HPV DETECTION IN URINE AND CERVICAL SAMPLES AND CYTOLOGICAL PROFILE OF WOMEN WITH HIV IN KENYATTA NATIONAL HOSPITAL: A COMPARATIVE CROSS-SECTIONAL STUDY

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H58/80970/2015

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A dissertation submitted in partial fulfillment for the award of Master of Medicine in Obstetrics and Gynecology in the Department of Obstetrics and Gynecology, School of Medicine, College of Health Sciences, University of Nairobi.

2021

DECLARATION

This is to declare that this dissertation is my original work, carried out with guidance from my supervisors, and references made to work done by others have been indicated.

CONFLICT OF INTEREST

The samples collected were processed at the main laboratory of Lancet Laboratories, a private ISO certified specialized tertiary laboratory. The machine that was used to analyse is Cobas 4800 (Roche). Neither the Lancet group nor Roche played a role in the development of the proposal leading to this dissertation and had no interest in the study. Thus the principal investigator (PI) bore the entire cost of the tests.

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LIST OF ABBREVIATIONS AND ACRONYMS

ASC-H	Atypical squamous cells cannot exclude HSIL
ASC-US	Atypical squamous cells of undetermined significance
BV	Bacterial Vaginosis
CD4	Cluster of Differentiation 4
CDC	Center for Disease Control and Prevention
CIN	Cervical Intraepithelial Neoplasia
HIV	Human Immunodeficiency Virus
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes Simplex Virus
KNH	Kenyatta National Hospital
LSIL	Low-grade squamous intraepithelial Lesion
NCI	National Cancer Institution
NGO	Non-Governmental Organization
NILM	Negative for intraepithelial lesion or malignancy
NPV	Negative predictive value
PCR	Polymerase chain reaction
PPV	Positive predictive value
SSA	Sub-Saharan Africa
STIs	Sexually Transmitted Infections
UoN	University of Nairobi
WHO	World Health Organization

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ABSTRACT

Background: Human immunodeficiency virus (HIV)–positive women are at higher risk of Human Papillomavirus (HPV) acquisition. While urine-based HPV testing could improve poor cervical cancer participant screening rates and circumvent the need for an annual Papanicolaou test (Pap test) for HPV negative women, it has not been studied to date in Kenya.

Objective: To compare the rate of detection of HPV in urine and cervical samples in HIV-positive women.

Design and Setting: A cross-sectional comparative study where HPV DNA was tested in a paired urine sample and cervical samples of women at the Comprehensive Care Centre between September 2019 and November 2019 at the Kenyatta National Hospital, Nairobi–Kenya.

Materials and methods: Roche Cobas 4800 Assay was used for testing 71 paired cervical and urine samples respectively for HPV DNA. The levels of agreement of the paired samples were assessed using kappa coefficient (k), with a 95% confidence interval (CI). The probability values (p-values) from McNemar's test were used for significance test. A multivariable Poisson regression model with a robust variance estimator was used to model the correlates of risks.

Results: HIV-positive women of mean age of 44.28±10.6 years were studied. The prevalence of HPV was 68% (95% CI: 55%–78%) in cervical samples and 35.2% (95% CI: 24% - 47%) in urine samples. The level of agreement between urine and cervical samples for any HPV was substantial (% agreement=84.5%, k= 0.69, 95% CI=0.5146–0.8563), fair for any HR HPV (% agreement = 67.6%, k=0.41, 95% CI=0.2160–0.6104, substantial agreement for HPV 16 and/or HPV 18 (% agreement=93.0%, k=0.70, 95% CI=0.4454–0.9534), and fair agreement (k=0.39, CI 95%=0.1922–0.5889) for other HR HPV (Non HPV 16/18). The McNemar's test and Chi-square tests showed that the performance of the urine and cervical samples were not equal (p-values <0.05). Sensitivity of urine for any HPV detection was high at 77% (95% CI=63% – 88%), while specificity was 100% (85%–1.00%). The pap smears were mostly negative for intraepithelial lesion or malignancy (NILM) (91.5%). Being married (monogamous) was associated with HR HPV prevalence that was 41% (adjusted Prevalence Ratio 0.59, 95% CI: 0.36–0.99) lower than those of divorced/separated women (p<0.05).

Conclusion: The detection rate in urine sample was a little lower than in cervical samples. These findings demonstrate that urine-based HPV test can be employed not as a single test as cervical sample test and certainly not as an alternative method but as a cotest with possible improvements of sample collection and processing.

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

The Human papillomavirus (HPV) is the most prevalent cause of sexually transmitted viral disease globally and the chief cause of cervical cancer in women (1). Persistence of HPV infections for between 10 and 15 years causes cervical squamous intraepithelial lesions (SIL) and Invasive Cervical Cancer (ICC) (2). Up to now, more than 200 genotypes of HPV have been characterized. Of which over 40 of HPV genotypes infect the mucosal and epithelial lining as well as other areas (3).

HPVs are categorized according to the risk they pose on various epithelial sites. For example, HPV types 16, 18, 31, are high risk (HR)/oncogenic types and involved in the etiology of majority of cervical cancers while HPV types 6, 11 and 44 are low risk (LR) types (4,5) and the basis of low-grade cervical lesions, recurrent respiratory papillomatosis, and genital warts.

The standard for the detection/screening for HPV and related cervical cancer and/or precancerous conditions comprise of the Papanicolaou (Pap) test, biopsy and Liquid Based Cytology (LBC), visual inspection using acetic acid and Lugol's iodine (VIA-VILI), in addition to HPV Deoxyribonucleic Acid (DNA) testing using a cervical brush. The disadvantages of these methods include the requirement for a pelvic examination, an invasive exam. It is uncomfortable for the patient as well as time-consuming for the health worker. (6). Detection methods for pathogenic microorganisms' DNA in patients' urine have been utilized in the diagnosis of other common STIs that are affecting the cervix, for instance, *Neisseria gonorrhea* and *Chlamydia trachomatis* infections (7). Therefore, the use of non-invasive urine-based samples for routine HPV detection may well afford a preliminary diagnosis for cervical cancer and at the same time circumventing the requirement for 3 yearly Pap test for HPV DNA negative women. One specimen may be enough to concurrently screen all the agents of infection.

HPV detection in urine can be used as an ancillary testing method for cervical cancer to triaged women having atypical squamous cells of undetermined significance (ASC-US). Several researchers have tried a urine sample for the detection of HPV DNA (8). However,

there has been no study up to now addressing the detective ability of HPV DNA in the urine among Kenyan women, more so Human immunodeficiency virus (HIV) –positive women.
HIV-positivity is linked with rising rates of incident HPV infections, multiple and persistent HR HPV incidences, dysplasia as well as cervical cancer. Once cervical cancer occurs in this population, it becomes more aggressive and less responsive to treatment. And it's these factors that underpin the significance of cervical cancer testing in these HIV-positive women. The non-invasive usage of urine in the detection of HPV DNA and comparing it with matching cervical swab specimens in the aforementioned population is the main aim of this study.

1.2 Epidemiology of HPV

1.2.1 A brief overview of the global prevalence of HPV

Global estimates demonstrate that HPV infection is the most common STI. However, the prevalence of HR HPV in women having normal cervical cytology differs among the world regions. It is estimated to vary from 2 to 44%, depending on the population, the severity of the injury and geographical region studied (9).

The Information Centre on HPV and Cervical Cancer (the HPV Information Centre) (10) report a global HPV 16/18 prevalence of 3.9% in normal cytology, in low-grade cervical lesions/Cervical intraepithelial neoplasia-1 (LSIL/CIN-1) 25.8%, in high-grade cervical lesions/Cervical intraepithelial neoplasia-2 (HSIL/CIN-2 /CIN-3 /carcinoma *in situ* (CIS)) 51.9% and cervical cancer of 64.9%. A high prevalence of 24% has previously been reported in sub-Saharan Africa, 21.4% in Eastern Europe and Latin America with 16.1% (11). This may suggest that the distribution of HPV genotypes may be area-specific.

1.2.2 Epidemiology of HPV in Kenya

The current report from HPV Information Centre (10) on Kenya indicates an HPV 16/18 normal cytology prevalence of 9.1%, 21.4% in LSIL/CIN-1, 45.0% in HSIL/CIN-2/CIN-3/CIS). Cervical cancer prevalence is 63.1% from the 2018 estimates and newly diagnosed cases being as high as 5,250. Cervical cancer is ranked the most common cause of cancer-related deaths among the females of ages 15–44 and the second chief cause of cancer among females.
Several individual studies have found a high prevalence of HPV in Kenya. These studies have reported area-specific distribution. According to Ngugi et al.(12), the prevalence of

HPV in the Thika district was 21.3% among women, with the prevalence of HR HPV 16 and type 18 reported as 43.7% and 17.2% respectively. In 2008, Yamada et al. (13) reported prevalence as high as 49% among HIV-positive women and 17% among HIV-negative women in Nairobi.

A recent study by Menon et al. (14) on the genotypic epidemiology in HIV-positive women reported an overall prevalence of HR HPV genotypes of 64%. In women with abnormal cytology, HPV 16 was found to be 26%, HPV 35 (21%) and 52 (18%) and is the most prevalent. However, in those with ICC, the Menon et al.'s study found that HPV 16 was 37% prevalent and HPV 18 was 24% being the most prevalent. In Kenya, ICC is the most frequent malignancy and is associated with less than 20% survival as findings from a prospective cohort study conducted by Maranga et al. (15) indicated.

Among outpatient women in a Nairobi Health facility, Omire et al.'s (16) study on risk predisposition reported that 35.3% of women were reactive for HPV L1 (gene) DNA. Omire et al.'s study also showed that 4.3% had abnormal cervical cytology with 3/8 being of HSIL, 1/8 of LSIL, 1/8 had adenocarcinoma while the other 3 were ASC-US.
Luchters et al. (17) in a community-based survey done in Mombasa found that more than half (55.6%) of female sex workers (FSW) were infected with HR HPV. Among these FSW, 54.9% had at least two or more different HR HPV types. Luchters et al. also reported that 22.8% were HPV 16/18, but more than twice as many had HR HPV types excluding HPV 16/18. Additionally, 25% of the FSW had LR HPV type 6/67. KNH research by Maranga et al. (18) on the HPV subtypes established that smear samples HIV-positive women had multiple HPV infections than HIV-negative women. Similar to Maranga et al., De Vuyst et al. (19) demonstrated that multiple-type infections are highly prevalent (37.2%) in HIV-positive compared to negative (13.7%) women but with similar type distribution.

A study done by He et al. (20) at KNH found strong correlation between Human T-cell leukemia virus type 1 or Human T lymphotropic virus type 1 (HTLV-1) infection (related to HIV infection) with numbers of sexual partners smoking and an unexpectedly high HTLV-1 DNA prevalence in HIV-positive women.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Disease Spectrum of HPV

2.1.1 Virology and Pathogenesis

2.1.1.1 Virology

HPV is a small, nonenveloped viral DNA that belongs to the Papillomaviruses in the *Papovaviridae* family. It has an icosahedral capsid composed of 72 capsomers. Each capsomer contains a pentamer of the major capsid protein, L1 and several copies of minor capsid protein L2 (21). The HPV genome has a single molecule of double-stranded, non-enveloped circular DNA with approximately 7,900bp. Over 100 HPV types have been characterized just on the basis of the DNA sequence divergence. Moreover, HPVs are categorized either as cutaneous types or mucosal types because of the epithelial sites they do colonize. The cutaneous HPV types are epidermitropic whereas the mucosal types are (highly) epitheliotropic.

2.1.1.2 Pathogenesis

Infection by HPV takes place at the squamous epithelial cells occurring in layers (stratified) in the basal membrane. The infection triggers differentiation in the epithelial cells and infected cells show a wide range of alterations, from benign hyperplasia, dysplasia and then invasive carcinoma. For effective replication, HPV uses and controls the cellular machinery of its host. During the process, the protein product of the virus that is encoded by E6 and E7 attaches to the genes that control the cell cycle known as the tumor suppressor gene p53, resulting in the p53 protein early disintegration. The E7 protein attaches to a tumor suppressor protein known as the retinoblastoma then alters and controls its function (22). These two proteins facilitate considerable oncogenesis of the virus and their assembly denotes the main distinction between LR and the HR HPV strains.

2.1.1.3 Types and Disease Spectrum

The HPV infection is linked with diverse clinical diseases from benign warts to invasive cervical lesions. Most of these infections are asymptomatic, or subclinical. Patients with a clinically evident disease have a variety of possible presentations that associate with the type of HPV and the host factors. Among these, the four most common manifestations include cervical dysplasia, anogenital warts, anogenital cancers, and anal dysplasia.

2.2 Cervical Cancer

Among Kenyan women, cervical cancer accounts for 18-23% of all cancers, being the second most prevalent among diagnosed cancers. The relationship between infection with HPV and cervical cancer was originally established in the early 1980s and the relationship is greater than that between lung cancer and smoking (23). HPV 16 accounts for 50% of the cervical cancer cases while HPV 18, 31 and 45 account for 25 – 30% (23). The cervical adenocarcinomas are also associated with HPV, but the association is less definite and is dependent on age.

2.3 HPV and HIV correlation

HPV and HIV interaction may in a vicious circle favor each other. Studies have found that HPV infection to be a significant HIV acquisition risk factor; alternatively, HIV amplifies the acquisition and persistence of HPV in individuals co-infected with HIV as Nowak et al. (24) found and is a prognostic indicator of poor treatment outcomes for ICC (Invasive Cervical Cancer). The World Health Organization also incorporated ICC to the clinical staging of the Acquired Immunodeficiency Syndrome or AIDS classification for resource-poor settings (25). In Kenya, Maranga et al. (18) established less than 20% survival in women with ICC.

A meta-analysis identifying HPV DNA by Houlihan et al. (26) revealed strong evidence of an elevated risk (2.06) contracting HIV with any strain of HPV genotype prevalent. This is the trend reported in global studies. For instance, in HIV-positive women, a prevalence as high as 47.5% has been reported in Brazil (27), of which correlates HPV infection were cytological alterations, advanced age, more than three sexual partners, < 200/mm³ CD4+ lymphocyte count, and alcohol abuse were correlates of infection. Typically, HIV status and HPV-type may affect the profile of lympho-mononuclear cells in the cervical lesions spectrum. Comparable study findings have been described by Jolly et al. (28) in the Kingdom of Swaziland in which cervical lesions were more in HIV-positive (22.9%) women than those HIV-negative (5.7%).

2.3.1 HIV and HPV as biological co-factors

HIV-infection favors HPV-infection both at cellular and clinical levels. HIV proteins (tat and gp120) in combination with host cytokines (TNF-α and IFN-γ) disrupt the epithelial tight junctions potentiating penetration of HPV into the basal cells, which are targeted by HPV. The same *tat* protein enhances HPV transcription and replication thus enabling expression of

HPV *E* oncogenes and L capsid proteins in the cells. Finally, in the AIDS stage, there is a persistence of HPV as HIV induces a shift of TH1 to TH2 leading to an escape of HPV from immune surveillance.

