# HUMAN PAPILLOMAVIRUS (HPV) TESTING AND PAP SMEAR CYTOLOGY CO-TESTING AS A 'TEST OF CURE' IN PATIENTS PREVIOUSLY TREATED FOR CERVICAL LESIONS BY LOOP ELECTROSURGICAL EXCISION PROCEDURE (LEEP) AT KENYATTA NATIONAL HOSPITAL

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# Human Papillomavirus (HPV) testing and Pap smear cytology co-testing as a 'test of cure' in patients previously treated for cervical lesions by LEEP at Kenyatta National Hospital

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**A thesis submitted in Partial fulfilment for the Degree of Master of Medical Laboratory Science in Clinical Histopathology & Diagnostic Cytology in the Jomo Kenyatta University of Agriculture and Technology**

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## DECLARATION

This thesis is my original work and has not been presented in any other institution for a degree or any other award.

Sign…….. Date……

**Raymond Chibvongodze**

This thesis has been submitted with our approval as University supervisors.

Signature……Date……

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Signature……Date……

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**University of Nairobi, Kenya**

## DEDICATION

I dedicate this work to my mother, brothers and sisters for believing in me in whatever I do.

Finally, I dedicate this work to all women who were diagnosed and treated for various uterine cervical lesions.

## ACKNOWLEDGEMENTS

I thank the almighty God for His strength grace and mercy that has enabled me reach this far.

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## OPERATIONAL DEFINITIONS OF TERMS

**Carcinoma -** A cancer arising in the epithelial tissue of the skin or of the lining of the internal organs.

**Cervical Cancer-** A type of cancer that occurs in the cells of the cervix, the lower part of the uterus that connects to the vagina.

**Colposcopy** – It's a procedure that allows illuminated and magnified viewing of the cervix and the vagina.

**Conventional Pap smear** – A technique used in screening women for pre-cancerous lesion and cervical cancer that involves collection of a smear from the cervix and the endocervical canal by the use of an Ayres spatula and cytobrush.

**Cryotherapy** – Freezing abnormal tissues with a probe cooled by liquid nitrous oxide or carbon dioxide.

**Dysplasia –** An abnormal tissue growth characterised by pleomorphism and hyperchromasia.

**Hysterectomy -** A surgery to remove a woman's womb (uterus). The uterus is a hollow muscular organ that nourishes the developing baby during pregnancy.

**LEEP -**One way to remove abnormal cells from the cervix by using a thin wire loop that acts like a scalpel (surgical knife). An electric current is passed through the loop, which cuts away a thin layer of the cervix.

**Liquid Based Cytology-** A method for preparing cytological specimens in particular from the cervix for microscopic evaluation in which the patient specimen is suspended in a liquid fixative, which is used to produce a thin layer of cells.

## ABBREVIATIONS

**AGC** Atypical Glandular Cells

**AIS** Adenocarcinoma in Situ

**ASC-H** Atypical Squamous Cells cannot exclude High Grade Squamous Intraepithelial lesion

**ASC-US** Atypical squamous cells of Undetermined Significance

**BTL** Bilateral tubal ligation

**BV** Bacterial vaginosis

**CIN** Cervical intraepithelial lesion

**CIS** Carcinoma in Situ

**CMI** Cell mediated immunity

**CKC** Cold knife conization

**DNA** Di-ribonucleic acid

**DPX** Distyrene Plasticizer Xylene

**EA** Eosin Azure stain

**HAART** Highly active antiretroviral therapy

**HC2** Hybrid Capture 2

**HI** Humoral immunity

| | |
|---|---|
| **HIV** | Human Immune-deficiency Virus |
| **HPV** | Human Papillomavirus |
| **HSIL** | High grade Intraepithelial Lesion |
| **ICC** | Invasive Cervical Carcinoma |
| **IUCD** | Intrauterine Contraceptive Device |
| **ISH** | In- situ Hybridisation |
| **KAVI** | Kenya Aids Vaccine Institute |
| **KNH** | Kenyatta National Hospital |
| **LEEP** | Loop Electrosurgical Excision Procedure |
| **LLETZ** | Large Loop Excision of the Transformation Zone |
| **LSIL** | Low Grade Squamous Intraepithelial Lesion |
| **NILM** | Negative for Intraepithelial Lesion or Malignancy |
| **NOS** | Not Otherwise Specified |
| **NPV** | Negative Predictive Value |
| **OG** | Orange Green |
| **ORF** | Open Reading Frame |
| **PCR** | Polymerase Chain Reaction |
| ***RB*** | Retinoblastoma |

**RNA** Ribonucleic acid

**SCC** Squamous Cell Carcinoma

**SIL** Squamous Intraepithelial Lesion

**SOP** Standard Operating Procedure

**SPSS** Statistical Packages for Social Sciences

**UoN** University of Nairobi

## ABSTRACT

Human Papilloma Virus infection is a pre-requisite for the development of the majority (99.7%) of precancerous cervical lesions. Treated women have five times risks of invasive cervical cancer compared to women who have always had a normal Pap smear, thus special follow-up measures are critical to reduce these risks. The main objective was to determine the utility of co-testing by conventional Pap smear cytology and HPV genotyping as a 'test of cure' in patients previously treated for cervical lesions by LEEP at KNH.This study was a cross sectional descriptive study. The study site and was clinic 66, KNH, for duration of 4 months**.** Twenty five women with prior history of cervical lesion treatment by LEEP were recruited into the study. The Papanicolaou staining protocol was used to stain the slides. Pap smears were reported using the Bethesda system 2014 by a Pathologist. HPV testing was done using the HPV Genotypes 14 Real-TM Quant test kit in KAVI laboratory. SPSS version 21 was used to analyze the data. In all tests, a p-value below 0.005 was regarded as statistically significant. Mean age of the study participants was 43.3 ±12.3 years. The average age at first intercourse was 21.4±3.9. Out of 25 participants, 22 (88%) had a report of NILM while 3 (12%) had a report of ≥ASCUS). 16 (64%) were positive for HPV with HPV 56 being the commonest subtype 11 (41%).The Cohen's Kappa correlation between Pap smear and HPV DNA test was statistically insignificant, k=0.143, 95% Confidence Interval: -0.17 to 0.46, p=0.166.The association between HIV status and Pap smear findings post LEEP was statistically insignificant, $X^2$=0.711, p=0.399. The association between HIV status and HPV positivity post LEEP was statistically insignificant, $X^2$=0.001, p =0.973. Approximately 10% of women have residual or recurrent cervical lesion post LEEP treatment at KNH. The majority of women were positive for high risk HPV post LEEP treatment**.** HPV 56 was the commonest HPV in post LEEP patients at KNH. HPV genotyping should be introduced in the monitoring of women post LEEP treatment as this helps to stratify women according to their risk of recurrent cervical lesions. HPV positivity did not correlate with clinical disease, a larger study which incorporate HPV-mRNA transcript which is more specific should be don

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background information

Cervical cancer is the second commonest cancer in women after breast cancer. In the year 2000, there were close to half a million incident cases and close to 300 000 deaths from cervical cancer globally. The majority of these fatalities ( 80%) occurred in third world countries like Kenya (WHO IARC, 2007; Parkin *et al*, 2003).

Infection by Human Papilloma Virus is a pre-requisite for the genesis of the majority (99.7%) of precancerous cervical lesions. Immunosuppressed patients like HIV positive women have a higher risk of HPV infection, higher risk of HPV persistence as well as increased possibility of infection by multiple HPV genotypes in comparison to HIV negative women (Anderson , 2012).

It is reported that HPV viral load is independently associated with HPV persistence hence prevalence and persistence of HPV is consistent with decreasing CD4+ count and increasing HIV RNA levels (Wang *et al*, 2011*)***.**

Approximately 68% of the estimated 33.3 million HIV infected people reside in Africa. The majority of these are women (60%). It is reported in literature that there are about 7,000 incident cases of HIV per day. These areas with high HIV prevalence are also overburdened with the highest cervical cancer rates ( UNIAIDS, 2010)**.** This

is attributable to the general unavailability of sound and effective screening programs in these low resource settings.

Residual or recurrent disease is more frequently reported in HIV infected as compared to HIV non infected women after treatment e.g. by cryotherapy, LEEP and cold knife conisation (CKC). Treated women have five times risks of invasive cervical cancer compared to women who have always had a normal Pap smear because there is a higher likelihood of recurrence, thus special follow-up measures are critical to reduce these risks (Kreimer et al, 2006; Kocken *et al*, 2012). Elfgren et al demonstrated that Human Papillomavirus will be undetectable 24 months post LEEP treatment. HPV vaccines (Gardasil and Cervarix) can greatly reduce incident HPV infections if administered to young women of ages 9-13 years, that is before initiation of coitus (Elfgren *et al*, 2002)**.**

### 1.2. Statement of the problem

Cervical cancer is the second commonest cancer in women and the leading cause of cancer deaths in women of reproductive age in Kenya. Kenyan hospital registries indicated that cervical cancer accounted for 70-80% of all cancers of the genital tract *(*NCCPP, 2012*)*. In 2017, 4802 new cases and 2451 deaths were recorded in Kenya (HPV and related cancers, 2017).

Treatment of cervical precancerous lesions significantly minimises invasive cancer risk by 90%; however treated patients still have a fivefold risk of developing invasive cancer compared with patients who have had repeated negative cytology

results because of higher likelihood of recurrence (Kocken et *al*, 2012; Kreimer *et al*, 2006). Thus it is essential to have special follow-up measures to try to reduce these risks.

Currently follow- up protocols in KNH involves screening with Pap smears only; however Pap smears have their own limitations. Pap smears have low sensitivity because cytological criteria for detection are not always present after infection with HPV. In addition the low sensitivity could be due to other reasons e.g. sampling, quality of smears including abundant inflammatory cells, subjectivity in cytology interpretation.

Furthermore it is reported that the sensitivity of Pap smear could be potentially lower in HIV infected women (Clifford *et al*, 2006.

### 1.3. Justification

In as much as Pap smear cytology is established as the method of choice for cervical cancer screening, it has its limitations with regard to reliability, sensitivity and specificity. The incorporation of oncogenic HPV genotype testing is proposed to enhance sensitivity and efficacy of gynaecological cytology.

In addition to improving sensitivity, women who repeatedly test negative for oncogenic HPV and do not have serious cervical lesions (≥ LSIL) on Pap smear will be screened after 3-5 years depending on age. This would greatly increase spacing of time between screenings and reduction in number of screening consultations. This would aide to better compliance and lower defaulter rate among patients.

Apart from improved accuracy and compliance, automated HPV genotype testing would reduce human errors due to subjectivity in interpreting Pap smears.

The proposed research was aimed at investigating the efficacy of new and improved strategies for the detection of recurrent and residual cervical lesions in patients previously treated for cervical lesions by LEEP using high risk HPV testing. These findings may be used to guide policy, strategy and guidelines formulation for better management of post treatment patients at KNH.

