ☐

4. Suspicious ☐

If other, specify……

History on ART

Less than 6 months ☐

6-12 months ☐

1-4 years ☐

5-9 years…….. ☐

>10 years…….. ☐

Section C: For Investigator's Only

1. Specimen Adequacy

   Satisfactory ☐

   Unsatisfactory ☐

   If Unsatisfactory, proceed to the end of the questionnaire.

2.Epithelial cell features

Negative ☐

ASCUS ☐

LSIL ☐

Inflammatory ☐

Reactive ☐

ASC H ☐

HSIL ☐

SCC ☐

AGC ☐

AIS ☐

Adenocarcinoma ☐

3.HIV Viral Loads, copies/ml

>1000……

500 1000……

<500……..

4. CD4 Cell counts, cells/mm^3

>500 ☐

200-500 ☐

<200 ☐

COMMENTS

Refer ☐

Call Back ☐

Other, specify**.....**

PRINCIPAL INVESTIGATOR'S NAME……

SIGN……..

PATHOLOGIST'S NAME……

SIGN…….

DATE…….

APPENDIX III: THE BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY (2014)

- The Bethesda System-2014 consists of several components, as outlined below, and is recommended for reporting cervical cytology.

SPECIMEN ADEQUACY

- Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone component and any other quality indicators, e. g, partially obscuring blood, inflammation, etc.)
- Unsatisfactory for evaluation (specify reason)
- Specimen rejected/not processed (specify reason)
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

GENERAL CATEGORIZATION (optional)

- Negative for intraepithelial lesion or malignancy
- Other: see Interpretation/Result (e.g., endometrial cells in a woman aged _45 years)
- Epithelial cell abnormality: see Interpretation/Result (specify "squamous" or "glandular," as appropriate)

INTERPRETATION/RESULT

- Negative for Intraepithelial Lesion or Malignancy
- (When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the
- report—whether or not there are organisms or other non-neoplastic findings)
- **Non-Neoplastic Findings (optional to report)**
- Non-neoplastic cellular variations
- _ Squamous metaplasia
- _ Keratotic changes
- _ Tubal metaplasia
- _ Atrophy
- _ Pregnancy-associated changes

- Reactive cellular changes associated with:
- Inflammation (includes typical repair)
- Lymphocytic (follicular) cervicitis
- Radiation
- Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

Organisms

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

Other

- Endometrial cells (in a woman aged _45 years)
- (Also specify if "negative for squamous intraepithelial lesion")

Epithelial Cell Abnormalities

Squamous Cell

- Atypical squamous cells
- _ of undetermined significance (ASC-US)
- _ cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
- (Encompassing: HPV/mild dysplasia/CIN-1)
- High-grade squamous intraepithelial lesion (HSIL)
- (Encompassing: moderate and severe dysplasia, CIS; CIN-2 and CIN-3)
- _ with features suspicious for invasion (if invasion is suspected)
- Squamous cell carcinoma

Glandular Cell

- Atypical

- _ Endocervical cells (NOS or specify in comments)
- _ Endometrial cells (NOS or specify in comments)
- _ Glandular cells (NOS or specify in comments)
- Atypical
- _ Endocervical cells, favor neoplastic
- _ Glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
- _ Endocervical
- _ Endometrial
- _ Extrauterine
- _ not otherwise specified (NOS)
- Other Malignant Neoplasms (specify)