Clinically HIV favors HPV infections since they both share common routes of transmission, and that progressive immune depression by HIV enhances HPV viral load (VL) and frequent, persistent HPV infection. This high HPV VL and persistent infection lead to the development of Squamous Intraepithelial Lesion (SIL) independent of CD 4 cell counts. HIV-infection also favors HPV-related dysplastic manifestations. For instance, a 38.3% prevalence of abnormal cervical cytology in HIV-infected patients higher than in HIV-uninfected women (16.2%).

2.4 Detection of HPV infection

2.4.1 Cytology testing

The conventional Pap test is the standard tool for screening of cervical cytological abnormalities and changes often due to HPV infection. The current Pap smear reporting classification is the Bethesda system (29). However, screening by this method has some limitations, including high false-negative rates. It has been reported that only 15–50% of HPV-infected patients are correctly identified by Pap test (30). Newer methods of collection (in a preservative solution) and preparation of Pap smear specimens have been developed. Among these are the PrepStain system and Thin Prep Pap Smear technique approved by the Food and Drug Administration (FDA).

2.4.2 VIA/VILI

After application of Acetic acid and Lugol's iodine, comprehensive visual inspection of the cervix is done. The findings from the colposcope are then graded depending on the degree of the aceto-white lesion, mosaic pattern, surface contour, and punctuation. Higher levels of abnormalities are associated with lesions severity.

2.4.3 Histopathology

Colposcopy is able to identify high-grade and low-grade dysplasia. However, it cannot detect micro-invasive disease. In case abnormalities aren't established or if the whole squamo-columnar junction is not visualized, then a cervical cone biopsy is obtained. The presence of abnormal koilocytes, or koilocytosis, is distinguishing features of HPV-infection. Additional markers of HPV-infection comprise dyskeratosis, acanthosis, as well as multinucleation.

Monoclonal antibodies can be used to demonstrate HPV antigens in the biopsy sample. Moreover, both HPV DNA and messenger Ribonucleic acid (mRNA) could well be established in biopsies done through in situ hybridization.

2.4.4 HPV DNA methods

The Hybridization of nucleic acids of the virus from the cervical swab is the established routine detection technique. Two HPV DNA detection methods currently are the Polymerase chain reaction (PCR) method (either as type-specific or general primer PCR) and the liquid hybridization assay (Hybrid Capture Kit). An HPV DNA test is favored in the management of Pap smear showing ASC-US. An HPV DNA test can be used singly for primary screening of cervical cancer in women of 25 years or above.

1.2.2.1 HPV DNA in Urine

Urine-based HPV DNA detection presents a reasonable alternative to cervical specimen-based HPV DNA detection. It's a simple and non-invasive approach to screening (31). HPV DNA urine testing is employable in the identification of abnormal cells in young women and adolescents not wishing to have pelvic examination (32).

2.5 HPV prevention

Prevention of HPV infection entails both behavioral risk reduction and the use of a vaccine. Abstinence from sexual activity is the most dependable method for genital HPV infection prevention. Patients can reduce their likelihood of acquiring and transmitting HPV by consistently and correctly using a condom and limiting the number of sexual partners.

The HPV vaccine, made from the L1 major protein through recombinant technology has made a significant impact in reducing HPV-infection related morbidity and mortality (33). Studies in the United States have revealed a 64% prevalence reduction of the four vaccine-targeted HPV types among females aged between 9 and 14 years and a 34% reduction among 20–24-year-old females (34). Other countries including Australia and Canada have reported a similar impact (35).

Indications include boys and girls between ages 11 and12 years, females between ages 13 and 26 and males between age 13 and 21 who haven't began or finished the HPV vaccine series, previously unvaccinated, immune-compromised persons (as well as HIV-infected individuals) including men who have sex with men or MSM past age 26 years. It hasn't been

recommended for women or men aged above 26 as well as in pregnant women.
The two licensed vaccines in Kenya are *Cervarix®*, from GlaxoSmithKline, a bivalent virus-like particle (VLP) HPV vaccine acting against HPV 16 and HPV 18 and *Gardasil®* from bioCSL/Merck & Co Inc., which is a quadrivalent vaccine effective against HPV types 16, 18, 6 and 11. In Kenya, vaccine trials have shown a positive response among women.

2.6 Studies on paired urine and cervical samples by HPV DNA Detection Assays

Several authors cited by Nilyanimit et al. (36) report varying concordance rates ranging from 65.2% to 100% between the cervical swab and urine specimen from different detection assays. Specifically, of values above 85%, Daponte et al. (37) reported 85.7%, Hagihara et al. (38) (98.4%), while Gupta et al. (39) found 100% concordance. Concordances of 76% or below were reported by Cuschieri et al. (40) (59.8%), and Nilyanimit et al. (41) (75%). Another study revealed an agreement of 78.90% (42).

In all these studies cited in Nilyanimit et al. (36), only Bernal et al. (43) used Cobas 4800HPV test and found 88% concordance with a sensitivity of 90.5% and specificity of 85%. Bernal et al.'s value is close to that reported by Khunamornpong et al (44) (86.2%) from The Cobas 4800 assay but had a kappa statistic of 0.65 which they concluded was a substantial agreement.

Scientific literature also reveals some variations in prevalence patterns in the paired samples depending on the study goals and setting. Considerable variation in distribution is quite apparent such as HPV types found in Thai women and those found in Kenyan women by Maranga et al. (45). Equally, in terms of prevalence, Jong et al. (46) found an HPV urine sample prevalence of 81.5% and in cervical smear samples prevalence of 52%; concordance was 71%. Comparatively, a study in Colombia found 70.6% and 63.2% infection in corresponding samples of cervix and urine.

Some studies have reported a high HPV detection sensitivity for urine-based assays, while other studies have reported a low HPV detection sensitivity from urine-based assays. It is a noninvasive method as it also permits the simultaneous detection of various infectious agent.

CHAPTER THREE

3. STUDY JUSTIFICATION, RESEARCH QUESTION, HYPOTHESIS, AND CONCEPTUAL FRAMEWORK

3.1 STUDY JUSTIFICATION

HPV is the most likely etiological agent in cervical cancer. In sub-Saharan Africa HIV, endemic in this region, augments HPV infection. The complex interplay between these two viruses could influence progression to Invasive Cervical Cancer.

The Pap smear is a cost-effective method for cervical cancer screening. Its limitation includes the inability to detect asymptomatic HPV infection, sociocultural barriers as it involves a pelvic examination. In addition, it has limited accuracy in the detection of cell changes due to HPV infection. These limitations warrant alternative and complementary HPV detection tests. Urine-based HPV DNA testing methods offer simple non-invasive alternative or complementary methods to Pap smear.

This study purposed to determine how urine-based HPV DNA testing compares with testing in paired cervical samples of HIV positive women attending the Comprehensive Care Centre (CCC) of the Kenyatta National Hospital (KNH) within Nairobi–Kenya. This study assessed the sensitivity and specificity which are components of detection rates useful in diagnostic medicine (bioassays).

3.2 RESEARCH QUESTION

How comparable is the detection rate of human papillomavirus in urine and in cervical samples in human immunodeficiency virus-positive women attending the Comprehensive Care Centre at the Kenyatta National Hospital?

3.3 HYPOTHESIS

3.3.1 Null Hypothesis

There is no difference in HPV detection rate between using urine and cervical samples in HIV-positive women.

That is, H_0: Kappa ≥ 0.84

3.3.2 Alternative Hypothesis

There is a difference in the HPV detection rate between using urine and cervical samples in the HIV-positive women.

That is, one-sided alternative hypothesis, H_1: Kappa value < 0.84.

3.4 OBJECTIVES

3.4.1 Broad objective

To compare the detection rate of human papillomavirus in urine and cervical samples in HIV-positive women visiting the Comprehensive Care Centre within the Kenyatta National Hospital.

3.4.2 The specific objectives

i) To determine the prevalence of HPV by urine and cervical samples and assess the concordance of detection of HPV between the paired samples.

ii) To establish the demographic and clinical correlates of risks associated with HPV infection using cervical samples as the gold standard.

iii) To evaluate the cytology profile of the HIV positive women by HPV status of the urine and cervical samples.

iv) To determine the prevalence of abnormal cytology and HPV type-specific prevalence by cervical cytology status of the urine and cervical samples.

3.5 CONCEPTUAL FRAMEWORK

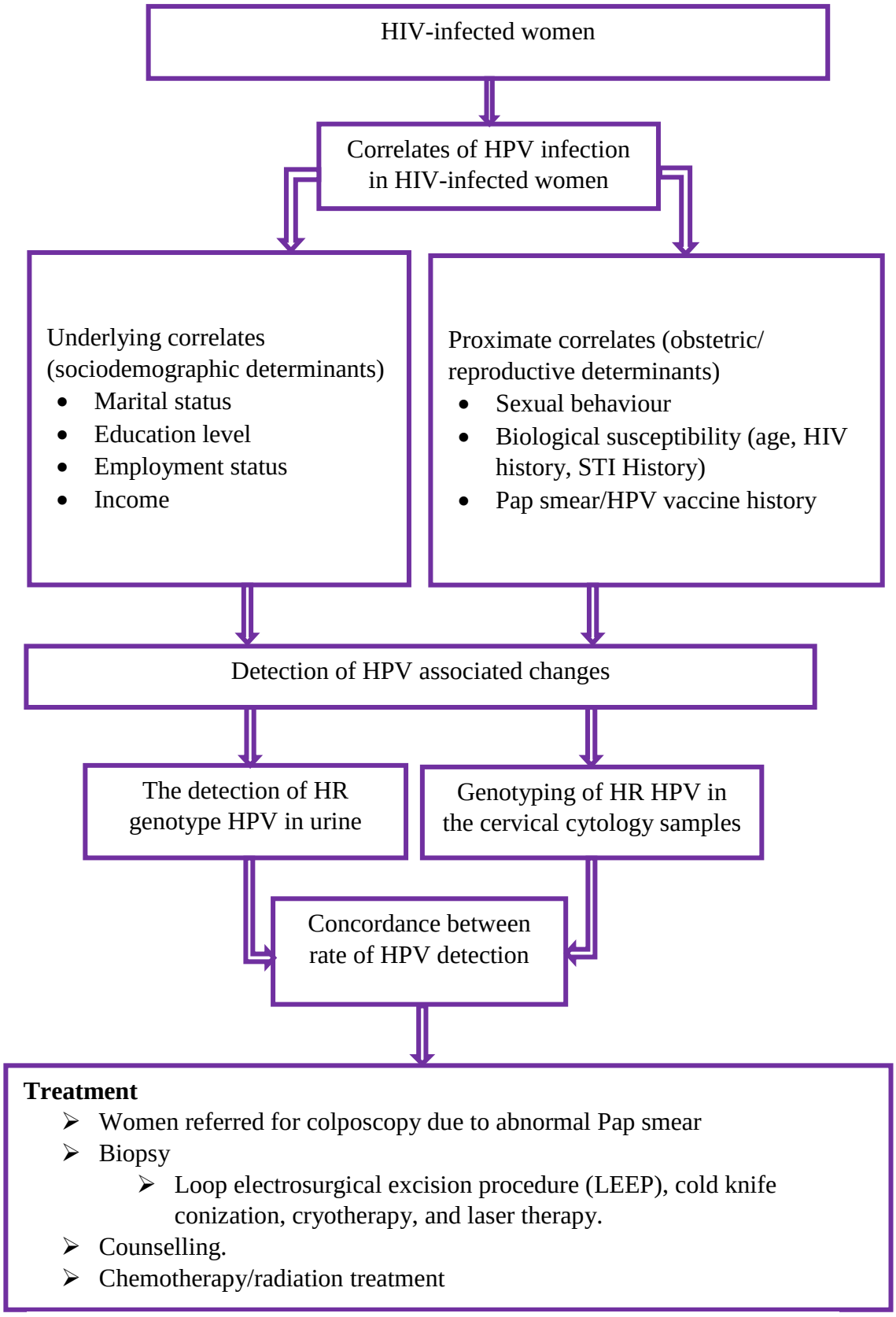

Figure 1. Conceptual framework

As illustrated in the conceptual framework, correlates of HPV infection in HIV-infected women who are usually at higher risk for cervical dysplasia were analyzed.
For clarity of terms, a correlate is an associated term to a risk factor. While a risk factor is a type of correlate though distinct from other correlates, a correlate could come about before the outcome (47). Risk factors usually increase the probability of an outcome, in this case cervical HPV or Urine-based HPV. Importantly and as a correlate, the association with or extent of Urine-based HPV DNA detection in an abnormal cervical cytology to that of HPV DNA detection in a cervical swab specimen is of importance.

From the framework, the proportion of abnormal cervical cytology observed in the women, could present a significant association with HPV-DNA being identified in urine sand cervical samples. In the conceptual framework, it is also shown that variables that correlates maybe positively or negatively associated with cervical HPV DNA or Urine-based HPV DNA detection, which are the outcomes, for instance young age and old age or age at sexual debut. In every HIV-infected woman, the absence or presence of a correlate is measurable. The correlate, as with clinical correlates in this framework, may be measured simultaneously as the cervical HPV or urine-based HPV outcome and therefore be a concomitant of it, or it may be resulting from the outcome if subsequently measured.

Study participants who were positive cytology were referred for colposcopy. They were then treated for cervical cancer through treatment modalities recommended by physicians such as loop electrosurgical excision procedure (LEEP), cryotherapy, cold knife conization as well as laser therapy. As appropriate they were referred for chemotherapy/radiation treatment for cases of cervical neoplasia.

CHAPTER FOUR

4. RESEARCH METHODOLOGY

4.1 Study Design

This was a facility-based cross-sectional comparative study in which HPV DNA was tested in paired urine and cervical samples from HIV-positive women attending the Comprehensive Care Centre (HIV care Centre) at the Kenyatta National Hospital.

4.2 Study Site

The study was conducted at the KNH CCC. The hospital (KNH), was founded in 1901 and became later known as King George VI hospital in 1952. It's situated in Nairobi County in Kenya and is the largest public national tertiary referral and teaching hospital hosting College of Health Sciences of the University of Nairobi (UoN) and the Kenya Medical Training College. It has a heterogeneous population that caters to patients of all walks of life, from Nairobi County, its environs, referrals from other hospitals in the country and the greater East African region.

Several medical specialist departments are hosted here including the department of Obstetrics and Gynecology, which conducts screening and treatment/management of cervical cancer, in addition to a lot of other services, among diverse patients both HIV-positive as well as HIV-negative. This study was, therefore, carried out in the hospital's CCC where complete HIV care, prevention, and treatment services are offered.