### 1.4. Hypothesis

Co-testing by conventional Pap smear and HPV DNA genotyping can be used as a 'test of cure' in patients with prior history of cervical lesion treatment by LEEP at KNH?

### 1.5.Objectives

#### 1.5.1. General objective

To determine the utility of co-testing by conventional Pap smear and HPV genotyping test as a 'test of cure' in women previously treated for cervical lesions by LEEP at KNH.

#### 1.5.2. Specific objectives

1. To determine the prevalence of residual or recurrent cervical lesions in women previously treated for cervical lesions by LEEP at KNH.

2. To evaluate HPV prevalence in women previously treated for cervical lesions in KNH.

3. To determine frequencies of HPV subtypes in women previously treated for cervical lesions by LEEP at KNH.

4. To correlate Pap smear findings and HPV test results

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Human Papilloma Virus (HPV) and its characteristics

HPV is a member of Papovavirus family and is a small circular, double stranded virus. Each of the strands is composed of approximately 7900 nucleotides which are encapsulated by an icosahedral capsule (Wilbur *et al*., 2008). The HPV genome is represented as a single strand of DNA in the form of genes (ORFs) that contain protein synthesis messages (Carter *et al*, 2011).

There are six early ORF's *E1, E2, E4, E5, E6 and E7*. These play a pivotal role in the replication of the virus. L1 and L2 are the two late ORFs. These are responsible for inscribing viral capsular proteins. *L1* is a component of HPV vaccines (Schiffman *et al*, 2007).

## 2.2. Classification of Human Papilloma Virus

HPV are a group of DNA virus in excess of 130 genotypes that infect human epithelial cells. HPV types are subdivided into two categories (Mesher *et al*, 2010):

### 2.2.1. High risk or Oncogenic HPV genotypes

These are implicated in the causation of low, high-grade squamous intraepithelial lesions, glandular lesions. The most important are 16, 18, 31, 45 and 52. Others include 33, 35, 39, 51, 56, 58, 59 and 68. HPV 16 is more common in squamous lesions while HPV 18 is more commonly identified in glandular lesions (Wilbur *et al*, 2008).

### 2.2.2. Low risk HPV genotypes

These are commonly associated with benign genital warts. These include 6, 11, 42, 43, 44, 53, 54, 57 and 66 (Carter *et a*, 2011).

## 2.3. Human Papillomavirus and cervical cancer

### 2.3.1. HPV persistence.

HPV is the commonest sexually transmitted infection in women as they become infected with HPV within 18 months of sexual intercourse onset. The majority of these infections are asymptomatic and are short lived with the majority being cleared within a further 18 months. HPV infection persists only in a minority of women with the ultimate result being, the abnormal proliferation of epithelium (Cater *et al*, 2011).

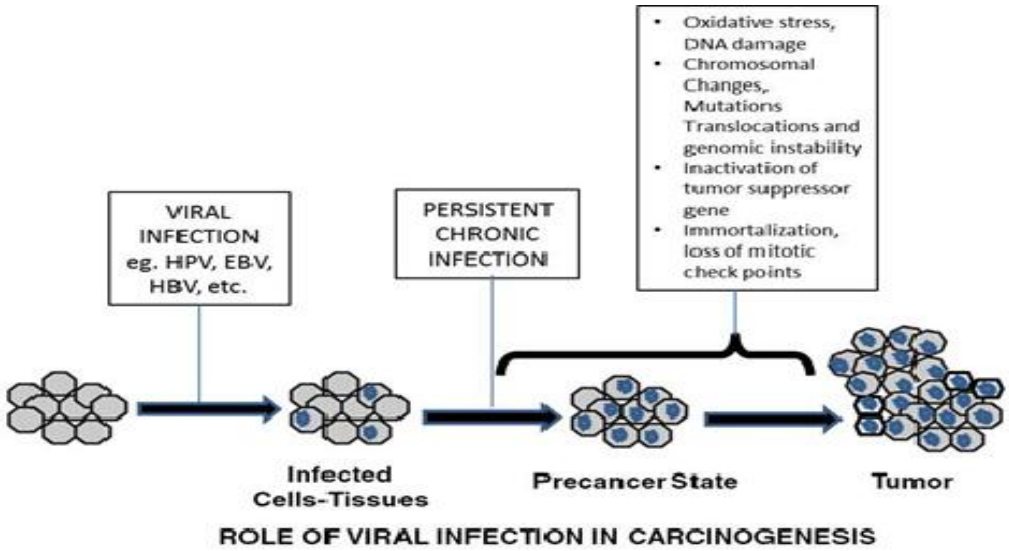


**Figure 2.1: Role of persistent HPV infection in carcinogenesis. (**Alexandros *et al*, 2010).

### 2.3.2. HPV pathogenesis

HPV pathogenesis involves the following stages:

#### 2.3.2.1. Infection

Cervical cancer development starts with the infection of the basal and reserve cells of the stratified non keratinised squamous epithelium by HPV during sexual intercourse. HPV penetrates the superficial stratified squamous epithelium through breaks and ulcers on the epithelium (Wilbur *et al*, 2008).

Once inside host cell, there is the over expression of early ORF genes that facilitates the replication of the virus as well as progressive movement of the infected cells to the surface of the epithelium. Replication occurs via two phases:

***Latent phase*:** The viral DNA is present as an episome outside host cell DNA and replicates in tandem with host cell DNA, thus no new viral particles are produced in this phase.

***Production phase**:* Viral DNA replication occurs independently of host DNA resulting in production of virions capable of infecting other cells. The virions are capable of filling up intermediate or superficial cells producing a cytopathic effect known as koilocytosis (Cibas *et al*, 2009).

#### 2.3.2.2. Integration

In advanced lesions, the HPV genome is integrated into the human genome and this disrupts the *E2* region whose function suppress the formation of E6 and E7 oncoprotein . This results in the increased levels of *E6* and *E7* proteins (Bibbo *et al*, 1997).

#### 2.3.2.3. *E6* and *E7* genes in carcinogenesis

*E6* and *E7* oncoproteins distort the cell cycle and its regulatory mechanisms in order to enhance the replication of the HPV virus. The *E6* proteins target the tumor suppressor gene *p53* and target it for degradation after conjugation to ubiquitin. The loss of *p53* gene causes defective apoptosis, defective DNA repair and G1 arrest of cells (Cibas *et al*, 2009)

*E7* protein targets the *RB* gene protein *(pRB).* Upon binding, the RB protein there is dissociation of the *pRB-E2F-1*complex and *E2F-1*is set free. The free E2F-1 then drives the formation of proteins that are vital for cells to progress to the S phase of the cell cycle (Cibas *et al*, 2008).

#### 2.3.2.4. Outcome

Outcome is increased cell proliferation, decreased apoptosis and defective DNA repair which cause genomic instability. Consequently, the cervical cell accumulates increased amounts damaged DNA that is irreparable leading to malignant transformation (Wilbur *et al*, 2008).

### 2.3.2.5. Role of immunosuppresion

The host cell mediated immunity (CMI) and humoral immunity (HI) prevents and slows down the development of cervical cancer as evidenced by the increase in cervical cancer in HIV patients and patients on immunosuppressive therapy (Mesher *et al* , 2010).

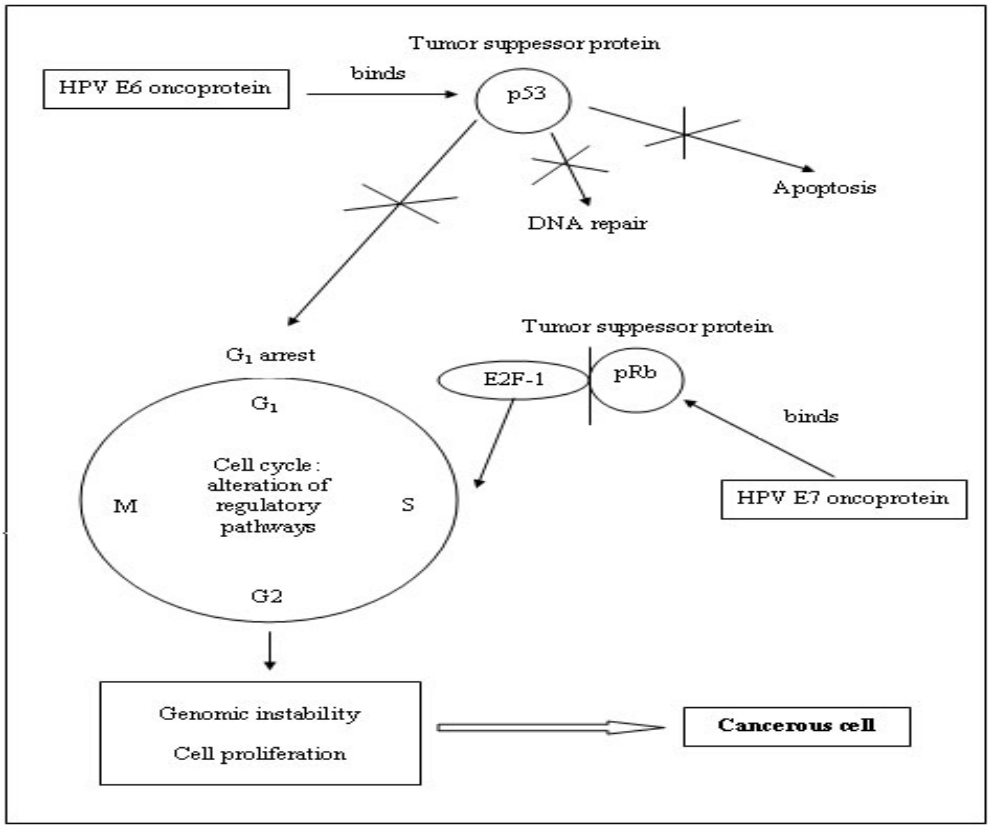


**Figure 2.2. Illustration of HPV pathogenesis.** (Gomez et al, 2007).

## 2.4. HPV, HIV and Cervical dysplasia

### 2.4.1. Interrelationship of HIV and HPV

Immunosuppressed patients like HIV positive women have a higher risk of HPV infection, higher risk of HPV persistence as well as increased possibility of infection by multiple HPV genotypes in comparison to HIV negative women (Clifford *et al*, 2006; Cibas *et al*, 2009).

In a cross-sectional survey done in South Africa of 109 HIV positive women on HAART, oncogenic HPV was detected in almost 80% of the cases (Moodley *et a*l., 2006). In a cohort by Wang et al in South Africa, of more than 123 HIV infected women, the likelihood of infection by HPV doubled a few years of contracting the HIV virus(Wang *et al*, 2011).