ADJUNCTIVE TESTING

- Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY

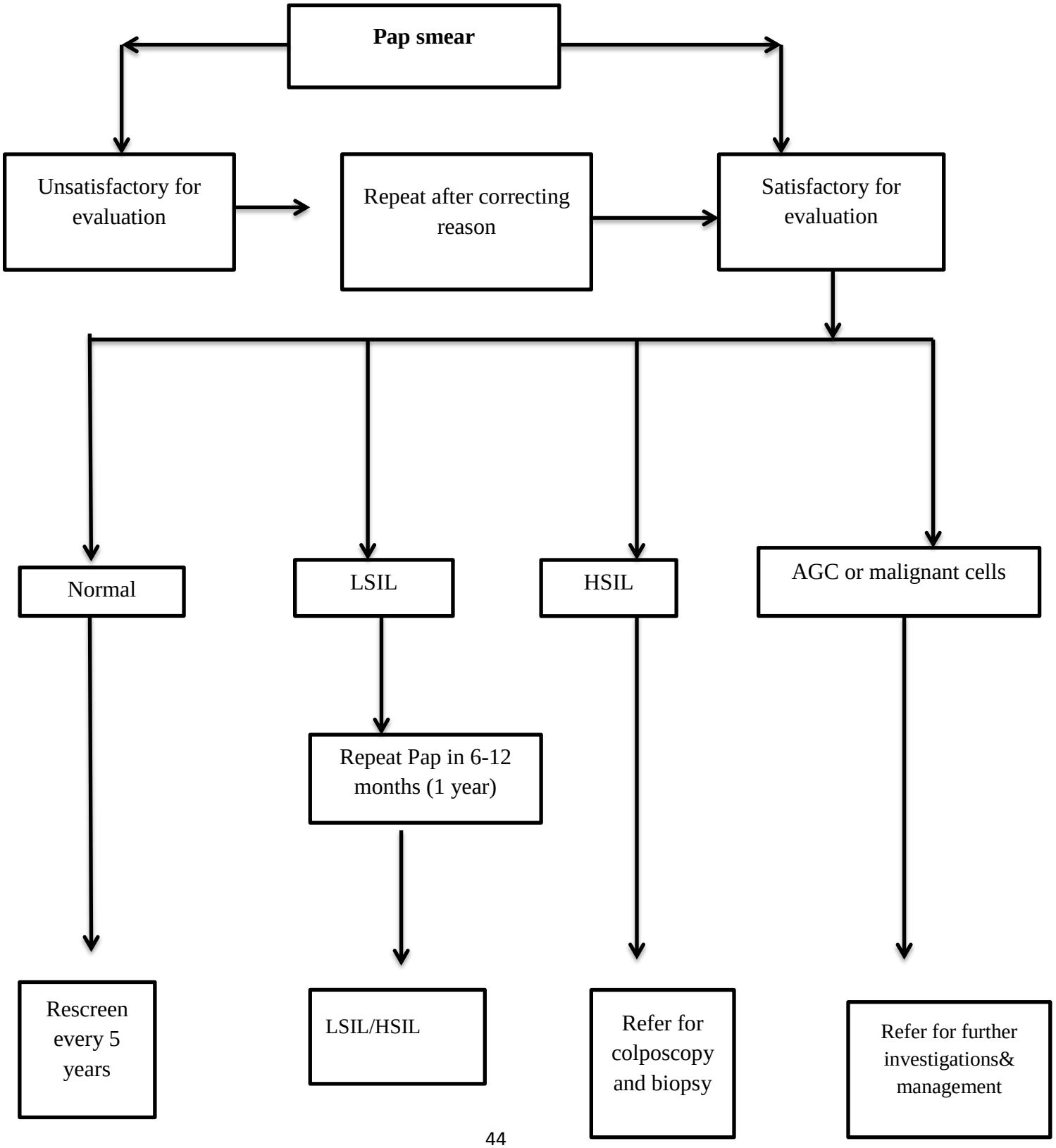
- If case examined by an automated device, specify the device and result

EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (optional)