The CCC is a global model for HIV/AIDS care. Following the development of a strategic plan of action for 2005-2010, KNH started CCC with the support from the United States Agency for International Development/Family Health International to provide a comprehensive HIV care, management, and support that includes counseling, nutrition, Prevention of Mother-To-Child-Transmission, pharmaceutical care, laboratory diagnostics and monitoring of patients. About 200 patients attend the facility daily.

In addition, the hospital also draws its clientele countrywide, serving a population with diverse cultural and socioeconomic backgrounds. At the CCC over 9000 patients are registered with daily patient visits of about 200, with a male to female ratio of 1:2. Viral

Load assays are offered free of charge once a year but CD4 is not done freely. Cervical cancer screening and HPV vaccine services are not offered routinely at CCC.

The samples were analyzed at The Lancet pathologists' laboratories, a private entity with high standard quality diagnostics that have a close working relationship with both public and private healthcare facilities. Lancet Laboratories is a leading reference laboratory. As such, KNH laboratories and Lancet hospital-independent reference laboratories can be complementary in the provision of comprehensive, medically appropriate laboratory services for optimal patient care and research.

Lancet Laboratories usually do high volume routine and specialty testing including HR HPV and therefore would be useful in providing the diagnostic capacity to support this research study of HPV detection in urine and cervical samples among women with HIV in Kenyatta National Hospital. Hence all samples were shipped to Lancet Laboratories. Additionally, gatekeeper permission was sought from Lancet Laboratories for laboratory analysis. Lancet laboratories, in addition to the above, provided free Pap smear kits to this particular study. In addition, Lancet has over the years supported research by registrars, including, the principal investigator in this study by subsidizing fees for diagnostics.

4.3 Study Population

The study enrolled women aged 18 years and above who are HIV-positive and are receiving service at the HIV clinic in Kenyatta National Hospital and have given consent.

4.3.1 Inclusion criteria

i) Women who are older than 18yrs.
ii) HIV positive.
iii) Informed consent.

4.3.2 Exclusion Criteria

i) Women diagnosed with cancer of the cervix
ii) Women with past or on treatment of cancer of the cervix.
iii) Pregnant women.
iv) Women too ill to participate.

4.4 Sample Size Calculation

The calculation of the sample size (N) was performed using R (48) as:

>N.cohen.kappa(rate1 = 0.62, rate2 = 0.687, k1 = 0.65, k0 = 0.84, alpha=0.05, power=0.8, twosided=FALSE) #library(irr), from the sample size formula for conducting Cohen's kappa (k) agreement test by Cantor (49) below:

$$N = \left[\frac{Z_{\alpha}\sqrt{Q_0} + Z_{\beta}\sqrt{Q_1}}{k_1 - k_0}\right]^2 = 71$$

Where rate2 is the facility-based HPV prevalence in Kenya reported by De Vuyst et al (50), k1 is the true Cohen's Kappa statistic from Cobas 4800 HPV (Roche Diagnostics, Indiana, USA) previously reported by Khunamornpong et al. elsewhere. The study's purpose was to determine whether or not a concordance is observed. Substantial agreement for this study would be k ≥0.84 guided by classifications reported by Munoz et al. (51) and McHugh's. (52) recommendation of the minimum values. The desire was to test the null hypothesis of H_0: K ≥ 0.84 against the one-sided alternative H_1: K < 0.84 with significance level 0.05 and power 0.80. Since no HPV urine prevalence studies have been done in Kenya, the probability of detection in urine (rate1=0.62) was estimated by taking the ratio of HPV urine to HPV cervical sample's prevalence from several studies done elsewhere (41-43) – which was found to be quite consistent – and imputed to the value obtained by De Vuyst et al. in their study.

4.5 Sampling Procedure

The study participants were identified from the Comprehensive Care Centre registry. Potential respondents were approached individually and sampled consecutively in the facility and those willing to participate were invited for an interview.

Patient flow at KNH CCC was affluent. In a hospital setup such as KNH, sampling design would be dependent on the patient burden concerns and the sample size calculated. For a probability sampling in the KNH set-up, the total target patient population would be required but was unknown at CCC. Whereas random sampling is largely the best for avoiding bias, systematic-random sampling would allow more statistical power and therefore let a smaller total sample (N) be acceptable. With this in mind systematic random sampling was adopted.

So, while recruiting the calculated sample (N=71), the total sample was divided equally for 30 days and everyday interviews and subsequent data collection done by approaching the 5th patient at the CCC assuming an average of 3 patients per day.

The principal investigator (PI) and the trained research assistants did the interviews individually and privately. Once it was clear to the participant what the benefits and risks of the study were and that they could withdraw voluntarily, both written and verbal informed consent (Appendix A) were obtained from those who opted to participate.
Once recruitment was done and informed consents obtained, a structured questionnaire (Appendix B) was then administered to the participants by either the principal investigator or the research assistants. The questionnaire bore a unique study number that was allocated to a particular participant.

After completing the questionnaires, all consented women were expected to give paired samples (cervical and urine samples). The first void urine (FVU) sample of about 30mls was collected by the participant using a sterile container and stored in ambient temperature. FVU was stable for 72 hours before analysis. The Principal Investigator and trained research assistants then collected the cervicovaginal sample from the participant using a speculum (for Pap smear analysis) and Viba brush (for HPV DNA analysis). The patients underwent the following tests in sequence: (i) self-collection of urine sample for testing HPV DNA, (ii) conventional Pap smear and (ii) clinician/provider-collected cervical sample for HPV DNA testing.
The cervical samples were stored at ambient temperature not >25^{o}C and were generally stable for months. It required no transport media and shall be collected and maintained in a sterile manner.
At the laboratory, before HPV DNA detection, five mL of each urine sample was mixed with five mL Roche medium, then it was centrifuged at 3000 revolutions per minute for 15 minutes. After removal of the supernatant, the pellet was re-suspended in two mL of the aforementioned Roche medium and then used for testing HPV. The collected urine and cervical samples were matched with the respective questionnaires to avoid mix up then transported to Lancet Laboratory headquarters where they were analyzed with Roche's Cobas 4800 (Appendix C).

The Cobas® HPV Test is an FDA- approved qualitative in vitro test for the detection of HPV in clinician collected cervical samples by use of an endocervical spatula/brush and then put in the ThinPrep® Pap smear Test™ PreservCyt®Solution or by using a cervical broom and put in SurePath™ Preservative Fluid. This test uses amplification of the target DNA by the PCR besides nucleic acid hybridization to detect 14 HR HPV types in one investigation. This

diagnostic test specifically identifies HPV types 16 and 18 while simultaneously detecting 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 HR HPV types.

4.6 Variables and Measures

4.6.1 Independent variables – the underlying correlates

The underlying correlates or the sociodemographic covariates included age, years of education completed, highest education level, marital status, employment, and household income per month.

4.6.2 Independent (exposure) variables – the proximate correlates (obstetric or reproductive covariates)

Reproductive History: Having children; the number of children; use contraceptive; contraceptive frequency of use; contraceptive type.

Pap smear and HPV vaccine history: Ever had Pap smear; the number of times had Pap smear (once, twice, thrice); vaccinated against HPV.

Sexual behavior: Sexually active – current/ever been; sexual debut (<18 years, 19-24 years, >25 years); Number of partners have you had in the past (0, 1, ≥2); use of protection during sexual intercourse. Sexually active here refers to engaging in sexual activity of any form with at least one partner.

History of sexually transmitted infections (STI): Ever been diagnosed with an STI (yes/no); STI (Gonorrhea, Syphilis, Chlamydia, other).

HIV History: HIV Stage; CD4 Level; Viral Load; duration of HIV; How was HIV detected, current use of antiretroviral medications, medications used currently, duration on medications; reason for antiretroviral medication initiation.

4.6.3 Outcome variable

- The concordance rate of HPV detection in matched samples of urine and cervical samples
- HPV-infection (an individual was considered positive if one or more of the HR-HPV was detected).

4.7 Data Collection Instruments

The investigator and the research assistants administered a structured questionnaire to the consented participants. The questionnaire contained questions about the participant's socio-demographic characteristics, reproductive history, Pap smear, and HIV history. The questionnaire bore a unique number that matched the collected urine and cervical samples.

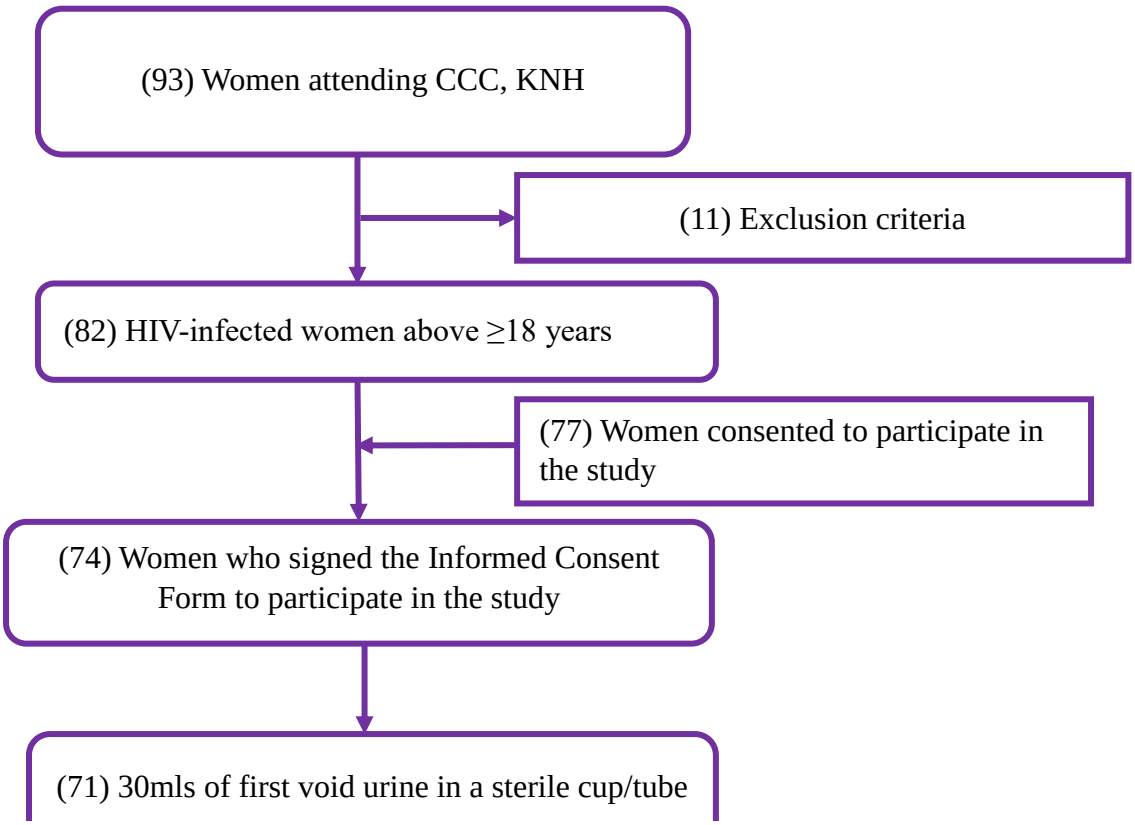

Figure 2. Study flow

The research assistants underwent half-day training on how to apply the questionnaire and take the appropriate samples. The research assistants consisted of nurses working at the colposcopy clinic and the HIV clinic in KNH thus benefitting from their clinical experiences. The recommended procedures for collecting, preparing and storing specimen was keenly followed to remove pre-analytical errors. The results were recorded onto data sheets, which were then be counter checked by the principal investigator to eliminate any post-analytical transcriptional errors.

4.9 Data Management

All participants' data were de-identified by removing all unique identifiers/serial numbers (codes) and kept safely. Data were entered into an Excel database and counterchecked against the hard copies to eliminate or harmonize any inconsistencies that may exist.

4.10 Statistical Data Analysis

4.10.1 Descriptive Statistics

The categories were compared with regard to underlying correlates (sociodemographic characteristics) and proximate correlates (obstetric/reproductive determinants) and reported as proportions, means and standard deviations (SD), appropriately.

The overall prevalence for any HPV genotype was computed as urine negative/cervical sample positive (neg/pos) plus urine positive/cervical sample positive (pos/pos). HR HPV prevalence was calculated as urine positive/cervical sample negative (pos/neg) plus urine positive/cervical sample positive (pos/pos) while type-specific HPV prevalence was assessed as the proportion of women testing positive for an HPV genotype.

4.10.2 McNemar's X^2 test for paired nominal data

The statistical methods chosen depended on the setting of this study. The HPV detection rates were not studied on two independent participant categories, hence the two-sample tests for binomial proportions (Chi-square and Fisher's exact test) were rendered inappropriate for statistical analysis. Applying a Chi-square test, therefore could have led to erroneous conclusions. Given that the HPV detection was performed on each HIV positive woman enrolled, paired data subsequently resulted and as such statistical methods accounting for the correlated dichotomous (binary) outcomes were essential. So since the outcomes were classified in categories (HPV positive and HPV negative) and the same patients tested by the two different diagnostic methods, the appropriate statistical method was by McNemar's test. The test was done using the Epicentre package in R software (epiR) and p-values obtained and ouput displayed in tables.

4.10.3 Kappa statistics (k) for agreement test

With the aim of evaluating comparability of urine sample test method to cervical sample method and therefore reliability of a nominal HPV observation, for instance whether a woman had a particular HPV test finding, Cohen's kappa ("kappa statistics") was used. This kappa statistic denoted level of concordance (agreement) that existed between the urine

sample and cervical sample methods than would have been expected to be observed by chance alone.

The kappa statistic was used to compare an observed agreement (OA) also called the observed accuracy with chance agreement (CA) or the expected accuracy or simply the random chance.

The kappa statistic was computed from a filled 2x2 contingency table of the HPV results.