### 2.4.2. HIV and cervical dysplasia

Abnormal Pap smear findings are usually associated with a combination of HPV infection and HIV associated immunosuppresion. A cohort study done by Wang et al in South Africa in 2011, two thirds of HIV infected patients initiating HAART had abnormal cervical cytology findings (Wang *et al*, 2011). Heard et al in 2002 documented increased likelihood of regression of cervical abnormalities with initiation of HAART. However, in total contrast to Heard et al findings , a study by Paramsothy et al in USA in 2009 (seven years later) showed that effective HAART correlated with better clearance of HPV rather than cervical neoplastic lesion regression (Paramsothy *et al*, 2009). These differences may be attributed to

differences in study designs, the HAART medication used and the duration of the therapy.

### 2.4.3. Association between HIV and cervical cancer

According to Centre for Disease Control (CDC), cervical cancer is an AIDS defining condition. A study done by Chaturvedi in 2009 in USA showed a statistically significant correlation between AIDS and cancer registries several regions ( Chaturvedi *et al,* 2009). Studies done in various countries from the sub- Sahara region demonstrated that invasive cervical cancer and HIV were strongly associated (Kahesa *et al*, 2008; Stein *et al*, 2008; Gichangi *et al*, 2003).

### 2.4.4. Recurrence of cervical lesions in HIV infected patients

There is increased persistence and recurrence after treatment in HIV positive patients with certain studies documenting a recurrence >50% (Tebeu *et al,* 2006). The followed situations result in increased recurrence rates: positive margins with LEEP, glandular involvement, greater immunosuppresion and lack of suppressive therapy (Shah *et al*, 2008; Robinson *et al*, 2001; Lima *et al*, 2009 ). The majority of recurrences appear to be LSIL in HIV infected patients which are usually associated with new HPV infections (Ginelmaier *et al,* 2007; Massad *et al,* 2008; NCCPP, 2012-2015).

## 2.5. Overview of Cervical Neoplasia

Cancer of the cervix arises from the cervical squamous and glandular epithelium (Bibbo *et al,* 1997). The cervix is covered by both simple columnar and stratified non-keratinizing squamous epithelia. The junction between these epithelia is called

the squamo-columnar junction (SCJ). This SCJ is composed of squamous metaplastic cells and is the place where oncogenesis usually starts from. The development of invasive cervical cancer is preceded by a number of precancerous lesions (Bibbo *et al*, 1997).

### 2.5.1. Squamous lesions

#### 2.5.1.1. Atypical squamous cells (ASC)

Atypical squamous cells are characterised by borderline nuclear and cellular changes that are inadequate for a definite classification into a squamous intraepithelial lesion (SIL).The uncertainty of the classification can be due to cellular sparsity or degeneration ( Nayar R *et al*, 2015) .

#### 2.5.1.1. Low grade squamous intraepithelial lesion (LSIL)

Low grade squamous intraepithelial lesion (LSIL) is a squamous epithelium lesion that is well differentiated and occurs mainly in intermediate cells. Atypia is confined in the lower third of the stratified squamous epithelium. Characterised by cells with cytoplasmic cavitations (koilocytosis), dysplastic nuclei and bi or multinucleation ( Nayar R *et al*, 2015).

#### 2.5.1.2. High grade Squamous Intraepithelial Lesion (HSIL)

High grade Squamous Intraepithelial Lesion (HSIL) is a more advanced and severe lesion than LSIL that predominantly occurs in cells with immature cytoplasm (parabasal cells). These cells can be singly dispersed, exfoliate as two dimensional

syncytial aggregates or hyperchromatic three dimensional clusters. Studies have demonstrated that the majority (97%) of HSIL lesions test positive for oncogenic HPV subtypes (Smith *et al*, 2011; Cibas *et al*, 2009).

### 2.5.2. Glandular Lesions

The Pap smear is not as good as a screening tool for lesions from glandular epithelium compared to squamous lesions. There are several factors that contribute to this low sensitivity but chief among them are sampling and reporting errors. There is enormous interobserver discrepancy in interpreting these lesions (Nayar R *et al*, 2015).

#### 2.5.2.1 Atypical Glandular Cells (AGC)

An atypical glandular cell is a diagnostic category for cells exhibiting nuclear and cytoplasmic features that are border line between benign reactive changes and adenocarcinoma in situ or adenocarcinoma. Based on parenchymal cells affected, atypia observed in endocervical is referred to atypical endocervical whereas those in endometrial cells are referred to as atypical endometrial cells. There is also an option of not further clarifying the affected cell type, this category is reported as atypical glandular cells not otherwise specified.

There is mild nuclear enlargement (3-5 times), hyperchromasia and pleomorphism. The cell boarders are distinct and well defined ( Demay *et al*, 2012) .

Atypical glandular cells, favour neoplastic are endocervical cells with cell morphology that is qualitatively and quantitatively inadequate for a definite classification into adenocarcinoma in situ (AIS) adenocarcinoma. Composed of cells

with ill defined boarders, occasional mitosis, moderate nuclear enlargement and moderate hyperchromasia (Nayar R *et al*, 2015 , Demay *et al*, 2012).

### 2.5.3. Endocervical adenocarcinoma in situ (AIS)

These are characterised by nuclear anisocytosis, coarse hyperchromatic chromatin, normal and abnormal mitotic figures, stratification and no evidence of invasion. The cells can have the following arrangements: sheets and clusters, palisaded strips, acinic, rosetting and feathering architectures. Histologically AIS can have endocervical, intestinal, endometroid and clear cell variants (Demay *et al*, 2012).

### 2.5.4. Adenocarcinoma

Adenocarcinoma of the uterine cervix is categorized into endocervical and endometrial adenocarcinoma.

#### 2.5.4.1. Endocervical adenocarcinoma

Constitutes of 25% of all cervical cancer cases and usually occurs in the fifth decade of life. The prognosis of endocervical adenocarcinoma is poorer than that of squamous cell carcinoma. Over half of cases of invasive adenocarcinoma are associated with AIS. HPV 18 is implicated more in the causation of adenocarcinoma compared to HPV 16 (Demay *et al*, 2012).

##### 2.5.4.1.1. Cytomorphology of endocervical adenocarcinoma

The cells are arranged as single cells, two dimensional loosely cohesive groups, three dimensional hyperchromatic crowded groups and syncytial aggregates. These cells can have acinic, rosetting or feathery architecture. The nuclei are enlarged,

pleomorphic, oval or elongate in shape, with irregular nuclear margins, irregular chromatin distribution and parachromatin clearing. Diminished vacuolated cytoplasm and a macronucleoli may be present. Necrotic tumor diathesis may be present in the background (Demay *et al*, 2012).

### 2.5.4.2. Endometrial adenocarcinoma

Endometrial adenocarcinoma evolves through several stages:

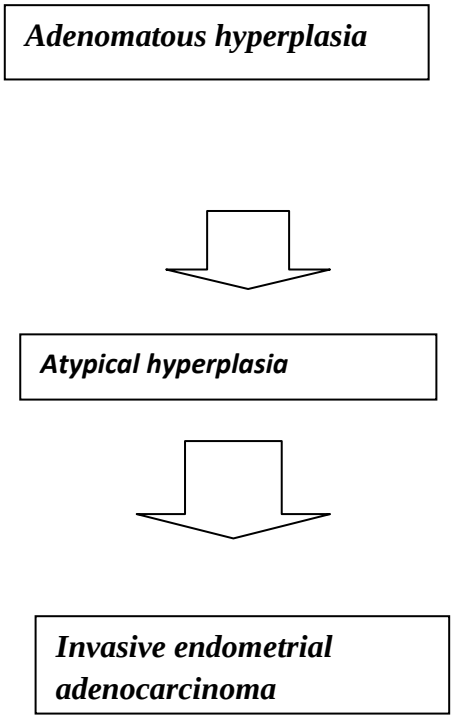


**Figure 2.3: Evolution of Invasive endometrial carcinoma. (**Demay *et al*., 2012)

#### 2.5.4.2.1. Cytomorphology of endometrial adenocarcinoma

In a well differentiated endometrial adenocarcinoma, the cells are singly scattered or in dyshesive small clusters comprising of 5 -10 cells. The size of the cells depends on the grade on the tumor. Well differentiated tumors the cells are slightly enlarged with mild anisonucleosis and slight hyperchromasia. In high grade tumors, there is

irregular chromatin distribution and parachromatin clearing. In most cases there is a finely granular tumour diathesis (Nayar *et al*, 2015).

### 2.5.3. Small Cell Carcinoma

These are carcinomas with poor differentiation. These closely mimic malignancies that arise from lungs. HPV subtype 18 has been demonstrated to be more associated with these tumors. These tumors are aggressive with higher likelihood of distant metastasis. The most diagnostic feature is the identification of nuclei with atypical features exhibiting nuclear moulding ( Demay *et al*, 2012).

## 2.6. Screening methods for cervical cancer

### 2.6.1. Pap smear

The introduction of Papanicolaou (Pap) test in 1949 has led to the reduction in incidence and suffering secondary to cancer of the cervix in the past 60 years in USA. The Pap test is based on simple scratching of cervical cells using a brush followed by cytologic examination. However the Pap smear has its own limitations. Sampling and interpretation errors cause false positive rates of 10-25% of all cases. Most of the demerits of Pap smears can be addressed by using newer screening tests using liquid based techniques (Anderson *et al*, 2012) .

The Bethesda system is currently being used for classification of Pap smears. This system includes specimen adequacy, diagnosis and recommendations. Pap smear screening is recommended to commence at the age of 21years and can be discontinued after the age 65-70 years, if no more risks such as unprotected sexual intercourse (Anderson *et al*, 2012).

Frequent screening is recommended in HIV infected women (once every year) compared to HIV negative women (once in three years). Screening should be done two in the first year of diagnosis and, if normal annual screening thereafter is recommended. Patients previously diagnosed with cervical abnormalities or treated for cervical dysplasia should have more frequent Pap smears (ACOG, 2009).

### 2.6.2. VIA/VILLI

Pap smear has its own barriers such as poor health infrastructure, shortage of competent cytologists and pathologist and cost. Furthermore, Pap smears do not use the 'See and treat approach', as women will need to be followed up for treatment. This usually results in high rates of loss of follow- up. This led to the popularity of VIA and (VILLI) (Palanuwong *et al*, 2007).

This technique is based on application of 3 to 5% acetic acid (VIA) or Lugol's iodine (VILLI) followed by optical observation. The results are negative, suspicious or positive (Phongsavan *et al*, 2011).

#### 2.6.2.1. Advantages and disadvantages of VIA/VILLI

The major merit of VIA/VILLI is the 'See and Treat' approach where patients are assisted on a single visit. Others include: it is inexpensive, acceptable, feasible and safe. However the major limitation is that it is less reproducible and is inherently subjective. In addition VIA/VILLI is not able to evaluate the transformation zone in

postmenopausal women which often recedes into the endocervical canal (Cremer *et al*, 2011).

### 2.6.3. HPV screening

HPV genotyping is currently used to stratify women with an initial diagnosis of ASCUS and LSIL and in developed countries where it complements cytology as a primary screening test (ACOG, 2009.; Masad *et al*, 2004*)*.