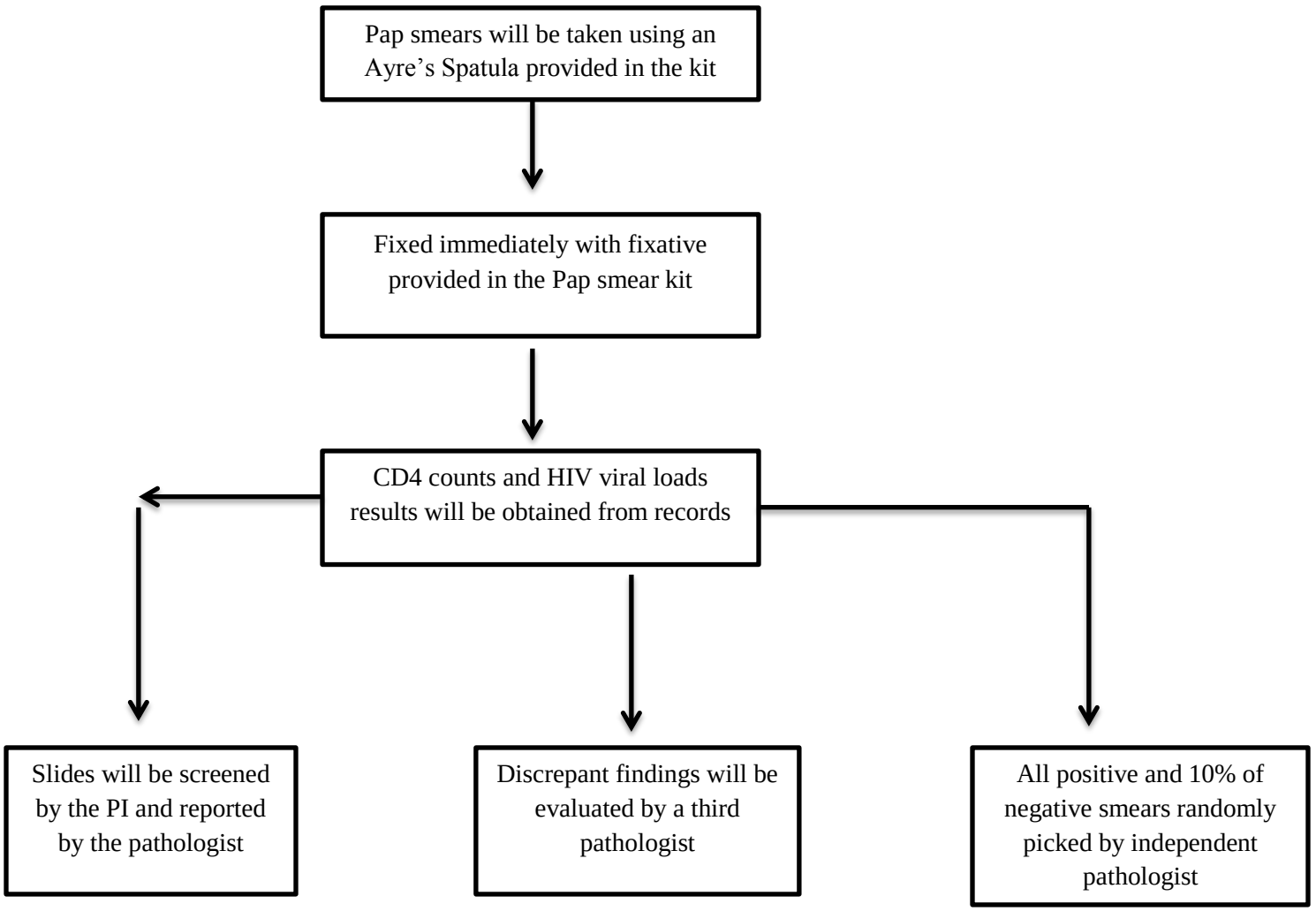
- Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included)

Source: The Pap test and Bethesda 2014, Ritu Nayar, MD and David C. Wilbur, MD

APPENDIX IV: KENYA GOVERNMENT GUIDELINES FOR MANAGEMENT OF WOMEN AFTER PAP SMEAR TEST



APPENDIX V: Sample Collection Procedure



Women CIN1

APPENDIX VI: SAMPLE COLLECTION AND HANDLING FOR CD4 CELL COUNTS AND HIV VIRAL LOADS.

Blood samples were collected by phlebotomists and put in potassium EDTA vacutainer tubes. 3-5 ml of whole blood is adequate for analysis of CD4 cell counts and HIV viral loads. Tubes were labeled accurately with patient's identification number, date and time of collection. Blood was mixed properly by inverting the EDTA tube six to eight times to prevent formation of clots that interfere with accuracy of the counts and ability to run the instrument. Specimens were processed within a maximum of 48 hours after phlebotomy. Whole blood with EDTA and cell-free EDTA plasma were stored at room temperature for up to 30 h, at 4°C for up to 14 days, and at −70°C for extended periods of time without significant decreases in viral load signal. If blood sample is to be transported to a laboratory elsewhere; specimens were placed in a leak proof container preferably a sealed plastic bag with zip lock, or can be placed in a tube rack packed in a cool box container for safe transport.