Diagnostic test method	HPV detection in cervical samples			
		Positive	Negative	Total
HPV detection in urine samples	Positive	A	B	A+B
	Negative	C	D	C+D
	Total	A+C	B+D	N=A+B+C+D

$$\textbf{kappa statistic (k)} = \frac{\text{Observed Agreement (OA) – Chance Agreement (CA)}}{1 - \text{Chance Agreement (CA)}}$$

From the 2x2 table for every HPV category, the observed percentage (%) agreement (OA) was obtained as: $\frac{A+D}{N}$. This was then expressed as percent. The CA was computed by taking the prevalence rates (probabilities), that is, for HPV detection in cervical samples it was $\left(\frac{A+C}{N}\right)$x100% while for HPV detection in urine samples it was $\left(\frac{A+B}{N}\right)$x100%. Therefore, the probability that both test methods were "positive" to HPV was $\left(\frac{A+C}{N}\right) x \left(\frac{A+B}{N}\right)$. The probability that both test methods were "negative" for HPV was $\left(\frac{B+D}{N}\right) x \left(\frac{C+D}{N}\right)$. The overall probability of CA was $\left[\left(\frac{A+C}{N}\right) x \left(\frac{A+B}{N}\right)\right] + \left[\left(\frac{B+D}{N}\right) x \left(\frac{C+D}{N}\right)\right]$. Therefore,

$$\textbf{kappa statistic (k)} = \frac{OA - CA}{1 - CA} \quad k = \frac{\left(\frac{A+D}{N}\right) - \left[\left(\frac{A+C}{N}\right) x \left(\frac{A+B}{N}\right) + \left(\frac{B+D}{N}\right) x \left(\frac{C+D}{N}\right)\right]}{1 - \left[\left(\frac{A+C}{N}\right) x \left(\frac{A+B}{N}\right) + \left(\frac{B+D}{N}\right) x \left(\frac{C+D}{N}\right)\right]}$$

The k computations were implemented using the epiR using the epi.kappa function while the OA were done using the Interrater Reliability and Agreement (irr) package from R software as well. Agreement of the matched samples was evaluated using (k), the kappa coefficient, with 95% CI, classified as in Table 1 below. k assesses the level of agreement when

comparing two dissimilar assessments from a categorical outcome (55). The statistic is less deceptive than merely utilizing accuracy as a means of measurement. For instance, an OA of 0.80 is very much less striking with a CA of 0.75 contrasted with a CA of 0.50.

Table 1: Kappa statistic (k) classification criterion

Kappa statistics[1]	Accuracy
< 0	Less than chance agreement
0.01 – 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 0.99	Almost perfect agreement

The p-values from McNemar's test for paired proportions were also used to assess the concordance of HPV results of the two samples.

4.10.4 Poisson regression model with a robust variance estimator for analysis of correlates

The correlates of risk associated with HPV-infection were assessed by estimating the relative risk or prevalence ratios and were correctly interpreted following the recommendation of Martinez et al. (53). A multivariable regression model was used to adjust the estimates for potential confounders as well as variables (the correlates) of clinical importance (the proximate correlates) and those correlates whose distribution indicated a clinically significant difference between the categories. The decision to use this approach rather than the probability value (p-value) dependent variable selection approach alone was because of the relatively small sample in this study, and consequently, the use of statistically based variable entry criteria may well exclude clinically important confounders (54).

Exploratory data analysis ruled out appropriateness of logistic regression since the estimates given as odds ratios (OR), if used in this study, would have not given good measures of effect (exaggerated risks) since HPV as an outcome was common (highly prevalent) and would

[1] From: Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam med. 2005 May 1;37(5):360-3.

have given wrong impression of very large effect. A log-binomial model was used as an initial method of doing the multivariable analysis, with an option if convergence failed due to sparseness of data, to use a Poisson regression model with a robust variance estimator to be implemented in R software. The significance of the statistical tests was at $p<0.05$ and 95% confidence intervals (CI) were used.

The output reported was from the robust Poisson regression model. The coefficients of PR were directly obtained from glm_coef function of R Software's Public Health and Epidemiology (pubh).

When PR > 1, then % increase = (PR – 1) x 100 increase in prevalence, for instance, 1.52 would be (1.52 – 1) x 100 = 52% increase in prevalence. Interpretation: The prevalence of HPV was 52% greater in "X category" than the HPV prevalence in the reference group. Or, the prevalence of HPV in the "X category" was 1.52 times the reference group. When PR < 1, then % decrease = (1 – PR) x 100 decrease in prevalence, for instance, 0.4 would be (1 – 0.4) x100 = 60% decrease in prevalence. Interpretation: The prevalence of HPV in the "X category" was 60% less than the prevalence of HPV in the reference group. Or, the prevalence of HPV in the "X category" was 0.4 times the prevalence of HPV among the reference group.

4.10.5 The descriptive and statistical analysis of the cervical cytology

The cytological profiles of ASC-US, atypical squamous cells of high grade, LSIL, HSIL and invasive adenocarcinoma of HPV infection results from the two samples were provided as frequencies and proportions. Equally, overall and type-specific HPV distribution of HPV from the matched samples by normal and abnormal cytological results were provided. The differences in the percentage of the women with abnormal or normal cytological results and HPV-negative and HPV-positive in both samples were assessed with McNemar's p-values. Sensitivity and specificity of HPV DNA detection in urine for prediction of abnormal cytology and cervical HPV were evaluated. In predicting cervical HR HPV DNA, the sensitivity of detection in the urine sample was calculated by taking the number positive in urine sample divided by the positives in the reference (detection in the cervical sample) multiplied by 100, while specificity was number negative in urine divided by number positive in cervical sample divided by 100. The analyses were done in a 2x2 table.

Similar to the prediction of cervical HR HPV DNA, in predicting abnormal cytology (ASC-US/ASC-H/LSIL/HSIL/Invasive adenocarcinoma), the sensitivity and specificity of HR HPV

detection in the urine sample was by having the cytology as the reference test. The formulae are shown below.

The 95 % CI for prevalence, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive value (NPV) was obtained from the formula below computed in Epicentre package of R software, where $\hat{p}$ is the estimate, and *n* could take either the number of true-positive or the true-negative in either case. $\hat{p} \pm 1.96 = \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$.

4.11 Ethical Considerations

The research protocol was submitted to the Ethical Review Committee of the Kenyatta KNH and UoN ERC for consideration, comment, guidance, and approval before the study commences. The study was done according to the principles encompassed in the Helsinki declaration. The precaution was taken to safeguard the study participants' privacy and guarantee the confidentiality of their personal information at all times.

Only the study participants who signed an informed consent to participate were enrolled, and had the freedom to withdraw at any time of the study period and not discriminated upon. Information gathered from the participants was kept confidential. Participants also received brief health messages from the investigative team. After approval from the KNH/UoN ERC, an introductory letter to Comprehensive Care Centre in-charge of Kenyatta National Hospitals was delivered and the research team introduced to the staff at the care center. The Questionnaire was translated to Kiswahili and validated to enable full comprehension of the items.

The diagnostic testing was done at the Pathologists Lancet Group of Laboratories, Nairobi, Kenya (PLK) and South Africa - Lancet Labs. The pathology laboratory samples/specimens were retained under appropriate storage conditions as per the Lancet Laboratories standard operating procedures (SOPS). The cervical cytology was done in PLK while the genotyping by Cobas 4800 Assay done at the South Africa - Lancet Labs.

Afterward, all materials used in the assay, including reagents and specimens, were thoroughly decontaminated/disposed of in a manner that would inactivate infectious agents. Solid Wastes: Autoclave, trashed in biohazard bins tagged for incineration according to Lancet Laboratories SOP. Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0% (1:5 dilution of household bleach). Allow 30 minutes for decontamination before disposal.

The laboratory results of the tests performed on the participants were appropriately communicated to them and their primary physician, therefore advice given depending on the

results. Follow-ups were done to care for clinical conditions detected in patients has referred to appropriately.

4.12 Limitations

This study involved some patient-reported outcomes, hence the possibility of recall, and social desirability biases could have arisen. As with sexual reproductive health, the sensitivity of this study might have led to underreporting given the socio-cultural norms. Invasive nature of cervical samples collected by the use of an endocervical spatula/brush could have led to a selection bias (with some declining to have the Pap smears taken). The urine samples were self-collected therefore the risk of the inadequate sample was contemplated; however, the procedure was well explained to the participants who signed an informed consent so as to minimize collection error and all the samples were verified by Lancet Laboratory as adequate.

This was a blinded study, that is, a test result was interpreted without the knowledge of a reference standard or a previous test results of the reference standard. That is, the personnel performing the tests were unaware of the true diagnosis. The experienced cytologists and physicians, who independently performed and interpreted the cervical cytological evaluation, were blinded to the findings of the reference standard, Cobas 4800 Assay. And where the personnel disagreed, the cytological tests were repeated by different set of personnel.

CHAPTER FIVE

5. RESULTS

A total of 71 HIV positive women from KNH CCC were enrolled in this study. They had a mean age of 44.28±10.6 years. All the women provided adequate samples of urine and cervical samples for HPV detection and for cytology. The reference/gold standard was HPV DNA detection in cervical samples. The overall detection rate was 67.6% from the gold standard. The results are presented in the subsequent sections of this Chapter.

5.1 The prevalence and concordance of detection of HPV between urine and cervical samples

5.1.1 Prevalence of HPV by urine and cervical samples

Table 2 displays the prevalence by sample type and HPV genotype. The prevalence of HR HPV was 67.6% (95% CI: 55%–78%) in cervical samples and 35.2% (95% CI: 24% - 47%) in urine samples. The prevalence of urine-based HPV was slightly more than half that of the Cervical HPV. The non-HPV 16/18 (HR genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) accounted for 63.4% and 29.6% in cervical and urine samples respectively. The prevalence of HR HPVs followed a similar trend for all the types considered.

Table 2:Prevalence of HPV genotypes by the method of detection and by carcinogenicity categories

HR HPV Genotype	Cervical sample	Urine sample
HPV 18	5.6%	2.8%
HPV 16	14.1%	7.0%
HPV 16/18	16.9%	9.9%
Non-HPV 16/18	63.4%	29.6%
Any HR HPV	67.6%	35.2%

5.1.1 The specificity, sensitivity, PPV, and NPV of HPV DNA detection in urine for prediction in cervical samples

Tables 3 and 4 shows the 2x2 table for sensitivity, specificity, PPV and NPV of urine sample's HR HPV for predicting cervical sample HR HPV were calculated from.

Table 3: The 2x2 table for calculation of sensitivity, specificity, PPV and NPV for any HR HPV in urine and cervical samples

Diagnostic test		Any HR HPV DNA detection in cervical samples		
	HPV result	Cerv+ve (48)	Cerv-ve (23)	Total
Any HR HPV DNA detection in urine samples	Urine+ve (25)	A=25	B=0	A+B=25
	Urine-ve (46)	C=23	D=23	C+D=46
	Total	A+C=48	B+D=23	A+B+C+D=71

From Table 3, the calculations are as below – the CIs included were computed from epiR.

- Prevalence of HR HPV:

  = Total $_{Disease}$/ Total × 100

  $$= \left(\frac{A + C}{(A + B + C + D)}\ x\ 100\right)$$

  $= \left(\frac{48}{71}\ x\ 100\right) = 67.6\% =$ 0.68 (0.55, 0.78).

- Sensitivity:

  = A/ (A + C) × 100

  $= \left(\frac{25}{48}\ x\ 100\right) = 52.1\% =$ 0.52 (0.40, 0.64).

- Specificity:

  = D/ (D + B) × 100

  $= \left(\frac{23}{23}\ x\ 100\right) = 100\% =$ 1.00 (0.85, 1.00).

- Positive Predictive Value:

  = A/ (A + B) × 100

  $= \left(\frac{25}{25}\ x\ 100\right) = 100\% =$ 1.00 (0.86, 1.00).

- Negative Predictive Value:

  = D/ (D + C) × 100

  $= \left(\frac{23}{46}\ x\ 100\right) = 50\% =$ 0.50 (0.35, 0.65).

Table 4: The 2x2 table for calculation of sensitivity, specificity, PPV and NPV for any HPV in urine and cervical samples

Diagnostic test		Any HPV DNA detection in cervical samples		
	HPV result	Cerv+ve (48)	Cerv-ve (23)	Total
Any HPV DNA detection in urine samples	Urine+ve (37)	A=37	B=0	A+B=37
	Urine-ve (34)	C=11	D=23	C+D=34
	Total	A+C=48	B+D=23	A+B+C+D=71

From Table 4, the CIs included below were computed from epiR as well.

- Sensitivity:

  = A/ (A + C) × 100

  $= \left(\frac{37}{48}\, x\, 100\right) = 77.1\% = 0.77\ (0.63, 0.88).$

- Specificity:

  = D/ (D + B) × 100

  $= \left(\frac{23}{23}\, x\, 100\right) = 100\% = 1.00\ (0.85, 1.00).$

- Positive Predictive Value:

  = A/ (A + B) × 100

  $= \left(\frac{37}{37}\, x\, 100\right) = 100\% = 1.00\ (0.91, 1.00).$

- Negative Predictive Value:

  = D/ (D + C) × 100

  $= \left(\frac{23}{34}\, x\, 100\right) = 67.6\% = 0.68\ (0.49, 0.83).$

In predicting any of cervical (HR) HPV DNA, the sensitivity of urine was 77.0% (95% CI=63%-88%) and specificity was 100.0% (95% CI=85.0%-100.0%). In contrast, the specificity of HR HPV in urine for predicting cervical HR HPV was high (100.0%, 95% CI=85.0%-100.0%).

The probability that women with a positive HPV screening test result indeed had the condition of interest (PPV) was 1.00 (95% CI: 0.91, 1.00) and 1.00 (95% CI: 0.86, 1.00) for any HPV and HR HPV respectively. Similarly, the probability that women with a negative HPV DNA screening test result indeed do not have the HPV DNA of interest was 0.68 (95% CI: 0.49, 0.83) and 0.50 (95% CI: 0.35, 0.65) for any HPV and HR HPV respectively. The PPV was similar in the two cervical sample types.

From the prevalence data aforementioned in Table 2, it can be gleaned from Table 4 that the higher the HPV prevalence (0.676 – any HPV for urine samples), the higher the PPV (1.00; 95% CI: 0.91, 1.00) – with smaller confidence interval, this means that it would be more likely to have a positive test outcome predicting the presence of HPV. When the prevalence of HPV is low (0.35 – any HR HPV for urine samples), the PPV will also be low as that of 1.00 (95% CI: 0.86, 1.00) – with wider confidence interval, even when using a high sensitivity and specificity test.

5.1.2 Concordance of detection of HPV between urine and cervical samples

Table 5 shows the results from epiR from which the kappa values and their 95% CI as well as carried out the McNemar's p-value were obtained. The results for percent agreement were obtained from Interrater Reliability and Agreement (irr) package from R software. The % agreements were obtained from the summarized values in the contingency table of Table 5. For instance, for HPV 16/18, % agreement denoted by the diagonal cells from upper left to lower right, was obtained as: (7+59) ÷71 x100=93.0%, while disagreement is represented by lower left to upper right. The computation behind the output from epiR are demonstrated subsequently for paired urine and cervical samples.