#### 2.6.3.1. Advantages and disadvantages of HPV testing

The major advantage of HPV is that it is reproducible and objective. Furthermore, it has a high NPV because test that are negative are associated with a cervical cancer risk of up to 2% (Lonky *et al*, 2010). In addition, studies by Balasubramanian et al in 2010 demonstrated that patients who perform self testing yield accurate results thus reducing the need for clinical examination. In as much as it offers reliable and valid testing, it has some drawbacks for low resource countries as the test still has prohibitive costs. Secondly, results may not be available instantly as the tests are usually run in batches.

#### 2.6.3.2. HPV testing methods

The following methodologies can be used for the detection of HPV:

#### 2.6.3.2.1. Polymerase chain reaction (PCR)

It is the standard reference method. PCR is based on *in vitro* replication of uncommon DNA fragments that can then be detected when their numbers are high. It is used for specific viral typing e.g. (HPV 16 and 18), viral load quantification, and DNA sequencing and mutation analysis. Disadvantages include lack of specificity for clinical disease, lack of inter-laboratory standardization, and by nature they are subject to environmental contamination of negative specimens giving rise to false positive results. However, closed system automation markedly reduces this problem (Smith *et al*, 2011).

#### 2.6.3.2.2. Hybrid capture 2 (HC2)

A Hybrid capture 2 is designed to test the presence of most high risk HPV subtypes. In addition, it can also detect low risk subtypes. Hybrid Capture 2 is an immunoassay based on hybridization of specific genomic RNA probes to targeted DNA fragments containing the HPV subtypes. The formed hybrids are then detected in a chemiluminescent assay.

HC2 can semi-quantitate the amount of HPV in the sample as well as classifying the HPV genotype according to their risk. However, PCR is incapable of identifying the specific HPV genotypes.

The method is not as sensitive as PCR but it does not have a problem of false positives inherent as in PCR since it does not depend on amplification of signals. The cocktail approach of the HPV HC2 test provides an excellent tool for the triage of

patient with minor cytologic abnormalities on Pap test though it cannot determine the specific HPV type present (Wilbur *et al,* 2008).

#### 2.6.3.3.2. In situ hybridization (ISH)

ISH is done on cellular specimens and thus permits direct visualisation of infected cells. The method is not as sensitive as HC2 however; it is more sensitive for the presence of clinical disease (Wilbur *et al*, 2008).

#### 2.6.3.3.3. Immunocytochemistry (ICC)

ICC is based on the use of the antibodies that can detect HPV capsid antigen; however it is not specific for clinical disease (Cibas *et al*, 2009).

#### 2.6.3.3.4. HPV wave invader technology

This is a signal amplication technology that utilises a proprietary isothermal process. Its major merit is that it is more sensitive than HC2 and it has an internal control (human anti actin) to ensure that adequate sample has been obtained ( Wilbur *et al*, 2008; Hubbard *et al,* 2003).

### 2.6.4. Comparison of specificity and sensitivity of the screening methods.

A pooled analysis of several previous studies, using histopathological diagnosis on a cervical biopsy as the gold standard and CIN 111 as the target lesion. The mean sensitivity for VIA almost 80%, VILLI had a sensitivity of 91.2%, whereas Pap smear had a lower sensitivity of 57% and HPV HC 2 assay had a fair sensitivity of 62%. The specificity of visual methods (both VIA and VILLI) was comparable at

85%.The specificity of  both Pap smear and HPV testing were comparable 93% and 94% respectively (Alexandros G *et al*, 2010).

### 2.7. Treatment

For high grade SILs, excisional or ablative treatment is recommended. Before hysterectomy can be done it is important to rule out the presence of invasive cervical cancer with an excisional biopsy. The commonly used procedures for treatment are Loop Electrosurgical Excisional Procedure (LEEP), cryotherapy or cervical conization. The table below summarises the characteristics of each treatment option ( Arbyn *et al*., 2008).

**Table 2.1. Summary of methods of treatment for cervical dysplasia.** (Arbyn et al, 2008)

| | **CERVICAL CONIZATION** | **LEEP** | **CRYOTHERAPY** |
|---|---|---|---|
| **ANAESTHESIA** | Regional | Local | Not required |
| **OTHER RESOURCES NEEDED** | Operating room supplies, instruments, personnel, anaesthetics | Electrical generator, wire loops (different sizes) | Cryoprobes (different sizes), carbon dioxide gas. |
| **EFFECTIVENESS** | 96% | 96%+ | 88% |
| **TECHNICAL DIFFICULTY** | Highest | Intermediate | Lowest–nurses, midwives can safely and effectively perform |
| **COMPLICATIONS** | Highest:bleeding, stenosis,adverse pregnancy outcomes most common. | Intermediate: dependent on amount of tissue removed; excessive bleeding during or after procedure most common | Lowest: <1–2%, generally minor |
| **PATHOLOGIC SPECIMEN** | Yes | Yes | No |
| **COST** | Highest | Intermediate | Lowest |

## 2.8. Management of women post CIN treatment.

Recurrent high grade SILs are proven to be associated with persistence of oncogenic HPV, thus it is crucial for increased surveillance in these women. Clearance of HPV post treatment is regarded as a valuable marker for low current disease risk. It is recommended that all women should have HPV testing and a Pap smear at 6 months and 18 months post treatment (CDCP, 2009).

Patients with repeated HPV negative results and do not have dysplasia on Pap smear (≥LSIL) after 18 months of surveillance should be discharged from colposcopy with routine follow up at 3-5 years depending on age. However, on the other hand, women with a positive HPV test and/or cervical dysplasia on a Pap smear either at 6 or 18 months of monitoring will need to undergo further colposcopy possibly with repeat treatment. Those women who do not require repeat treatment should be on ongoing surveillance for 10 years (CDCP, 2009).

## 2.9. Primary prevention of HPV and cervical cancer prevention

The high prevalence of HIV and HPV infections in sub Sahara Africa makes the primary prevention of HPV infections a priority. The correct and consistent use of condoms results in a lower risk of acquisition of genital HPV infection which in turn lowers the risk for genital warts, cervical pre-cancerous lesions and invasive cervical cancer (Winer *et al*, 2006).

Randomised control trials in Uganda have shown that male circumcision reduces risk of sexually transmitted infections including HIV and HPV by 60% and increased

oncogenic HPV clearance in cases as compared with controls (14.8% vs. 22.3%, respectively) (Gray *et al*, 2010). Moreover, male circumcision has long been associated with reduced cervical cancer risk in the spouses of circumcised males.

Finally, HPV vaccines can offer 95-100% protection against infection for young girls prior to the initiation of sexual activity or adults not previously infected with high risk HPV. There are two types of vaccines: quadrivalent which provides protection against HPV types 16, 18, 6, 11, and bivalent that provides protection against HPV 16 and 18 only. With improved access to HAART treatment and longer survival rates, more girls perinatally infected with HIV will live into adulthood. This group of individuals may hugely benefit from HPV vaccination. However, the major drawback is that the HPV vaccine is the most expensive vaccine ever produced. This would interfere with accessibility of the vaccines in low resource countries (Villa *et al*, 2005).

### 2.10. Summary of previous studies done in women post cervical lesion treatment

A follow-up study by De Vuyst et al in 2014 to assess residual disease and HPV persistence after cryotherapy for Cervical Intraepithelial Neoplasia Grade 2/3 in HIV-positive women at Coptic Hope Centre for infectious diseases for HIV related conditions in Nairobi, Kenya demonstrated that 77.2% (95%CI: 66.4–85.9) of post cryotherapy women had eliminated CIN 2/3 on six month follow up, 22.8% had residual CIN 2/3. HPV was found in 77.5% of women and was associated with residual CIN2/3 (OR = 8.1, 95% CI: 70-90). The sensitivity, specificity and negative predictive value (NPV) of oncogenic HPV test in detecting residual CIN2/3 were

0.94, 0.36, and 0.96 respectively. The study by De Vuyst et al focused on follow up post cryotherapy treatment, however, this study aimed at following up women post LEEP treatment (De Vuyst *et al*, 2014).

A study done by Costa S et al in 2015 showed that oncogenic HPV DNA testing is more sensitive (95%) compared to cytology follow up alone whose sensitivity was to (70%) in detecting squamous intraepithelial lesions. Costa S also reported that combining HPV and cytology can reach a sensitivity of 90% in detecting recurrent and residual lesions (Costa S *et al*, 2015).

In another study by Ibanez R et al in 2012. Among the 611 women enrolled in the study oncogenic HPV was detected in 48.3%. Oncogenic HPV testing positivity was also reported to have decreased with age from 72.6% in women younger than 25 years of age to 31.6% in women older than 54 years of age. The p- value was ($p < 0.01$). Oncogenic HPV detection had a sensitivity of 97.2% with a 95% CI of 85.5-99.9 and a specificity with a 95% CI of 63.1-73.2) (Ibanez *et al*, 2012).

In a study done by Chirenje M et al in Zimbabwe in 2001, a failure rate of 40.5% and 15.8% respectively in HIV infected and uninfected patients was documented after one year follow up after cryotherapy treatment. In the same study, it is documented that LEEP had 14% and 0% failure rates, respectively, among HIV+ and HIV- women (Chirenje *et al*, 2001) .

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Study design

The study was a cross sectional descriptive study.

## 3.2. Study Site

The study was conducted in KNH Clinic 66, KNH Cytology laboratory and KAVI molecular laboratory.

Clinic 66 is located near the casualty department. The clinic provides routine pap smears services, services to patients referred with abnormal pap smears or positive VIA/VILI tests from other facilities in the country. The clinic also provides treatment services such as colposcopy, biopsy and LEEP procedures.

Kenyatta National Hospital Cytology laboratory processes both gynaecological and non gynaecological specimen where about 3000 specimens are processed annually.

KAVI-UoN molecular laboratory is located at University of Nairobi, College of Health Sciences in the third floor and runs several molecular tests such as Polymerase Chain Reaction (PCR) and hybridisation tests.

### 3.3. Target population

Women previously treated for cervical lesions by LEEP who came to clinic 66 for follow up after treatment.

#### 3.3.1. Inclusion criteria

- Women of any age above 18 years.
- Women previously treated by LEEP.
- Women who gave consent to participate in the study.

#### 3.3.2. Exclusion criteria

- Women who had had treatment within 6 months of the study.
- Women with cervical lesions without history of treatment.
- Patients treated by other methods e.g. cryotherapy or total abdominal hysterectomy

### 3.4. Sample size

The number of samples for the study population was calculated using Using R Studio Software V 3.2.2. A study done by Baloglu *et al* which had an HPV residual rate of 30% and a Pap smear cervical lesion residual rate of 25% was used in sample size estimation (Baloglu *et al*, 2010).

$$n = \left\{ \frac{Z_{\propto} * \sqrt{Pdisc} + Z_{\beta} * \sqrt{Pdisc - Pdiff^2}}{Pdiff} \right\}^2$$

$$n = \left\{ \frac{1.96 * \sqrt{0.55} + 0.80 * \sqrt{(0.55 - (0.15)^2)^2}}{0.15} \right\}$$

- CI= 95%
- Power =80%
- Minimum sample size (n) = 14.
- 25 samples were analyzed for both HPV DNA and Pap smear cytology to make use of the whole HPV kit that had been purchased.