HR HPV:

$$k_{\text{HR HPV}} = \frac{OA - CA}{1 - \text{CA}} = \frac{\left(\frac{25+23}{71}\right) - \left[\left(\frac{25+23}{71}\right)x\left(\frac{25+0}{71}\right) + \left(\frac{0+23}{71}\right)x\left(\frac{23+23}{71}\right)\right]}{1 - \left[\left(\frac{25+23}{71}\right)x\left(\frac{25+0}{71}\right) + \left(\frac{0+23}{71}\right)x\left(\frac{23+23}{71}\right)\right]}$$

$$k_{\text{HR HPV}} = \frac{\left(\frac{48}{71}\right) - \left[\left(\frac{48}{71}\right)x\left(\frac{25}{71}\right) + \left(\frac{23}{71}\right)x\left(\frac{46}{71}\right)\right]}{1 - \left[\left(\frac{48}{71}\right)x\left(\frac{25}{71}\right) + \left(\frac{23}{71}\right)x\left(\frac{46}{71}\right)\right]} = \frac{(0.676) - [(0.676)x\,(0.352) + \,(0.324)x\,(0.648)]}{1 - [(0.676)x\,(0.352) + \,(0.324)x\,(0.648)]} = 0.4131$$

HPV 16/18:

$$k_{\text{HPV16/18}} = \frac{OA - CA}{1 - \text{CA}} \text{ k} = \frac{\left(\frac{7+59}{71}\right) - \left[\left(\frac{7+5}{71}\right)x\left(\frac{7+0}{71}\right) + \left(\frac{0+59}{71}\right)x\left(\frac{5+59}{71}\right)\right]}{1 - \left[\left(\frac{7+5}{71}\right)x\left(\frac{7+0}{71}\right) + \left(\frac{0+59}{71}\right)x\left(\frac{5+59}{71}\right)\right]}$$

$$k_{\substack{\text{HPV}\\16/18}} = \frac{\left(\frac{66}{71}\right) - \left[\left(\frac{12}{71}\right)x\left(\frac{7}{71}\right) + \left(\frac{59}{71}\right)x\left(\frac{64}{71}\right)\right]}{1 - \left[\left(\frac{12}{71}\right)x\left(\frac{7}{71}\right) + \left(\frac{59}{71}\right)x\left(\frac{64}{71}\right)\right]} = \frac{(0.930) - [(0.169)x(0.09859) + (0.831)x(0.901)]}{1 - [(0.169)x(0.09859) + (0.831)x(0.901)]} =$$

Non-HPV 16/18:

$$k_{\text{non-HPV16/18}} = \frac{OA - CA}{1 - \text{CA}} = \frac{\left(\frac{21+26}{71}\right) - \left[\left(\frac{21+24}{71}\right)x\left(\frac{21+0}{71}\right) + \left(\frac{0+26}{71}\right)x\left(\frac{26+24}{71}\right)\right]}{1 - \left[\left(\frac{21+24}{71}\right)x\left(\frac{21+0}{71}\right) + \left(\frac{0+26}{71}\right)x\left(\frac{26+24}{71}\right)\right]}$$

$$k_{\text{non-HPV16/18}} = \frac{\left(\frac{47}{71}\right) - \left[\left(\frac{45}{71}\right)x\left(\frac{21}{71}\right) + \left(\frac{26}{71}\right)x\left(\frac{50}{71}\right)\right]}{1 - \left[\left(\frac{12}{71}\right)x\left(\frac{7}{71}\right) + \left(\frac{59}{71}\right)x\left(\frac{64}{71}\right)\right]} = \frac{(0.6620) - [0.4453]}{1 - [0.4453]} = 0.3906$$

The statistical methods of obtaining the results have been described in Section 4.10.1 and 4.10.2 of Chapter 4.

For the detection of HPV 16 and/or HPV 18, the proportion or level of agreements after chance has been excluded was 0.70 (95% CI 0.4454–0.9534), that is, the observed agreement is 70% of the way between chance agreement and perfect agreement indicating a substantial agreement. Similarly, detection of any HPV depicted substantial agreement with a kappa value of 0.6855 (95% CI 0.5146–0.8563). This suggests that there is a substantial agreement between the detection method in urine and the cervical test methods. In the detection of Non-HPV 16/18, the proportion of agreements is 0.3906 (95% CI 0.1922–0.5889) implying that there was a fair agreement between the two diagnostic approaches. The detection of any HR HPV also resulted in fair agreements in the diagnostic methods giving a kappa value of 0.4132 (95% CI 0.2160–0.6104).

The Null hypothesis (H_0) of the study was that: There is no difference in the HPV detection rate between using urine and cervical samples in the HIV-positive women. That is, H_0: Kappa value $\geq$ 0.84. The alternative hypothesis (H_1): There is a difference in the human papillomavirus detection rate between using urine and cervical samples in the human immunodeficiency virus-positive women. That is, one-sided alternative hypothesis, H_1: Kappa value < 0.84. According to Table 5, the kappa value obtained for the genotypes (0.69; any HPV, 0.41; any HR HPV, 0.70; HPV 16/18, and 0.39; HR Non HPV 16/18) are below

0.84, hence the null hypothesis is rejected and conclude that there is a difference in the HPV detection rate between using urine-based HPV detection and cervical HPV detection in the HIV-positive women.

From the 2x2 contingency tables also shown in the Tables 3 and 4 already described above, McNemar's test was used on paired sample data to assess marginal homogeneity (whether the marginal frequencies of the rows and the columns were equal) and check concordance. The p-values from McNemar's test for paired proportions were also used to assess the concordance of HPV results of the two samples.

H_0: HPV detection in cervical samples does not perform better than detection in urine samples.

H_1: The performances of the two sample are not equal.

Since the p-values were <0.05 as indicated in Table 5, there was a statistically significant result and therefore the H_0 was rejected to conclude that the performances of the two sample detection methods were not equal. That is, the HPV prevalence in urine samples were statistically significantly different from that cervical samples.

Although the p values from Chi square were lower than McNemar's, they corroborated them – suggesting non equality of the detection rates from urine and cervical samples.

Table 5: HPV DNA detection agreement in paired urine and cervical samples

		Cervical samples						
Urine Genotype		**HPV DNA+**	**HPV DNA−**	**% Agreement**	**Kappa value (k^2)**	**95% CI of k**	**McNemar P-value**	**X^2 p-value**
Any HR HPV[3]	HPV+	25	0	67.6%	0.41**[4]	0.2160–0.6104	0.00000620014	0.00005472
	HPV−	23	23					
HPV 16/18	HPV+	7	0	93.0%	0.70*	0.4454–0.9534	0.0253	0.00000001624
	HPV−	5	59					
HR Non-HPV 16/18[5]	HPV+	21	0	66.2%	0.39**	0.1922–0.5889	0.0000009634	0.0001041
	HPV−	24	26					

[2] k classification: < 0 = less than chance agreement, 0.01 – 0.20 = slight agreement, 0.21 – 0.40 = fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = substantial agreement, 0.81 – 0.99 = almost perfect agreement.

[3] HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

[4] ** Double star – fair agreement.

[5] HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

5.2 The correlates of risks associated with cervical HR HPV detection

5.2.1 Sociodemographic and clinical characteristics of the HIV-infected women

Table 6 below shows sociodemographic characteristics of the study participants. In terms of household income, 7 (10.0%) had no income while 28 (40.0%) had an income of 5001 – 10000 KES which was the highest. Most of the study participants were self-employed (62.0%) and never used protection during sex (39.4%). Of the 52 patients responding, 17.3% used contraceptives regularly.

Table 6: Sociodemographic characteristics

Characteristic	**n (%)**
Age (mean = 44.28±10.6), (N=71)	
≥ 50 years	20 (28.2)
18–34 years	12 (16.9)
35–49 years	39 (54.9)
Age at first sexual intercourse (N=71)	
<18 years	34 (47.9)
19 –24 years	36 (50.1)
>25 years	1 (1.4)
No. of biological children (N=71)	
0	4 (5.6)
1	17 (23.9)
2	19 (26.8)
3	17 (23.9)
≥ 4	14 (19.7)
Marital Status (N=71)	
Divorced/Separated	12 (16.9)
Single	21 (29.6)
Married (monogamous)	27 (38.0)
Married (polygamous)	2 (2.8)
Widowed	9 (12.7)
Employment (N=71)	
Casual labourer	4 (5.6)
Self-employed	44 (62.0)
Housewife	8 (11.3)

Table 6: Sociodemographic characteristics

Characteristic	**n (%)**
Salaried job	11 15.5)
Unemployed	4 (5.6)
Education (N=71)	
Above secondary	13 (18.3)
Secondary	36 (50.7)
Primary	22 (31.0)
Household income per month (N=71)	
None	8 (11.3)
< 5000 KES,	17 (23.9)
5001 – 10000 KES,	28 (39.4)
10001 – 15,000 KES,	6 (8.4)
>15,000 KES	12 (16.9)

Table 7 shows the clinical correlates. Nearly all (97.2%) were sexually active, that is, reported engaging in sexual activity of any form with at least one partner. Most of the women (43.7%) had 3 – 4 lifetime sexual partners. Majority of the women had a single history of Pap test but all had no history of being vaccinated against HPV. Above 50% of the women were on TDF/3TC/EFV.

Table 7: Clinical correlates

Characteristic	**n (%)**
Sexually active[6] (N=71)	
No	2 (2.8)
Yes	64 (97.2)
Number of Pap tests in the past five years (N=41)	
Once	28 (68.3)
Twice	3 (7.3)
Thrice	10 (24.4)
Contraceptive use (N = 71)	
No	44 (64.8)

[6] Sexually active – engaging in sexual activity of any form with at least one partner.

Table 7: Clinical correlates

Characteristic	**n (%)**
Yes	25 (35.2)
Contraceptive type (N=25)	
IUCD	3 (12.0)
Barrier methods	10 (40.0)
COCP	4 (16.0)
Other	8 (32.0)
History of Pap test (N=71)	
No	29 (36.6)
Yes	42 (59.2)
Lifetime no. of sex partners (N=71)	
≥ 10	2 (2.8)
3–4	31 (43.7)
5–9	8 (11.3)
1–2	30 (42.3)
Protection during sex (N=71)	
Never	28 (39.4)
Rarely	3 (4.2)
Sometimes	15 (21.1)
Often	25 (32.2)
History of STI (N = 71)	
No	60 (84.5)
Yes	11 (15.5)
CD4 cell count (cells/mm3) (n = 65)	
≤ 200	9 (13.8)
201–499	24 (36.9)
≥ 500	32 (49.2)
Viral load (n = 71)	
Undetectable	59 (83.1)
Detectable	12 (16.9)

Table 7: Clinical correlates

Characteristic	n (%)
Current ART[7] (n = 71)	
DTG/3TC/EFV	8 (11.3)
TDF/3TC/ATV/r	5 (7.0)
TDF/3TC/DTG	15 (21.1)
TDF/3TC/EFV	42 (59.2)
TDF/3TC/LPV/r	1 (1.4)

5.2.2 Results from regression analyses of correlates of risk associated with HPV-infection in HIV-positive women

From exploratory data analysis, appropriateness of logistic regression was ruled out since the estimates given as odds ratios (OR), if used in this study, would have given exaggerated measures of effect because HPV highly prevalent and would have given wrong impression of very large effect. So, a Poisson regression model with a robust variance estimator was utilized to model the data.

As shown in Table 8 below, on the Poisson bivariate model, there was a statistical association (p=0.011) between women who had 3 children and HPV infection, that is, women who had three children had a prevalence that was 0.35 lower than that of women who didn't. At the same time, women who had ≥ 4 children had a prevalence that was 50% lower that of women who didn't have (p=0.014).

Women who had 1–2 life number of life partners had a prevalence that was 0.37 lower than that of ≥ 10 life partners. (p-value=0.001) but wasn't statistically significant on a multivariable model after adjusting for covariates.

[7] First-line and second Antiretroviral Therapy regimens for adults

Table 8: Robust Poisson Bivariate regression and Multivariable regression for correlates of risks associated with cervical HPV Positivity in the HIV-positive women

			Poisson Bivariate regression[8]		**Poisson Multivariate regression[9]**	
Characteristic	**HPV+ (%)**	**HPV- (%)**	**PR[10] (95% CI)**	**p-value[11]**	**Adjusted PR (95% CI)**	**p-value**
Age group						
18–34 years	83.33	16.67	Reference[12]		Reference	
35–49 years	69.23	30.77	0.83 (0.59, 1.17)	0.292	1.93 (0.05, 73.98)	0.724
≥ 50 years	45.00	55.00	0.66 (0.4, 1.08)	0.724	0.87 (0.02, 48.37)	0.944
No. of biological children						
0	100.0	0.00	Reference		Reference	
1	82.35	17.65	0.82 (0.65, 1.04)	0.102	0.87 (0.05, 14.33)	0.921
2	38.89	61.11	0.61 (0.4, 0.93)	0.022	0.85 (0.03, 28.32)	0.928
3	64.71	35.29	0.65 (0.46, 0.9)	0.011	1.01 (0.06, 17.35	0.996
≥ 4	50.00	50.00	0.5 (0.29, 0.87)	0.014	0.14 (0, 5.85)	0.299
Marital Status						
Divorced/Separated	83.33	16.67	Reference		Reference	
Single	85.71	14.29	1.03 (0.73, 1.45)	0.873	0.3 (0.04, 2.36)	0.253
Married (monogamous)	51.85	48.15	0.62 (0.38, 1.02)	0.059	0.38 (0.05, 3.18)	0.372
Married	50.00	50.00	0.6 (0.13, 2.74)	0.510	0 (0, 0)[13]	< 0.001

[8] Bivariate regression – Output from single variable Poisson regression model with a robust variance estimator implemented in R.
[9] Multivariable regression - Output from multiple variable Poisson regression model
[10] PR – Prevalence Ratio with 95% confidence intervals from Poisson regression model with robust Poisson regression model with a robust variance estimator.
[11] p-value – Probability value for statistical significance. Variable which were deemed both statistically significant and clinically significant given their distribution, such as for the proximate correlates, were fed into the multivariable regression model to adjust the estimates for potential confounders.
[12] Reference – the reference category. A group of comparison for the other groups. That is, the other groups/categories are compared to the reference.