### 3.5. Sampling methods

Consecutive and snowball sampling methods were used. Patients were consecutively recruited into the study as they came. Recruited patients also encouraged others whom they knew to have been treated to enrol into the study (snowballing method). Participants who met the selection criteria and consented to the study were recruited until the desired sample size was achieved.

### 3.6. Data collection

#### 3.6.1. Research assistants

A nurse working in the Clinic 66 was recruited as a research assistant and worked with the Gynaecology resident doctor.

#### 3.6.2. Data collection tool

A pre-designed questionnaire was used (Appendix 4).

#### 3.6.3. Recruitment

Potential participants were identified by the research assistant who then introduced herself. The benefits and rationale of the study were explained to all the participants

by the principal investigator and the research assistant and thereafter signing of the consent form was done (Appendix 3).

### 3.7. Laboratory procedures

#### 3.7. 1. Sample collection procedures

The procedure used for sample collection is presented in Appendix 8. The Pap smears and the HPV DNA samples were well preserved to ensure optimum results. The Pap smears were immediately fixed with a 95% alcohol to avoid air drying. After collection of the HPV DNA sample, the brush was inserted into a transport tube which contained the preservative (methanol). The principle investigator preserved the samples for HPV DNA testing at an appropriate temperature i.e. -20 °C.

#### 3.7.2. Specimen processing

The Pap smears were stained using the Papanicolaou staining procedure (Appendix 5) in the KNH cytology laboratory. The HPV DNA testing was done by a technologist and the principal investigator in the KAVI laboratory using the procedure in Appendix 7. Refer to figure 3.1 for flow of procedures.

#### 3.7.3. Cytological evaluation of specimens

Stained cytological slides were first screened by the principal investigator and reported by supervisors. Discrepant findings were analyzed by a different pathologist. The results were reported according to the Bethesda system 2014 (Appendix 6) (Nayar et al, 2015).

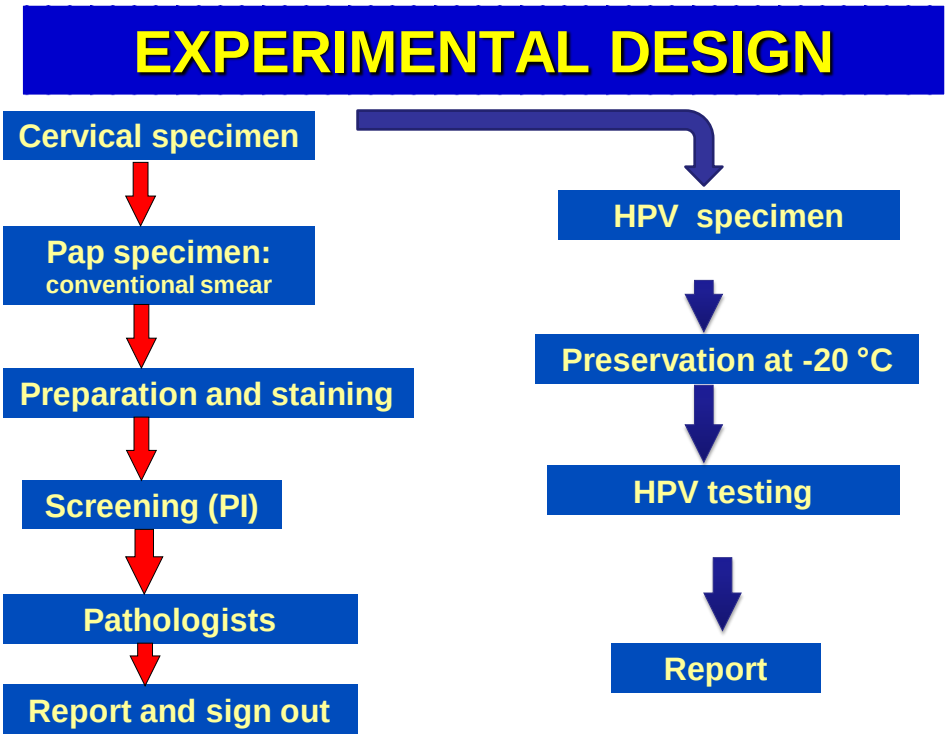


**Figure 3.1: Experimental design for the study**

### 3.8. Quality control

#### 3.8.1. Pap smear

The study was conducted by trained, qualified and competent personnel at KNH and UoN during specimen collection, sample processing and analysis of results. Standard operating procedures (SOP) were used and adhered to during the sample collection, specimen processing and reporting.

#### 3.8.1.1. Measures done to minimise pre-analytic errors

- All Pap smears were collected by a qualified and competent nurse or Gynaecology resident doctor in standard manner to assure quality of Pap smear.
- Standardization of sample collection**:** In order to standardize sample collection, the nurse recruited as research assistant, the Gynaecology resident doctor and the principle investigator held a half-day's meeting and went through the sample collection procedures.
- Immediate alcohol fixation was done to avoid air drying artifact that can affect cellular interpretation.
- SOPs were followed during the sample collection.
- Reagents stored at the correct temperatures were used to stain the Pap smears.
- Hematoxyllin stain was filtered before use.

#### 3.8.1.2. Measures done to minimise analytic errors.

- The Pap smears were interpreted by the principal investigator and signed out with the supervisors.
- All positive cases were shown to an independent pathologist.

#### 3.8.1.3. Measures done to minimise post- analytic errors.

- All reports were proof read before dispatch to avoid typing errors.
- The principal investigator confirmed that the results had been delivered to clinic 66.

### 3.8.2. HPV DNA SAMPLE

#### 3.8.2.1. Measures done to minimise pre-analytic errors

- The sampling was done by a qualified nurse and gynecologist
- DNA DAP sampler brush was inserted into a transport tube which contained the preservative immediately to avoid degeneration. The principle investigator preserved the samples for HPV DNA testing at appropriate temperature i.e. -20 °C.
- All the reagents were prepared using SOPs as per the manufacturer's instructions.
- HPV testing was performed by a qualified technologist and the principal investigator.

#### 3.8.2.2. Measures done to minimise analytic errors

- The test controls and calibrators incorporated in the test kit were used during the assay.
- The machines used were validated and had current service records.

#### 3.8.2.3. Measures done to minimise post-analytic errors

- All reports were proof read before dispatch to avoid typing errors.
- The principal investigator confirmed that the results had been delivered to clinic 66.

### 3.9. Data management

#### 3.9.1. Data collection

The data was collected and stored in hard cover register, Microsoft excel as well as SPSS software. Information stored in soft copies was protected from access from unauthorized persons by a password which was changed periodically. Data stored in hard copies was stored in well secured lockable cabinets where only authorized persons could access them. All records were identified by study identification numbers. Records were stored and identified using unique laboratory numbers.

#### 3.9.2. Data presentation

The cytological features were described and displayed using photomicrographs. The results were presented in tables and charts.

#### 3.9.3. Data analysis

All data was analyzed using SPSS version 21. All statistical tests were performed at 5% level of significance. Cohen's kappa test was done to determine the agreement between Pap smear results and HPV results. Chi square test used to determine associations between HIV status with Pap smear results post LEEP and HPV positivity post LEEP treatment. Descriptive statistics were presented as proportions and percentages in the form of tabulation charts and graphs.

### 3.10. Ethical considerations

- Permission to conduct the study in clinic 66 was sought from KNH/UON ethics review committee (Protocol number: P138/02/2016).
- Permission was sought from the manager in charge of clinic 66.
- All eligible clinic attendees were offered the equal opportunity (equity) to participate without discrimination.
- Informed consent was obtained from all the participants by the research assistant.
- Inclusion and participation by patients was voluntary.
- No payment or incentives were offered to the study participants.
- Patients' privacy and confidentiality were strictly observed.
- All procedures were performed in a standard manner to minimize harm, and maximize benefit to study participants.
- Results sent to the patients´ files in a timely manner to inform clinical decisions.

# CHAPTER FOUR

# RESULTS

A total of 25 study participants were recruited into the study.

## 4.1. Socio- demographic characteristics

### 4.1.1. Age distribution

The mean ± SD age of the study participants was 43.3±12.3 and the range were from 23-84 years. The figure 4.1 below shows a peak in the age group 40-49 years.

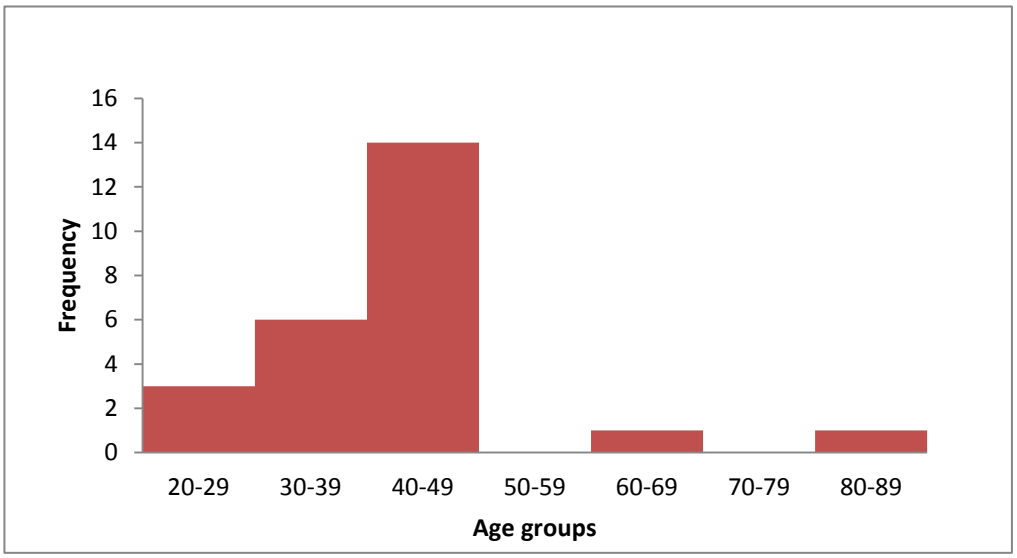


**Figure 4.1: Age distribution of study participants**

### 4.1.2. Parity and sexual history

Parity ranged from 0-15 with a mean ± SD of 2.72 ± 2.8 and a median of 2.8. The mean age of first sexual intercourse was 21.4 years and ranged from 15-30 years. This data is summarised in Table 4.1 below:

**Table 4.1: Parity and sexual history descriptive statistics**

| Variable | Minimum | Maximum | Mean | SD | Median |
|---|---|---|---|---|---|
| Age of first sexual intercourse | 15 | 30 | 21.4 | 3.9 | 21 |
| Parity | 0 | 15 | 2.7 | 2.8 | 2 |

### 4.1.3. Marital status

The majority of the women (68%) reported to be married, while 20%, 8% and 4% reported to be single, widowed and divorced respectively. The data is illustrated in Figure 4.2 below.

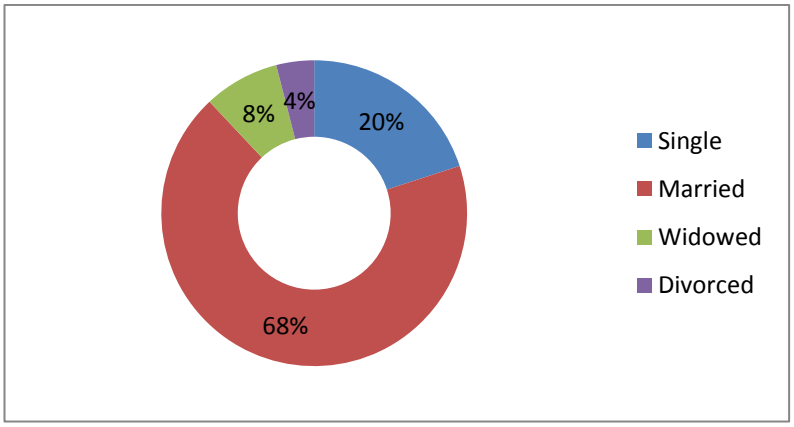


**Figure 4.2: Marital status of study participants**

### 4.1.4. Contraceptive use

Contraceptive use was high with sixteen (64%) women using it while the minority nine (36%) were not on contraceptives. The majority of women used condoms ten (40%), followed by oral contraceptive pills (OCP) three (12%), Intrauterine contraceptive device (IUCD), Implants and bilateral tubal ligation (BTL) all contributed 4%. This data is summarised in figure 4.3 below.

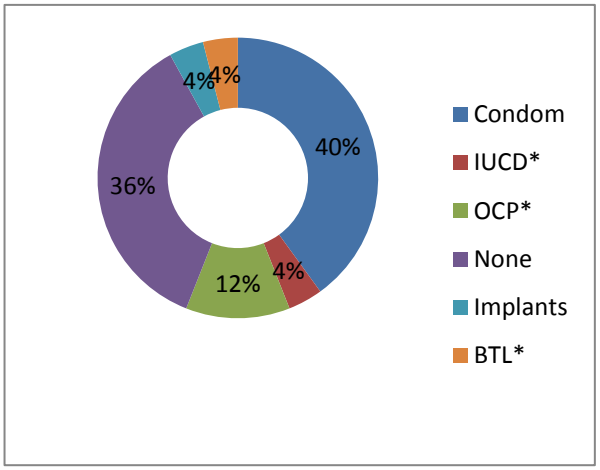


**Figure 4.3: Contraceptive method used by study participants.**

### 4.1.5. HIV status

Out of the 25 participants, eleven (44%) were HIV positive and fourteen (56%) were HIV negative. This data is illustrated in Figure 4.4 below.

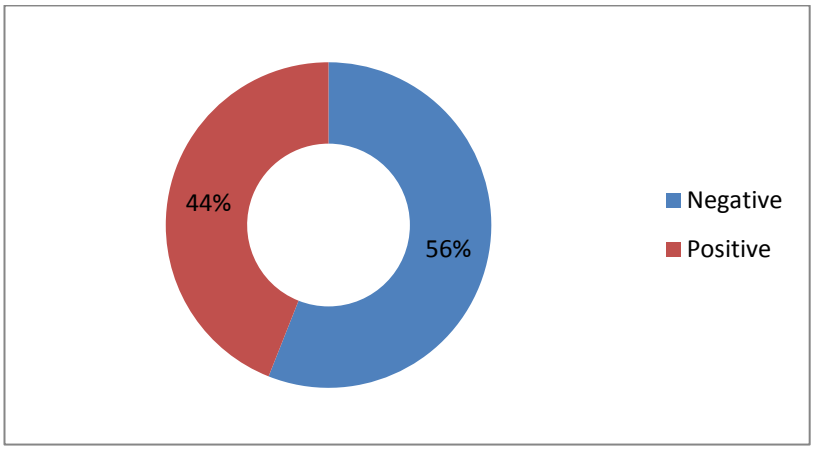


**Figure 4.4: HIV status of study participants**

### 4.2. Pap smear findings

#### 4.2.1. Prevalence of cervical lesions post LEEP treatment

A minority of the women three (12%) had abnormal Pap smear cytological findings (ASCUS and above).High grade squamous intraepithelial lesion (HSIL), Low grade squamous intraepithelial lesion (LSIL) and Atypical squamous cells cannot exclude High grade squamous intraepithelial lesion (ASC-H) contributed a single case (4%) each. These findings are illustrated in figure 4.5 below.

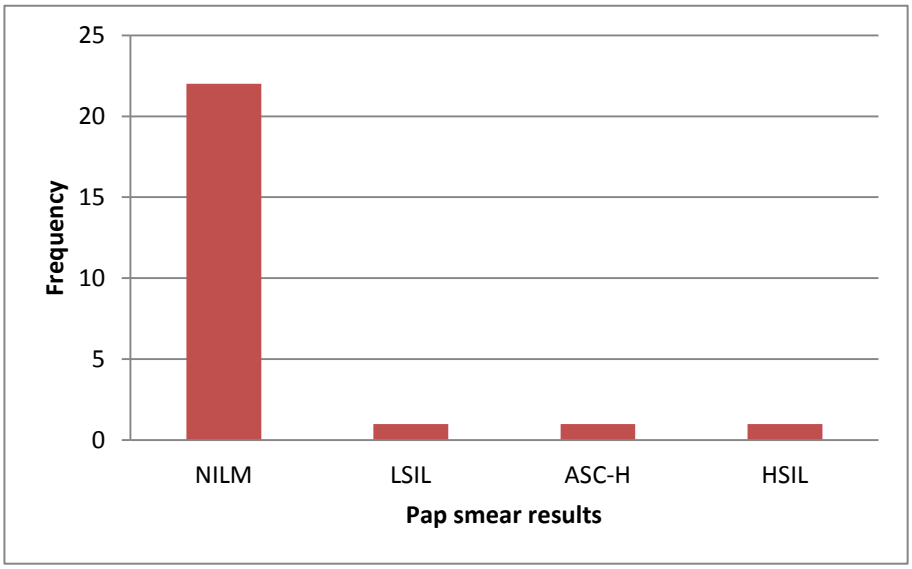


**Figure 4.5: Frequency of cervical lesions post LEEP treatment**

### 4.2.2. Prevalence of other non neoplastic findings

The commonest non neoplastic finding was inflammation in six (24%) of the cases followed by shift in flora suggestive of bacterial vaginosis (BV) three (12%) and age related atrophy accounted for two cases (8%).

### 4.2.3: Comparison of diagnosis pre and post treatment

Table 4.4 shows the comparison of diagnosis pre and post treatment. The majority of patients with abnormal results (88%) had successful treatments.

**Table 4.2: Comparison of diagnosis pre and post treatment**

| | | Post treatment | | |
|---|---|---|---|---|
| | | Neg | Pos | **Total** |
| **Pre-treatment** | normal | 1 | 0 | **1** |
| | abnormal | 21 | 3 | **24** |
| | **Total** | **24** | **3** | **25** |

### 4.3. High risk HPV DNA testing findings

#### 4.3.1. High risk HPV test results

Most of the women sixteen (64%) were positive for high risk HPV on follow up and a few nine (36%) were negative for high risk HPV. These proportions are shown in Fig 4.6 below.

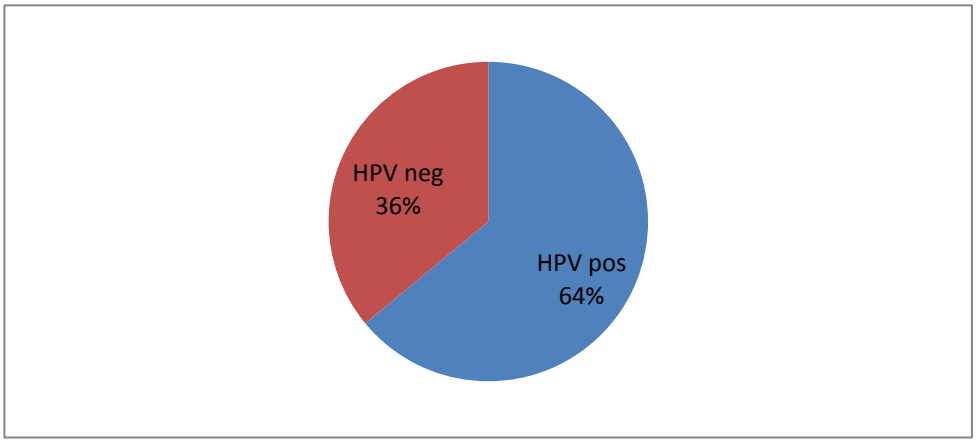


**Fig 4.6: High risk HPV results**

### 4.3.2. Age group and HPV positivity

HPV positivity peaked in the age group 40-49 and no cases were recorded in the age groups 50-59 and 70-79. These findings are summarised in Fig 4.7 below.

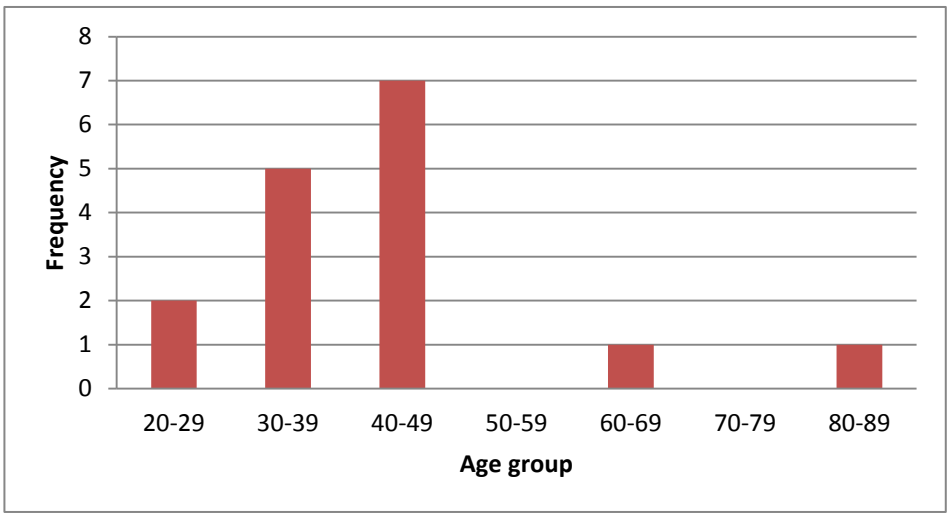


**Figure 4.7: Distribution of HPV positive patients by age group**

### 4.3.3. Frequency of HPV genotypes

Thirteen different high risk HPV genotypes were detected. The commonest genotypes were 56 and 51 which accounted for eleven cases (40.7%) and six (22.2%) respectively. HPV 16 and 18 accounted for one cases (3.7%) and two cases (7.4%) respectively. This information is depicted in Fig 4.8 below.