Table 8: Robust Poisson Bivariate regression and Multivariable regression for correlates of risks associated with cervical HPV Positivity in the HIV-positive women

			Poisson Bivariate regression[8]		**Poisson Multivariate regression[9]**	
Characteristic	**HPV+ (%)**	**HPV- (%)**	**PR[10] (95% CI)**	**p-value[11]**	**Adjusted PR (95% CI)**	**p-value**
(polygamous)						
Widowed	55.56	44.44	0.67 (0.36, 1.25)	0.207	1.15 (0.12, 11.24)	0.907
Employment						
Casual laborer	75.00	25.00	Reference		Reference	
Self-employed	68.18	31.82	0.91 (0.55, 1.51)	0.711	0.64 (0.01, 36.68)	0.831
Housewife	25.00	75.00	0.33 (0.09, 1.19)	0.090	0.92 (0, 186.91)	0.975
Salaried job	81.82	18.18	1.09 (0.59, 2.03)	0.783	2.07 (0.01, 705.25)	0.807
Unemployed	100.00	0.00	1.33 (0.81, 2.19)	0.254	**Wide CI[14]	< 0.001
Education						
Above secondary	76.92	23.08	Reference		Reference	
Secondary	58.33	41.67	0.76 (0.52, 1.1)	0.144	0.52 (0.12, 2.33)	0.392
Primary	77.27	22.73	1 (0.68, 1.48)	0.982	2.02 (0.09, 46.88)	0.662
Contraceptive use						
No	61.36	38.64	Reference		Reference	
Yes	76.00	24.00	1.24 (0.92, 1.67)	0.164	0.59 (0.01, 51.96)	0.818
Contraceptive type						
IUCD	63.27	36.73	Reference		Reference	
Barrier methods	80.00	20.00	1.26 (0.85, 1.87)	0.242	3.37 (0.02, 471.84)	0.63

[13] RR = 0 there aren't cases of disease among the Married (polygamous).
[14] ** = Few cases

Table 8: Robust Poisson Bivariate regression and Multivariable regression for correlates of risks associated with cervical HPV Positivity in the HIV-positive women

			Poisson Bivariate regression[8]		**Poisson Multivariate regression[9]**	
Characteristic	**HPV+ (%)**	**HPV- (%)**	**PR[10] (95% CI)**	**p-value[11]**	**Adjusted PR (95% CI)**	**p-value**
COCP	75.00	25.00	1.19 (0.65, 2.16)	0.579	0 (0, 0)	< 0.001
Other	75.00	25.00	1.19 (0.75, 1.88)	0.468	3.65 (0.02, 661.3)	0.626
History of Pap test						
No	62.07	37.93	Reference		Reference	
Yes	71.43	28.57	1.15 (0.82, 1.62)	0.424	2.54 (0.51, 12.74)	0.257
Lifetime no. of sex partners						
1–2	63.33	36.67	0.63 (0.48, 0.84)	0.001	0.88 (0, 359.39	0.968
3–4	67.74	32.26	0.68 (0.52, 0.89)	0.005	0.83 (0, 431.3)	0.953
5–9	75.00	25.00	0.75 (0.51, 1.09)	0.136	3.26 (0.01, 1252.35)	0.697
≥ 10	100.00	0.00	Reference		Reference	
Protection during sex						
Never	60.71	39.29	Reference		Reference	
Rarely	33.33	66.67	0.55 (0.1, 2.88)	0.479	**Wide CI	< 0.001
Sometimes	86.67	13.33	1.43 (0.98, 2.08)	0.065	2.47 (0.15, 40.09)	0.525
Often	68.00	32.00	1.12 (0.73, 1.71)	0.600	2.44 (0.26, 22.88)	0.436
History of STI						
No	66.67	33.33	Reference		Reference	
Yes	72.73	27.27	1.09 (0.72,	0.677	0.31 (0.03, 2.77	0.293

Table 8: Robust Poisson Bivariate regression and Multivariable regression for correlates of risks associated with cervical HPV Positivity in the HIV-positive women

			Poisson Bivariate regression[8]		**Poisson Multivariate regression[9]**	
Characteristic	**HPV+ (%)**	**HPV- (%)**	**PR[10] (95% CI)**	**p-value[11]**	**Adjusted PR (95% CI)**	**p-value**
			1.64)			
CD4						
≤200	88.89	11.11	Reference		Reference	
200 – 499	54.17	45.83	0.89 (0.7, 1.13)	0.328	1.44 (0.31, 6.66)	0.643
500 and above	65.62	34.38	0.66 (0.51, 0.85)	0.001	0.53 (0.07, 3.78)	0.528

Table 9 indicates the output of multivariable Robust Poisson regression. After adjusting for other covariates, being married (monogamous) was associated with HPV prevalence that was 41% (adjusted PR of 0.59) lower than those of women in divorced/separated category (p = 0.045).

As described in Section 4.10.4 of Chapter 4, A robust Poisson multivariable regression model was built with only clinically and statistically significant variables screened in the bivariate model – non significant covariates left out.

Table 9: Poisson Multivariate regression[15]

Characteristic	**Adjusted PR (95% CI)**	**p-value**
Marital Status		
Divorced/Separated	Reference	
Single	0.96 (0.65, 1.41)	0.820
Married (monogamous)	0.59 (0.36, 0.99)	0.045
Married (polygamous)	0.45 (0.1, 2)	0.296
Widowed	0.67 (0.35, 1.28)	0.225
Contraceptive use		
No	Reference	
Yes	1.34 (0.98, 1.84)	0.068

[15] Multivariable regression - Output from multiple variable Poisson regression model with variables screened from the bivariate model.

Table 9: Poisson Multivariate regression[15]		
Characteristic	**Adjusted PR (95% CI)**	**p-value**
Lifetime no. of sex partners		
1–2	0.91 (0.63, 1.33)	0.636
3–4	1.06 (0.81, 1.38)	0.691
5–9	1.05 (0.68, 1.62)	0.836
≥ 10	Reference	

5.3 Cervical cytology profile of the HIV positive women by HPV status of urine and cervical samples.

5.3.1 The cytological profile of HIV–positive women

Figure 4 below shows the cytological profiles based on abnormal.

The pap smears were mostly NILM (91.5%), HSIL (4.2%), and with ASC-US (1.4%), ASC-H (1.4%) and LSIL (1.4%) and had HR HPV 35/45/56 for urine, none HPV 16/18 for cervical samples as illustrated in Figure 4 above.

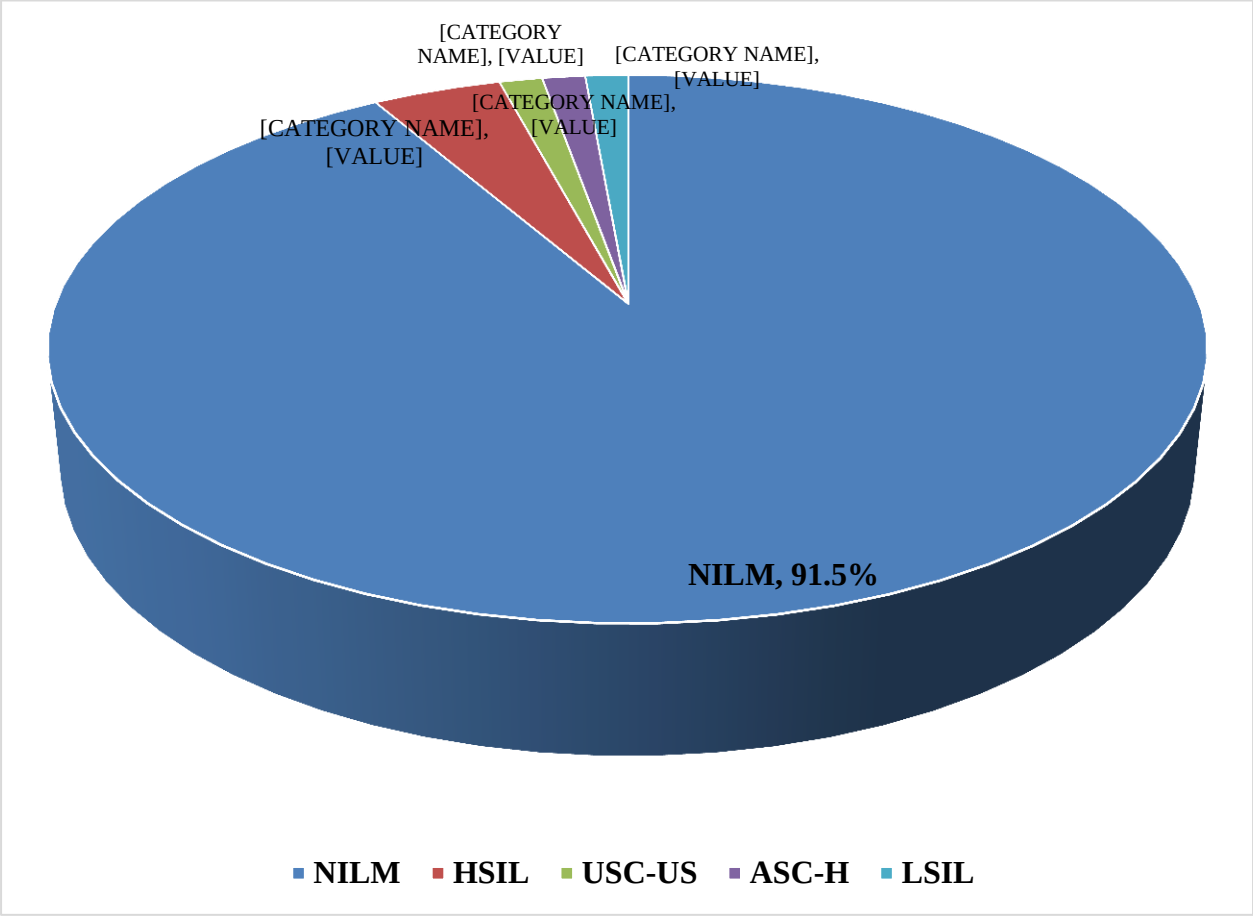

Figure 3: The cytological profiles based on abnormal Pap smears

5.3.2 NILM vaginal microecology by HIV viral load, HPV status and STI history

The results of the frequency of other cytological abnormalities are as shown in Table 10 below. Of the 65 NILM patients tested, 10 (15.4%) had bacterial vaginosis, 5 (7.6%) Candida spp., 1 (1.5%) Cytolytic vaginosis, 20 (30.8%) Cervicitis and 6 (9.2%) Cervicitis. Eleven (16.9%) of patients self-reported history of an STI. The results are as shown in Table 10 below. Comparatively, the cervical sample identified most of the positive HR HPV in NILM.

Table 10: Results of NILM vaginal microecology/cervical inflammation of various causes according to last viral load, HPV status, and STI history

Vaginal Microecology/ cervical inflammation of various causes	**Last HIV Viral Load**		**HPV status**				**STI history**			
			Cervical any HR		**Urine any HR**					
	Det.	Und.	+ve	-ve	+ve	-ve[16]	Yes (11)	No (54)	Chlamydia (4)	Syphilis (7)
Bacterial vaginosis	1	9	6	4	2	8	2	8	1	1
Candida spp.	0	5	5	5	3	7	0	5	0	0
Cytolytic vaginosis	0	1	0	1	0	1	0	1	0	0
Cervicitis	2	18	14	6	4	16	3	17	2	1
Atrophic vaginitis	1	5	3	3	1	5	2	4	0	2

5.3.3 HIV CD4/µL count, HIV viral load detection and HIV duration according to HR HPV prevalence and cytology findings

This study looked at KNH CCC HIV positive cohort, hence underscoring the importance of some clinical factors to HPV positivity. According to Table 11 below, CD4 count below 500 cells/µL (≤ 200 or 201–499) seemed suggestive of concurrent positivity with at least one HR HPV. Forty-five percent of the participants in ≤ 200 or 201–499 CD4 groupings were negative for NILM. HIV duration of 10 to 15 years appeared to predict HR HPV in both samples and in abnormal cytology samples. As depicted in the table, a higher percentage (76.1%) of women had undetectable HIV viral load cross tabulated by NILM and compared with those with detectable viral load., this appeared predictive of NILM. However, when Chi Square test was done between Cervical and Urine samples for the viral load, there was no

[16] Det. = Detectable, Und. = undetectable, +ve = positive, -ve = negative

difference in occurrence of HPV between the cervical samples and urine samples (p-value >0.05) (see Table 11). All the Chi Square p-values were not statistically significant for the HIV variable considered.

Table 11: Comparison of HIV CD4/μL count, HIV viral load detection and HIV duration by HR HPV positivity in cervix/urine and by cytology finding

	HR HPV positivity			**Cytology**		
	Cervix	**Urine**	**Chi Square p-value**	**NILM/ Inflammatory**	**HSIL/ASC-US/ASC-H/LSIL**	**Chi Square p-value**
CD4 cell count (cells/mm3) (n=65)						
≤ 200	11.6% (8)	2.8% (2)	0.5505	12.7% (9)	0.0% (0)	0.3309
201–499	18.3% (13)	8.4% (6)		32.4% (23)	1.4% (1)	
≥ 500	29.6% (21)	18.% (13)		39.4% (28)	5.6% (4)	
HIV Viral Load						
Detectable	14.1% (10)	11.3% (8)	0.4749	14.1% (10)	2.8% (2)	0.5933
Undetectable	52.1% (37)	23.9% (17)		76.1% (54)	5.6% (4)	
HIV Duration						
Less than 5 years	7.1% (5)	4.2% (3)	0.9235	12.7% (9)	1.4% (1)	0.147
5 to 10 years	25.4% (18)	11.3% (8)		32.4% (23)	1.4% (1)	
10 to 15 years	22.5% (16)	14.1% (10)		28.2% (20)	4.2% (3)	
15 to 20 years	11.3% (8)	5.6% (4)		16.9% (12)	0.0% (0)	
More than 20 years	1.4% (1)	0.0% (0)		1.4% (1)	1.4% (1)	

5.4 The prevalence of abnormal cytology and HPV type-specific prevalence by cervical cytology status of the urine and cervical samples

5.4.1 The prevalence, specificity and the sensitivity of HPV DNA detection in urine samples for prediction of abnormal cervical cytology

Table 12 shows the 2x2 table for the computation of Sensitivity, Specificity, PPV, and NPV of Detection of any HR HPV DNA in urine samples with reference to cervical cytology results.

The prevalence of ASC-US/LSIL/ASC-H/HSIL was 8.5% (CI: 3%, 17%). The sensitivity and specificity of urine sample HR HPV for predicting cytological pap tests were assessed (Table 12). For ASC-US/LSIL/ASC-H/HSIL, the sensitivity of HR HPV in urine was moderate (50.0%, 95% CI=12.0%-88.0%). In contrast to sensitivity, the specificity of HR HPV in urine for predicting ASC-US/LSIL/ASC-H/HSIL was higher (66.0, 95% CI=53.0%-77.0%).

Table 12: The 2x2 table for calculation of sensitivity, specificity, PPV and NPV for any HR HPV in paired urine and cervical cytology

Diagnostic test		ASC-US/LSIL/ASC-H/HSIL[17] (n=5)		
	HPV result	Abnormal (6)	NILM[18] (65)	Total
Any HR HPV DNA detection in urine samples	Urine+ve (25)	A=3	B=22	A+B=25
	Urine-ve (46)	C=3	D=43	C+D=46
	Total	A+C=6	B+D=65	A+B+C+D=71

From Table 12, the CIs included below were computed from epiR.

- Prevalence of abnormal cytological sample:

  = Total $_{Disease}$/ Total × 100

  $$= \left(\frac{A + C}{(A + B + C + D)}\ x\ 100\right)$$

  $$= \left(\frac{6}{71}\ x\ 100\right) = 8.45\% = \ 0.085\ (0.03, 0.17).$$

- Sensitivity:

  = A/ (A + C) × 100

[17] HR HPV – high risk Human papillomavirus, ASC-US – atypical squamous cells of undetermined significance, LSIL – low-grade squamous intraepithelial Lesion, HSIL – high-grade squamous intraepithelial lesion, ASC-H – atypical squamous cells cannot exclude HSIL.