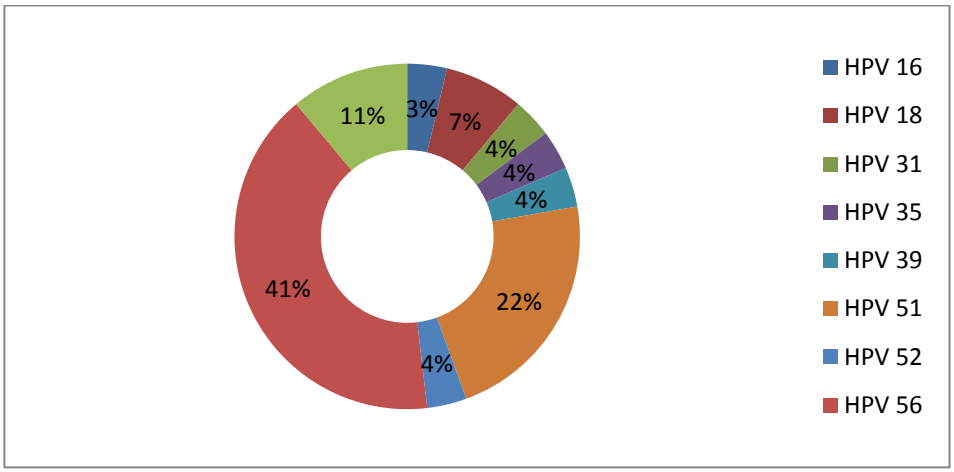


**Figure 4.8: Frequency of HPV subtypes**

### 4.3.4. Single vs. multiple infections

Single and multiple infections were equally distributed. Of the sixteen samples positive for HPV, eight (50%) were multiple infections. This information is shown in the table below.

**Table 4.3: Single vs. multiple infections**

| **Single vs. multiple infection** | **n=16** | **%** |
|---|---|---|
| Single infection | 8 | 50 |
| Multiple infections | 8 | 50 |

### 4.4. Correlation of Pap smear and HPV results

The majority of the women thirteen (52%) were positive for HPV but without any a detectable cytological abnormality. All women with abnormal Pap smear findings, three (12%) were also positive for high risk HPV. Table 4.4 below shows the cross tabulation of HPV and Pap smear results.

**Table 4.4: Cross tabulation of HPV and Pap smear results**

| Pap results | HR-HPV pos | HR-HPV neg | Total | Cohen's Kappa(k) | p-value |
|---|---|---|---|---|---|
| NILM | 13 | 9 | **22** | 0.143 | 0.166 |
| Abnormal(≥ ASCUS) | 3 | 0 | **3** | | |
| **Total** | **16** | **9** | **25** | | |

Cohen Kappa test was run to determine if there was an agreement between Pap smear and HPV results. There was poor (non to slight) agreement between the tests, k=0.143, 95% CI: -0.17 to 0.46, p=0.166.

### 4.5. Association between HIV status and Pap smear post LEEP findings.

Cervical lesions were more common in HIV positive patients two (66.6%) compared to HIV negative patients (33.3%). Table 4.5 below shows the cross tabulation of HIV status and Pap smear results.

**Table 4.5: Cross tabulation of HIV status and Pap smear results**

| Pap smear results | HIV neg | HIV pos | Total | Pearson value | p-value |
|---|---|---|---|---|---|
| NILM | 13 | 9 | **22** | 0.711 | 0.399 |
| Abnormal (≥ ASCUS) | 1 | 2 | **3** | | |
| **Total** | **14** | **11** | **25** | | |

A Chi- square test was done to determine if there is an association between HIV status and Pap smear findings. There was no statistically significant association HIV status and pap smear findings post LEEP, $X^2$=0.711, d.f= 1, p=0.399.

### 4.6. Cross tabulation of HIV status and HPV positivity.

The HPV positivity between HIV positive and HIV negative women was comparable each accounting for 63.6% and 64.3% respectively. These findings are summarised in table 4.6 below.

**Table 4.6: Cross tabulation of HIV status and HPV positivity**

| HPV results | HIV neg | HIV pos | Total | Pearson value | p-value |
|---|---|---|---|---|---|
| Negative | 5 | 4 | **9** | 0.001 | 0.97 |
| Positive | 9 | 7 | **16** | | |
| **Total** | **14** | **11** | **25** | | |

A Chi- square test was done to determine if there is an association between HIV status and HPV positivity post LEEP treatment. There was no statistically significant association HIV status and HPV positivity post LEEP, $X^2$=0.001, d.f= 1, p =0.973 (p>0.005).

# CHAPTER FIVE

# DISCUSSION

## 5.1. HPV and Pap smear results post LEEP treatment

In this study, follow up of women post LEEP treatment revealed that 12% had ASCUS or more severe cytological abnormalities. LEEP treatment procedures in this study were considered successful because the proportion of lesions post LEEP treatment in this study (12%) were comparable to those reported by Gosvig CF *et al* in Denmark (17%) and those reported by Chirenje *et al* in Zimbabwe (14%). In comparison to cryotherapy, LEEP yielded a lower lesion rate compared to De Vuyst *et al* in 2014 (22.8%) and Chirenje *et al* (40.1%).

HPV positivity in this study (64%) differs from the value reported by Gosvig *et al* (48%). This difference can partly be explained by earlier follow in that study (mean=3.4 months) compared to this study (mean=18 months). The delay in follow up in this study could have accounted for the higher HPV positivity due to new infections.

Infections with single and multiple HPV subtypes was equally distributed in this study (50% each) contrary to findings by Quint W *et al* and Van der MJ *et al* in which single infection dominated accounting for 62% (Quint *et al*., 2012; Van der MJ *et al*., 2012). Massad LS *et al* and Quint W *et al* also reported no increased probability of cervical lesions with multiple HPV subtypes, this is in contrast to our study in which all the abnormal cytological cases were associated with multiple HPV subtypes (Quint *et al*, 2012; Massad *et al*, 2007).

### 5.2. Pap smear findings post LEEP treatment and HIV status

This study showed that cervical lesions after treatment are more common in HIV positive women (1.8 %) compared to HIV negative women (0.7 %). This could be attributable to immune suppression. This is consistent to the one reported by Chirenje M.Z *et al* in Zimbabwe which showed cervical lesions in 3.8% and 1.8% respectively (Chirenje *et al*, 2001). These results are also supported by results by Heard *et al* who reported that 54% of HIV positive women previously treated for cervical lesions had residual or recurrent disease at 36 months (Heard *et al*, 2005 ).

Residual or recurrent disease in HIV negative women has been reported in many studies (Kocken *et al*, 2012; Hirsch *et al*, 2010). A study by Kreimer AR *et al* showed residual disease in 3% and 7% at 6 months and 18 months follow up respectively (Kreimer *et al*, 2006).

The findings by Kreimer *et al* were consistent with studies done by Jones *J et al* and Nobbenhuis MA *et al* which showed residual rates between 1 and 8 % (Jones *et al*, 2011; Nobbenhuis *et al*, 2001). The results from the three mentioned studies were consistent with findings in this study. However, a Chi- square test done in this study revealed no statistically significant association between HIV infection and presence or absence of cervical lesions (p=0.399).

### 5.3. HPV positivity post LEEP treatment and HIV status.

HPV positivity after LEEP in HIV positive patients was 63.6 %. This value was comparable to findings by Gingelmaier et al which showed a positivity rate of 57 % (Gingelmaier *et al*, 2007). HPV positivity in HIV negative patients was 64.3% in

this study. This figure is higher compared to figures reported by Kreimer AR *et al* and Kocken M *et al* which ranged from 10-37% (Kreimer *et al*, 2006; Kocken, 2011). This may be attributable to the detection of HPV at a fixed period post treatment in those studies unlike ours which had various periods with a mean of 18 months post treatment. This could explain the higher rates of HPV positivity possibly due to new infections rather than persistent infections.

The HPV positivity rates in HIV positive and HIV negative patients in this study 63.6% and 64.3% respectively were comparable. This is contrary to reports by De Vuyst H et al which reported that HPV persistence is more common in HIV positive women compared to HIV negative women (De Vuyst *et al*, 2014). However, a Chi-square test done in this study revealed no statistically significant association between HIV status and HPV positivity p=0.973.

HPV 56 (40.7%) was the commonest HPV subtype in this study. This differs from what Pirtea L *et al* reported in a study done in Romania in which they reported HPV 16 as the commonest subtype (Pirtea et al, 2016). This may be evidence to support the inclusion of HPV 56 in the vaccination programs in Kenya. However larger studies need to be done to validate this claim. Findings of this study are consistent with studies by Pirtea L *et al* which reported that HPV positivity is higher in women who are 30 years and above (Pirtea *et al*, 2016).

**5.4. Correlation of Pap smear and HPV results.**

A Cohen Kappa correlation was done to ascertain the agreement of HPV results and Pap smear results. However, the test was not statistically significant (k=0.143,

p=0.166).This can possibly be because most HPV infections detected were not yet associated with any cytological changes. This makes HPV testing in addition to Pap smear testing a useful and valuable combination as it helps to stratify women according to their risk for future recurrent disease.

### 5.5. Limitation of the study

Pre- treatment HPV testing was not available, thus it was difficult to distinguish persistent HPV infections from a new infections.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1. Conclusions

- Approximately 10% of women have residual or recurrent cervical lesion post LEEP treatment at Kenyatta National Hospital.
- The majority of women are positive for high risk HPV post LEEP treatment.
- HPV 56 is the commonest HPV in post LEEP patients at Kenyatta National Hospital.
- There poor correlation between Pap smear and HPV testing results post LEEP treatment at Kenyatta National Hospital.

## 6.2. Recommendations

- A minority of patients has residual or recurrent cervical lesions post LEEP treatment, thus closer follow up is necessary to detect and treat lesions before they become malignant.
- The majority of patients tested positive for HPV post LEEP treatment and have a high risk of recurrence, thus closer follow up is required.
- HPV 56 was the commonest subtype detected and not the usual HPV16 and 18, thus larger study should be done to characterize frequencies of HPV subtypes so that HPV vaccines administered to the population include the most frequent subtypes.

- HPV testing should be introduced in the follow up of patients post LEEP treatment as this helps to stratify women according to their risk of recurrent cervical lesions.
- HPV positivity did not correlate with clinical disease, a larger study which incorporate HPV-mRNA transcript which is more specific should be done.

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# APPENDICES

## Appendix 1: Informed consent explanation

My name is Raymond Chibvongodze, a postgraduate student pursuing a Master of Medical Laboratory Sciences (Clinical Histopathology & Diagnostic Cytology option) at Jomo Kenyatta University of Agriculture and Technology (JKUAT). I am carrying the following study.