[18] NILM – negative for intraepithelial lesion or malignancy.

$= \left(\frac{3}{6}\ x\ 100\right) = 50\% =\ 0.50\ (0.12, 0.88).$

- Specificity:

  = D/(D + B) × 100

  $= \left(\frac{43}{65}\ x\ 100\right) = 66.2\% =\ 0.66\ (0.53, 0.77).$

- Positive Predictive Value:

  = A/ (A + B) × 100

  $= \left(\frac{3}{25}\ x\ 100\right) = 12\% =\ 0.12\ (0.03, 0.31).$

- Negative Predictive Value:

  = D/ (D + C) × 100

  $= \left(\frac{43}{46}\ x\ 100\right) = 93.5\% =\ 0.93\ (0.82, 0.99).$

The probability that women with a positive HPV screening test result indeed do have the condition of interest (PPV) was very low at 0.12 (95% CI: 0.03, 0.31) for abnormal cytology. Similarly, the probability that women with a negative HPV DNA screening test result (NPV) indeed do not have the HPV DNA of interest was very high at 0.93 (95% CI: 0.82, 0.99).

The level of agreement between urine and cytology was very poor (k=0.06628 with HR HPV, 0.06913 with HPV 16/18 and 0.1045 with non-HPV 16/18). This means urine performed extremely better than cytology. Both McNemar's and Chi square p values indicated statistical significance, hence the null hypothesis that they are of equal performance was rejected. The calculations are shown below. Due to few cell counts in Table 13, Chi square was used together with its variant (Fisher's exact test) correcting for the small counts. However, Chi square and Fisher's Exact gave non-significant p values (see Table 13) meaning failing to reject the null hypothesis that he performance of urine and cervical samples are equal/same – this was wrong. The percent agreements were quite misleading (for instance, 84.57% for HPV 16/18). Kappa values and 95% CI including a negative showed indicated tests non-equality.

$$k_{\text{HR HPV}} = \frac{\left(\frac{46}{71}\right) - \left[\left(\frac{6}{71}\right)x\left(\frac{25}{71}\right) + \left(\frac{65}{71}\right)x\left(\frac{46}{71}\right)\right]}{1 - \left[\left(\frac{6}{71}\right)x\left(\frac{25}{71}\right) + \left(\frac{65}{71}\right)x\left(\frac{46}{71}\right)\right]} = \frac{(0.6479) - [0.6229]}{1 - [0.6229]} = 0.06628$$

$$k_{\text{HPV16/18}} = \frac{\left(\frac{60}{71}\right) - \left[\left(\frac{6}{71}\right)x\left(\frac{7}{71}\right) + \left(\frac{65}{71}\right)x\left(\frac{64}{71}\right)\right]}{1 - \left[\left(\frac{6}{71}\right)x\left(\frac{7}{71}\right) + \left(\frac{65}{71}\right)x\left(\frac{64}{71}\right)\right]} = \frac{(0.8450) - [0.8336]}{1 - [0.8336]} = 0.06913$$

$$k_{\text{nonHPV16/18}} = \frac{\left(\frac{50}{71}\right) - \left[\left(\frac{6}{71}\right)x\left(\frac{21}{71}\right) + \left(\frac{65}{71}\right)x\left(\frac{50}{71}\right)\right]}{1 - \left[\left(\frac{6}{71}\right)x\left(\frac{21}{71}\right) + \left(\frac{65}{71}\right)x\left(\frac{50}{71}\right)\right]} = \frac{(0.7042) - [0.6697]}{1 - [0.6697]} = 0.1045$$

The statistical methods of obtaining the results have been described in Section 4.10.1 and 4.10.2 of Chapter 4.

Table 13: HPV DNA detection agreement in paired urine and cervical samples

		Cervical samples						
Urine Genotype		**Abnormal**	**NILM**	**% Agreement**	**Kappa value (k[19])**	**95% CI of k**	**McNemar p-value**	**Chi Square/Fisher's exact p-value**
Any HR HPV[20]	HPV+	3	22	64.79%	0.06628	-0.2283–0.3609	0.0001447	0.7293/ 0.6582
	HPV−	3	43					
HPV 16/18	HPV+	1	6	84.57%	0.06913	-0.4366–0.5748	0.7630	1.0/ 0.4765
	HPV−	5	59					
HR Non-HPV 16/18[21]	HPV+	3	18	70.42%	0.1045	-0.2169–0.4259	0.001064	0.4977
	HPV−	3	47					

5.4.2 Type-specific prevalence of HR HPV by cervical cytology status

Table 14 below shows the type-specific prevalence of HR HPVs. The type-specific HPV identification indicated that HPV-52 was more prevalent in both samples and non-HPV 16/18

[19] k classification: < 0 = less than chance agreement, 0.01 – 0.20 = slight agreement, 0.21 – 0.40 = fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = substantial agreement, 0.81 – 0.99 = almost perfect agreement.
[20] HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
[21] HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

in cervical samples according to NILM and non-HPV 16/18 in cervical samples according to abnormal cytology results. For urine, HPV 16, HPV 35, HPV 45, HPV 52 and HPV 56 were prevalent. There were no variations in negative results.

Table 14: HPV type-specific distribution according to cervical cytology results

Normal cytology result (n = 65), NILM					**Abnormal cytology result (n = 6)**			
HR HPV types	**% Both positive**	**% Cervical sample only**	**% Urine sample only**	**% Both negative**	**% Both positive**	**% Cervical sample only**	**% Urine sample only**	**% Both negative**
16	5.6	12.7	5.6	78.9	1.4	0.0	1.4	7.0
18	2.8	5.6	2.8	85.9	0.0	0.0	0.0	8.5
31	5.6	57.7	5.6	33.8	0.0	4.2	0.0	4.2
33	0.0	57.7	0	33.8	0.0	4.2	0.0	4.2
35	2.8	57.7	2.8	33.8	1.4	4.2	1.4	4.2
39	2.8	57.7	2.8	33.8	0.0	4.2	0.0	4.2
45	5.6	57.7	5.6	33.8	1.4	4.2	1.4	4.2
51	4.2	57.7	4.2	33.8	0.0	4.2	0.0	4.2
52	9.9	57.7	9.9	33.8	0.0	4.2	1.4	4.2
56	1.4	57.7	1.4	33.8	1.4	4.2	1.4	4.2
58	1.4	57.7	1.4	33.8	0.0	4.2	0.0	4.2
59	0.0	57.7	0.0	33.8	0.0	4.2	0.0	4.2
66	0.0	57.7	0.0	33.8	0.0	4.2	0.0	4.2
68	0.0	57.7	0.0	33.8	0.0	4.2	0.0	4.2

CHAPTER SIX

6. DISCUSSIONS OF RESULTS

6.1 Discussions

Cervical cancer is among the most prevalent cancers in women, especially in Kenya with prevalence as high as 63.1% from the year 2018 estimates (18) and ranked the most common cause of cancer-related deaths among the females of ages 15–44 and the second chief cause of cancer among females. At present, the Pap test offers benefits and is the advised method for cervical cancer screening in Kenya, however, only a fairly small proportion of women are screened due to low attendance (56). Another detection method is to look for cervical cancer linked HPV genotypes in self-sampled urine, and this is an extensively accepted approach. In this study, the results that primarily aimed at comparing the detection rate of HPV in paired urine and cervical samples in HIV-positive women visiting the KNH CCC are presented.

This study found an overall (total) prevalence of any HPV as high as 67.6% (68% to 2 decimal places) for cervical samples (true prevalence). Such result has been reported previously by Munoz et al. (51) found 70.6% and 63.2% HPV infection in cervical and urine samples respectively. Similarly, Luchters et al. (17), Menon et al (14) and Combita et al. (42) reported comparable values of 73.3%, 64% and 60.0% respectively. This study also reports HPV urine sample prevalence of 35.2% which is a little lower than urine prevalence reported by Combita et al. (42) of 64.72% and considerably lower than that of 81.5% (46) in somewhat similar populations.

These prevalence findings suggest that when test results between HPV testing by cervical sample method and urine were discrepant, the cervical approach is inclined to depict more women as HPV infected. However, the urine method appeared quite predictive of the cervical method and may serve as a useful non-invasive method. Besides, it has been reported that (first-void) urine-based sampling is acceptable among women (73.5%) when compared with clinician collected samples (21.2%) (57).

The diagnostic tests used were Roche Linear Array/Roche Cobas 4800. The Roche Linear Array is comparable to the Roche Cobas 4800 test and have an excellent agreement (58) and has been widely used for HPV diagnostics. As the trend from prevalence estimates in this study, it has been established previously that HPV is highly prevalent in cervical samples than in urine samples for any HR HPV (58).

As the primary endpoint, this study computed percent agreement and kappa values across the paired sampling methods. The statistical significance of the agreement was evaluated using McNemar's p-values. The McNemar's test (p-values <0.05) gave statistically significant results across the HPV types for the study to conclude that the detection performances of the urine and cervical samples were not equal. The McNemar's p-values corroborates the results obtained from kappa statistics, implying that thresholds of levels of agreement of ≥0.84 cannot be attained (the levels set by this study) for it be used alternatively to cervical sample. This further implied that they don't have equal detection performances.

The level of agreement between detection in urine and cervical samples was reported for any HPV (k= 0.6855, 95% CI=0.5146–0.8563, McNemar p<0.001) were somewhat substantial but not comparable with cervical samples. This study also reported an overall % agreement of 67.6%) for any HPV. This percent agreement is consistent with a concordance of 65.2% reported by Nilyanimit et al. (41) and close to 71% reported by Jong et al. (46).
Among the women population of ages ranging from 21 to 65, just as in this present study, a study found a concordance of 88% using Cobas 4800 HPV detection assay (56). This current study also reports a percent agreement of 84.5% and 93.0% for urine in the detection of any HPV and HPV 16 and/or HPV 18 respectively. Other concordance values of between 60% to 100% have been reported by several other authors (51-53) in paired urine and cervical samples using varied detection assays.

Reporting the kappa coefficient (k), the level of agreement between the paired samples was 0.69 (95% CI=0.5146–0.8563) classified as substantial for any HPV, 0.41 (95% CI=0.2160–0.6104) fair for any HR HPV, 0.70 (95% CI=0.4454–0.9534) substantial for HPV 16 and/or HPV 18, 0.39 (CI 95%=0.1922–0.5889) and fair for other HR HPV (Non-HPV 16/18) and were all statistically significant on McNemar's p-values, according to classifications of k.
The k reported in this study agrees with previously reported k of 0.660 (95% CI=0.486–0.833) for any HPV and 0.688 (95% CI=0.542–0.835) for HR HPV DNA (89). Prior studies have also found agreement (86.2%) in the HR HPV detection, with k of 0.65 classified as substantial agreement (59) and moderate agreement with cervix-based sampling (k=0.55) (60). Since urine-based sampling has found acceptance in women, the concordance results of this study suggest that, although urine-based HPV DNA testing may not be excellently equivalent to HPV DNA cervical testing head-to-head, it provides an opportunity to enhance coverage of cervical cancer screening.

This study found that the overall sensitivity of urine for any HPV detection is high at 77%, specificity and PPV of 100% and NPV are 68% respectively. These findings are comparable with Hernandez et al.'s 60) sensitivity and specificity of 51.0% and 96.2%, respectively; Tshomo et al.'s (61) 80% and pooled values reported by Pathak et al. (62) and suggest the utility of urine-based sampling in HPV detection.

The pap smears were mostly NILM (91.5%), HSIL (4.2%) with ASC-US, ASC-H (1.4%), LSIL all being 1.4%. In urine, HR HPV 35/45/56 occurred while cervical samples had mostly none HPV 16/18. The findings appear consistent with Maranga et al. (45) and Yamada et al. (13). When pap smears are the reference, the urine samples demonstrated better diagnostic performance relative to the cytological results. The NILM cytology was largely comprised of bacterial vaginosis 15.4% among other vaginal microecology/cervical inflammation of various causes and is associated with HPV genotypes (63).

Most of the NILM had undetectable viral load but with high HPV positivity in the paired urine and cervical samples as unexpected, this is probably due to HPV risk factors such as bacterial vaginosis, cervicitis among others. From the NILM perspective, it was inferred that urine-based HPV testing showed revisions to positive from negative results for the Pap test readings.

It is established in the medical literature that HIV amplifies the acquisition and persistence of HPV in women co-infected with HIV (41). In terms of the present study's population, HIV duration of 10 –15 years seemed to be a predictor of HR HPV in the paired samples and in non-normal cytological samples. The undetectable HIV viral load appeared more predictive of NILM as expected.

6.2 Conclusions

Although, detection rate in urine samples is a little lower than that of cervical samples, the findings of this study demonstrate that urine-based HPV test, although simple and reliable than the cervical sample test, can be employed not as a single test as cervical sample test and an certainly not as an alternative method but as a cotest with possible improvements of sample collection. The detection of HPV 16/18 was quite comparable but not by percent agreement alone. However, with pap smears as the reference, and as a non-invasive method as well, the urine samples provide better diagnostic performance than the cervical cytology. This study implies that urine-based HPV testing, if used, provides quite a higher specificity but a lower sensitivity with cervical samples and can detect cervical cancer much earlier than

the Pap test. The urine-based HPV DNA detection was almost corroborative with the cervical samples in all findings from pap smear cytology, with very few exceptions and exhibited substantial diagnostic accuracy for possible usage only in cytology triage.

6.3 Recommendations

Given the non-invasiveness and better clinical/diagnostic performance, urine-based HPV sampling can be used as a better alternative for women who decline to undergo a Pap test. The non-invasive urine-based sampling for HPV DNA can potentially address the cultural and logistical barriers associated with Pap test and offers substantially comparable HPV testing to cervical samples, especially when considering the low screening levels and the high incidence and prevalence of cervical cancer reported in Kenya and elsewhere.

While it is not the reference standard, the use of this sampling approach would increase the cervical cancer screening coverage and adherence in programmes meant to mitigate the disease. However, going forward, it would require standardizing sample collection and processing methods.

The results of this study also underscore the need for additional and/or larger-scale research studies to assess urine-based sampling as alternatives to cervical HPV sampling for cervical cancer screening interventions and to clarify the gynaecologic implications of the discordant test results between the paired sampling approaches.

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APPENDICES

APPENDIX A: INFORMED CONSENT FORM

APPENDIX A.1 INFORMED CONSENT FORM - ENGLISH

Study number…… Sex……

Name…… Age...……

Introduction

Hello. I am Dr. Fatma Taher, a post-graduate student in the Department of Obstetrics and Gynecology, University of Nairobi. This information form seeks informed consent for your participation in the study that seeks to compare the detection rate of HPV in urine and cervical samples in HIV-positive women attending the Comprehensive Care Centre at the Kenyatta National Hospital.

Purpose of the study

1. Determine the prevalence of HPV genotypes and correlates of HPV-infection in the HIV-positive women in the paired urine and cervical samples.
2. Determine the concordance of detection of HPV genotypes between urine and cervical samples.
3. Determine the Pap smear cytology profile of HIV-positive women.

List of investigators

Name	**Role**	**Institution/Affiliation**
Dr. Fatma Taher	Principal Investigator	UoN
Dr. I.S Orora. Maranga	Co-investigator	KNH
Dr. Francis X.O. Odawa	Co-investigator	UoN

Procedure

If you agree to participate in this study, you will receive an identification number. A trained interviewer will ask you several questions on risk factors associated with cervical cancer. The questions will be about socio-demographic characteristics, reproductive history, HIV history, and Antiretroviral therapy (ART) status. After that, you will be given a container to put some urine for sampling purposes. This will then be followed by a pelvic examination that will allow us to collect a sample from the opening of the uterus (the cervix).

Risks/ Discomforts

This study does not have any physical risks though there could be a minor invasion to your privacy when sensitive questions are being asked. There is slight discomfort when collecting a sample from your cervix.

Benefits

You will benefit from the study by knowing your HPV status and learning more about the risk factors associated with cervical cancer caused by HPV. You will also benefit from free Pap smear testing. In case we detect problems in your samples we shall refer you for more appropriate management.

Alternatives to participation/withdrawal from the study

If you decide not to take part in this study no one will force you to, so you will be free to make your own decision. You are free to withdraw from the study, and this shall not affect your care in any way, and you will not be discriminated in any way. You can also choose to take part in any other studies in the future.

Confidentiality

Any information you provide during the study will be kept strictly confidential. Your name will not appear on any study document and instead, we shall use a unique number assigned to your questionnaire that will match your collected sample as well.

Voluntariness

Your participation in this study, which will be in the form of an interview, pelvic examination, and provision of urine and cervical samples, is completely voluntary. You are free to choose whether or not to participate in this study. You are also free to withdraw from the study at any time you wish to do so.

Who to contact

You are encouraged to ask any questions to clarify any issues at any time during your participation in the study. If you need more information on the study, here are the contacts of persons coordinating the study.

Name	**Mobile phone number**	**email address**
Dr. Fatma Taher	0721128197	fatmataher@yahoo.com

For more information about your rights, research problems or questions about your rights as a research participant, you may contact the KNH/UoN ERC through the Secretary/Chairperson,

Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

Declaration

I have read and understood the study information. I have been given the opportunity to ask questions about the study. I understand that my taking part is voluntary; I can withdraw from the study at any time and I will not be asked any questions about why I no longer want to take part. I understand my personal details will be kept private. I hereby consent to participate in the said study as has been explained and as I have understood.

Participants' name: .....

Participants' signature: .....

Date: .....

Name of the Investigator: Dr. Fatma Taher

Signature of the Investigator: .....

Date: .....

APPENDIX A.2: INFORMED CONSENT FORM - KISWHAILI

Fomu ya Kuomba Ridhaa

Utangulizi

Hujambo, naitwa Dkt. Fatma Taher, kutoka chuo Kikuu cha Nairobi Idara ya Uzazi na Afya ya wanawake kwa jumla. Fomu hii ina habari inayoomba ridhaa yako ili ushiriki kwenye utafiti utakaofanywa ilikubaini virusi aina ya Human Papilloma kwenye mikojo ya wagonjwa ambao wanahudhuria kliniki ya UKIMWI Kenyatta National Hospital, Nairobi

Madhumuni au lengo

1. Kuamua kuenea kwa aina-jeni ya virusi inayosababisha saratani ya kizazi kwa sampuli ya mkojo na ya kizazi na sababu ya hatari ya maambukizi kwa kina mama wanaoishi na virushi vya UKIMWI.
2. Kuamua makubaliano ya kutambua aina-jeni ya virusi inayosababisha saratani ya kizazi kati ya sampuli ya mkojo na ya kizazi.
3. Kuamua maelezo mafupi ya uchunguzi ya seli inayosababisha saratani ya kizazi baada ya kupimwa kwa kina mama wanaoishi na virusi vya UKIMWI.

Orodha ya watafiti

Jina	Jukumu	Taasisi
Dr. Fatma Taher	Mtafiti mkuu	UoN
Dr. I.S Orora. Maranga	Mtafiti	KNH
Dr. Francis X.O. Odawa	Mtafiti	UoN

Utaratibu

Ukikubali kushiriki kwenye utafiti huu utapata namba yako ya kitambulizi halafu mtafiti mtaalamu atakuuliza maswali kuhusu hatari zinazohusishwa na saratani ya mfuko wa kizazi. Maswali yatakuwa kuhusu tabia ya kushiriki ngono, sifa ya mambo ya kijamii kidemografia, kuhusu ugonjwa wa ukimwi n.k.

Madhara au changamoto

Utafiti huu hauna hatari zozote za kimwili , ila tu kutakuwa na uvamizi wa mambo madogo ya kibinafsi wakati wa kuulizwa maswali magumu. Na pia kuhisi usumbufu wakati ambao uchunguzi wa sehemu nyeti unafanyiwa ili kuchukua sampuli kutoka kizazi.

Manufaa

Utanufaika kushiriki utafiti huu kwa kujifunza mengi kuhusu hatari zinazohusishwa na saratani ya mfuko wa kizazi inayoletwa na Human Papilloma. Juu ya hio habari utakayotupatia itasaidia ufahamu zaidi ya virusi papilloma. Pia itakuwa muhimu kwa madktari kufanya matibabu na uzuiaji wa ugonjwa huu.

Njia mbadala za kushiriki

Ukiamua kutoshiriki utafiti huu hakuna mtu yeyote ambaye atakulazimisha, kwa hivyo utakuwa huru kufanya uamuzi wako mwenyewe na pia utajichagulia kushiriki kwenye utafiti mwingine wa siku zijazo.

Usiri

Habari yoyote ile utakayotupatia wakati wa utafiti huu utawekwa kwa siri kikamilifu. Jina lako halitajulikana popote bali tu utapatiwa namba yako ya kitambulizi.

Hiari

Ushirikiano wako kwenye utafiti huu ambao utakuwa kwa njia ya mahojiano ya moja kwa moja ni kujitolea kwa hiari yako. Utakuwa huru kuchagua kama utashiriki au kutoshiriki utafiti huu pia, utakuwa huru kujiondoa kwenye utafiti huu wakati wowote utakaotaka

Nani wa kuwasiliana nayo.

Unashauriwa kuuliza maswali yoyote ilikubaini maswala yote yanayoibuka wakati wa kushiriki kwenye utafiti. Kama utahitaji habari au mambo mengine kuhusu utafiti huu haya ndiyo majina ya wale ambao watahakikisha utafiti huu utafanyika bila tashwishi.

Jina	**Nambari za simu**	**Barua pepe**
Dkt. Fatma Taher	0721128197	fatmataher@yahoo.com

Kwa habari zaidi kuhusu haki zako, matatizo ya utafiti au maswali kuhusu haki zako kama mshiriki wa utafiti, unaweza kuwasiliana na KNH/UoN ERC kupitia Katibu/Mwenyekiti, Hospitali ya Taifa ya Kenyatta-Chuo Kikuu cha Nairobi Maadili na Utafiti Namba ya 2726300 Ext. 44102 barua pepe uonknh_erc@uonbi.ac.ke.

Mkataba

Nimesoma na nimeelewa habari inayohusiana na utafiti huu. Nimepatiwa nafasi ya kuuliza maswali yanayohusiana na utafiti huu. Nimeelewa kwamba kushiriki kwangu ni wa kujitolea kwa hiari na ninaweza kujiondoa kwenye utafiti wakati wowote na sitaulizwa maswali kama vile mbona haushiriki tena kwenye utafiti. Ninaelewa kuwa mambo yanayonihusu yatawekwa kwa siri kikamilifu kwa hivyo nimekubali kushiriki kwenye utafiti huu kama vile nimeelezwa na kuelewa.

Jina la mshiriki: .....
Sahihi ya mshiriki: .....
Tarehe: .....
Jina la mtafiti: Dkt. Fatma Taher
Sahihi ya mtafiti: .....
Tarehe: .....

APPENDIX B: SOCIODEMOGRAPHIC QUESTIONNAIRE

Study ID Number__ __ __ **Interviewer Number__ __ __**

Date of interview (day/month/year) ___ ___/___ ___/___ ___ ___ ___

Study eligibility

Please confirm that the patient meets the study eligibility criteria and has read the information sheet and consented to the study

Age over 18 years	☐
Confirmed HIV positive status	☐
Patient information sheet	☐
Informed consent	☐

Agreed to collect samples: Yes □ No □

A: Socio-demographic details

1. Date of birth (day/month/year) ___ ___/___ ___/___ ___ ___ ___
2. Age _____ years
3. How many years of education did you complete? _____ years
4. What is highest education level you have completed? None □
   Primary □
   Secondary □
   Higher Education/ University □
   Don't know □
   Refused □
   Other, specify
   _____
5. Marital status (tick one): Married (monogamous) □
   Married (polygamous) □
   Single □
   Divorced/Separated □
   Widowed □
   Refused □
   Cohabiting □
   Other, specify

_____

6. Employment (tick one):

Salaried job	(1)
Self-employed	(2)
Housewife	(3)
Unemployed	(4)
Casual laborer	(5)
Refused	(6)
Other, specify	(7)

_____

7. Household income per month (tick one):

None	(1)
< 5000 Ksh	(2)
5001 – 10000 Ksh	(3)
10001 – 15,000 Ksh	(4)
>15,000 Ksh	(5)
Don't know	(6)
Refused	(7)

B: Reproductive History

Do you have children? Yes () No ()

If yes, how many? _____

Do you use contraceptive? Yes () No ()

If yes, how often? Regular () Sometimes () Rarely ()

If yes, what type? COCP () Barrier methods () IUCD ()

Others, specify_____

C: Pap smear and HPV vaccine history

Have you ever had Pap smear? Yes () No ()

If yes, how many times? Once () Twice () Three times ()

Have you been vaccinated against HPV? Yes () No ()

D: Sexual behavior

Are you currently sexually active or have you ever been?

(Yes) (No)

What was your age at first sexual intercourse?

Below 18 years (1) 19-24 years (2) Above 25 years (3)

How many partners have you had in the past?

1–2, 3–4, 5–9, ≥ 10

Do you use protection during sexual intercourse with your partner?

Never (1) Rarely (2) Sometimes (3) Often (4

E: History of STIs

Have you ever been diagnosed with a sexually transmitted disease/infection?

Yes (1) No (2)

If yes, which one below here

Gonorrhea (1) Syphilis (3) Chlamydia (4)

Others (specify) ……

F: HIV History

HIV Stage: CD4 Level: Viral Load:

When were you diagnosed as having HIV? (day/month/year) ___ ___/___ ___/___ ___

How was HIV detected? (tick only one)

- At the occasion of a VCT: Yes □
- During pre-natal check-up: Yes □
- Because of a sickness, Yes □

Specify: _____

- Other, specify: _____
- Don't know: □
- Refused: □

Are you currently on antiretroviral medications? Yes □ No □

Don't know □

Refused □

Other, specify

_____

If yes,

a) Specify current medications: _____

b) Original start date: ___ ___/___ ___/___ ___ ___ ___ Don't know □

c) Do you know why you were started on antiretroviral medication?

- Because of sickness, Yes □

  Specify: _____
- Because of low CD4 count: Yes □
- Because of high viral load: Yes □
- Other, specify: _____

- Don’t know: □
- Refused: □

E: Urine and Cervical Sample

1. Urine sample obtained Yes () No ()
   If no, specify reason _____
2. Cervical Sample obtained Yes () No ()
   If no, specify reason _____

APPENDIX C: LABORATORY METHODS

DETECTION OF HIGH RISK (HR) GENOTYPE HPV USING THE COBAS 4800 SYSTEM AND THE COBAS 4800 HPV ASSAY

Purpose

The detection of HR genotype HPV using the Cobas 4800 automated system.

Scope

This procedure outlines the method for extraction amplification and detection of HPV genotype using the Cobas 4800 platform.

Specimens are limited to cervical cells collected in **Cobas** PCR Cell Collection Media (Roche Molecular Systems, Inc.), PreservCyt Solution (Cytyc Corp.) SurePath Preservative Fluid (BD Diagnostics-TriPath) and Evalyn® Brushes.

Principles of the Procedure

The Cobas 4800 Human Papillomavirus (HPV) Test is a qualitative *in vitro* test for the detection of Human Papillomavirus in patient specimens. The test utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies (types) HPV16 and HPV18 while concurrently detecting the rest of the high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) at clinically relevant infection levels.

The Cobas 4800 HPV Test is based on two major processes:

(1) Automated specimen preparation to simultaneously extract HPV and cellular DNA;

(2) PCR amplification of target DNA sequences using both HPV and β-globin specific complementary primer pairs and real-time detection of cleaved fluorescent-labeled HPV and β-globin specific oligonucleotide detection probes.

Specimen preparation for the Cobas 4800 HPV Test is automated with the use of the Cobas x 480 instruments. Cervical specimens collected in Cobas PCR Cell Collection Media, PreservCyt Solution or SurePath Preservative Fluid are digested at elevated temperatures and then lysed in the presence of chemotropic reagent. Released HPV nucleic acids, along with the β-globin DNA serving as an internal control, are purified through absorption to magnetic glass particles, washed and finally separated from these particles, making them ready for PCR amplification and detection.

The Master Mix reagent for the Cobas 4800 HPV Test contains primer pairs and probes specific for the 14 high-risk HPV types and β-globin DNA. The detection of amplified DNA (amplicon) is performed during thermal cycling using oligonucleotide probes labeled with

four different fluorescent dyes. The amplified signal from twelve high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), is detected using the same fluorescent dye, while HPV16, HPV18, and β-globin signals are each detected with their own dedicated fluorescent dye.

All samples, if refrigerated, must be brought to ambient temperature before loading on the Cobas x 480 instruments.

The amplification process takes about 2 hours to complete.

- Analysis: the brush is re-suspended in medium to allow testing on Cobas 4800

APPENDIX D: STUDY TIMELINES

Activity	**March-June 2019**	**July 2019**	**August 2019**	**September 2019**	**Oct 2019**	**November 2019**	**November 2019**
Proposal writing and presentation							
Ethical committee approval							
Data collection							
Data consolidation and analysis							
Compilation of report							

APPENDIX E: STUDY BUDGET

Component	**Duration/Number**	**Cost (KES)**	**Total (KES)**
Research assistant	2	20000	40,000/=
Statistician	1	30000	30,000/=
HPV Charges	140	7000	980,000/=
Printing			
Consent form	150	20	3,000/=
Questionnaires	150	20	3,000/=
Final report	150	10	1,500/=
Miscellaneous	10,000		10,000/=
TOTAL			**1,067,500/=**