**Research title:**

Human Papillomavirus DNA testing and Pap smear cytology co-testing as a 'test of cure' in patients previously treated for cervical lesions by LEEP at Kenyatta National Hospital

**Objective:**

To determine the utility of Human Papillomavirus (HPV) DNA testing and Pap smear cytology co-testing as a 'test of cure' in previously treated for cervical lesions at Kenyatta National Hospital

**Benefits and Risks of the study to you:**

Potential benefits:

Patients will benefit from the study because HPV testing will detect recurrent or residual HPV infections which might cause cervical lesions in the future.

Potential risks:

Bearable pain and mild bleeding may occur during the procedure

**Procedure**

After agreeing to participate into the study, a speculum will be inserted into the vagina followed by gentle scrapping of cells from the cervix. These cells will be examined under the microscope.

**Confidentially**

Names will not be required in the study and you will be identified by study numbers. Questionnaires will be kept under key and lock and only the principal investigator will access it. Questionnaires will be kept for one year then destroyed. Any information given to us will remain confidential and will be for your benefit. You will get your results for both conventional Pap smear and HPV testing through your physician.

**Participation and withdrawal from study**

Participation in this study will be voluntary. You will not be forced or paid to participate in this study and you are free to withdraw any time without losing the benefits to which you are entitled in this institution.

**Contact information**

If you have any question regarding the study please contact me Raymond Chibvongodze on mobile number 0714376431, Jomo Kenyatta University of Agriculture and Technology,
P.O. Box 62,000 – 00200 NAIROBI, KENYA or my supervisor Dr Ndung'u : University of Nairobi, P.O BOX 19676-00202 Nairobi or telephone number Tel: 726300 Ext. 43774. And if you have any ethical issue please contact Prof. M. L.

CHINDIA, the Secretary, KNH/UON Ethical Research Committee, Tel: 726300-9 Ext 44102.

**Appendix 2: Ufafanuzi  wa cheti cha kukubali**

Jina langu ni Raymond Chibvongodze, mwanafunzi wa shahada la udhamili katika sayansi ya chembechembe za mwili, kitengo cha magonjwa ya binadamu, katika chuo kikuu cha Jomo Kenyatta of Agriculture and Technology (JKUAT). Ninajishughulisha na utafiti ufuatao.

**Kichwa cha utafiti**

Kipimo cha DNA cha Human Papillomavirus na Pap smear cytology co-testing kama kipimo cha kutibu kwa wagonjwa waliotibiwa apo hawali kwa Cervical lesions katika hospitali ya rufaa ya Kenyatta.

**Lengo kuu la utafiti huu**

Kuonyesha matumizi ya kupima Human Papillomavirus (HPV) DNA na Pap smear cytology co-testing kama kipimo cha kuponya kwa wagonjwa waliotibiwa cervical lesions apo hawali  katika hospitali ya rufaa ya Kenyatta.

**Faida na hatari ya utafiti huu kwako**

Faida zinazotarajiwa:

Wagonjwa watanufaika kutokana na utafiti huu kwa sababu utafiti utagawa wagonjwa kulingana na hatari za ugonjwa kutokea tena ama ugonjwa uliobaki kulingana na matokeo ya HPV na ii itapunguza kupimwa kwa kundi moja na kufuatilia maendeleo ya ugonjwa kwa makundi mengine.Matokeo yatakua kamili na ya ukweli kwa kutumia vipimo viwili.

<u>Hatari zinazotarajiwa:</u>

Wakati wa kupimwa kutakua na uchungu ambao unaweza stahimili na pia kumwaga damu wakati wa kupima.

**Utaratibu**

Baada ya kukubali kuhusika katika utafiti**,** chombo kitaingishwa kwa sehemu nyeti ambapo watatoa muhusika.

**Usiri**

Matumizi ya majina hayatatutumiwa katika utafiti huu. Nambari za usajili itatumika Ili kuihifadhi usiri wa wahusika. Hati ya maswali yatawekwa mahali salama palipo na usiri na kufungiwa. Mhusika msukuu ndiye tu atapata kuona hati za maswali. Hati izo zitaekwa kwa muda wa mwaka mmoja kabla ya kutupiliwa mbali. Habari yeyote itakayotolewa itakuwa siri na itakua na manufaa kwako.Utapata matokeo yako ya pap smear na pime ya HPV kutoka kwa daktari.

**Kujitoa kwa utafiti**

Kuhusika katika utafiti huu ni kwa kujitolea na waweza kujiondoa wakati wowote ambapo unahitaji bila kupoteza haki yako yote ya matibabu katika hospitali kuu.

**Mawasiliano:**

Ukiwa na swali lolote kuhusu utafiti huu tafadhali nipigie mimi Raymond Chibvongodze nambari la simu 0714376431, pia Chuo kikuu cha Jomo Kenyatta University of Agriculture and Technology. P.O. Box 62,000 – 00200 NAIROBI , pia waweza kuwapigia wahadhiri wakuu wangu Dr Ndung'u chuo kikuu cha Nairobi, P.O BOX 19676-00202 Nairobi, nambari la simu 726300 Ext. 43774. Ukiwa na swali lingine linalohusu mahadili ya utafiti, tafadhali mpigie Prof. M. L. CHINDIA,

karani, KNH/UON kitengo cha mahadili ya utafiti, nambari la simu: 726300-9 Ext 44102.

**Appendix 3: Consent form**

I ……. after reading and being explained the study purpose and what it involves do hereby give informed consent to participate in the diagnostic study fully aware of the benefits and risks. I have not been pressurized to participate in this study in any way or baited by any incentive. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without loss of any benefit or quality of management to which I am entitled. I am fully aware that the results of this study will be used for scientific purposes and may be published.

Participant Signature: ……..……..Date……..

Doctor/Nurse Signature…….. Date ……..

Principal investigator Signature**.....**.… Date ……..

**Appendix 4: Questionaire**

**Project title:**Human Papillomavirus (HPV) DNA testing and Pap smear cytology co-testing as a 'test of cure' in patients previously treated for cervical lesions by LEEP at Kenyatta National Hospital

**Study number**----------

1. Age …… years
2. HIV status.....
3. Are you currently using modern family planning method(1) Yes… (2)No…If yes which one?

- Natural…...
- OCP……
- IUD…….
- Injectable…….
- Condoms……

Others ….Specify……..

4. Experience contact or post coital bleeding 1) Yes……2) No……..
5. Date of treatment..... Method of treatment.....
6. Condom use 1.always/ 2.sometimes/ 3.never
7. Sex in the last 6 months 1) Yes……. 2) No.……
8. Age at first intercourse……

9. Marital status

- Single …….
- Widowed.....
- divorced/separated……
- married…….

10. Number of pregnancies……

11. L.M.P……

**12. Appearance of the cervix**

- normal……..
- eroded…….
- inflammed…….
- suspicious……..
- others……..specify…….

**DATE OF SPECIMEN COLLECTION**: ……..

Previous Pap/ Histology result…….Date…….

**LAB REPORT**

**BETHSEDA CLASSIFICATION**

Adequacy 1.Yes……2. No……3.Specify…….

4. Negative…….5.ASCUS……6.LSIL…….

7. Inflammatory/ 8.Reactive……..9.ASC H…….10.HSIL……11.SCC……

12. AGC……13.AIS……14.ENDOCERVICAL ADENOCARCINOMA……

**HPV DNA RESULTS**

1. HR-HPV Positive……  2.HR-HPV Negative……

**COMMENTS**

**Principal investigator's name……Sign……..**

**Pathologist's name……. Sign…….**

**Date…….**

## Appendix 5: Procedure of Pap staining

### Principle of the stain

Haematoxylin stains the nuclei blue by dye-like formation. The eosin azure solution being acidic stains the cytoplasm which is basic so that the eosin has affinity for the mature cells while light green has affinity for the young endocervical cells. Orange G also being an acidic dye has an affinity for the cytoplasm of the oldest superficial cells (64).

1. Smears are fixed in 95% ethanol.

2. They are hydrated through ethanol grades of 80%, 70% and then 50%.

3. Smears are rinsed with distilled water.10dips.

4. They are stained in Harris haematoxylin for 4minutes.

5. They are rinsed with tap water.

6. Smears are differentiated in 0.05%acid water 10dips

7. They are rinsed in tap water and blued in running tap water 10 dips.

8. They are rinsed in 95% ethanol.

9. Smears are stained with O.G for 2 minutes.

10 They are rinsed in 95% ethanol 10 dips.

11. They are stained in EA50 for 4 minutes

12. Smears are rinsed in 95% ethanol10dips

13They are dehydrated in 3 changes of absolute alcohol.

14. They are cleared in 2changes of xylene 10 dips.

15. They are mounted in DPX.

**Results:** Pap smear results will be reported using the Bethesda System 2014.

**Appendix 6: Bethesda system for reporting cervical cytology (2014)**

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

1. **Specimen type**

- Indicate conventional (Pap smear) vs. liquid-based preparation

**2. Specimen adequacy**

- Satisfactory for evaluation (describe presence or absence of endocervical or transformation zone component and other quality indicators), e.g., partially obscuring blood, inflammation, etc.)
- Unsatisfactory for evaluation (Specify reason).

✓ Specimen rejected/not processed (specify reason).

✓ Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason).

**3. General categorization** (Optional)

- Negative for Intraepithelial Lesion or Malignancy.
- Epithelial Cell Abnormality:
- Others: see /Result (e.g. endometrial cells in a woman (40 yr. of age or older).

**4. Interpretation/result**

**Negative for Intraepithelial Lesion or Malignancy**

- When there is no cellular evidence of neoplasia, state this in the General
- Categorization above and/or in the Interpretation/Result section of the report whether or not there are organisms or other non-neoplastic findings.

**2. Organisms:**

- ✓ *Trichomonas vaginalis*
- ✓ Fungal organisms morphologically consistent with *Candida spp.*
- ✓ Shift in flora suggestive of bacterial vaginosis.
- ✓ Bacteria morphologically consistent with *Antinomyces spp.*
- ✓ Cellular changes consistent with herpes simplex virus
- ✓ Reactive cellular changes associated with
- ✓ Inflammation (includes typical repair)
- ✓ Radiation
- ✓ Intrauterine device (IUD).
- ✓ Glandular cells status post hysterectomy
- ✓ Atrophy.

**3. Others**

- Endometrial cells (in a woman 40 years of age or older)
- Specify if (negative for squamous intraepithelial lesion)

**B. Epithelial Cell Abnormalities**

**Squamous cell:**

- Atypical squamous cells
- Of undetermined significance (ASC-US).
- Cannot exclude HSIL (ASC-H).
- Low-grade squamous intraepithelial lesion (LSIL)
- Encompassing: HPV/mild dysplasia/CIN1).
- High-grade squamous intraepithelial lesion (HSIL)
- Encompassing: moderate and severe dysplasia, CIS, CIN 2 and CIN 3).
- With features suspicious for invasion (if invasion is suspected).
- Squamous cell carcinoma

**2. Glandular Cell:**

- Atypical

  Endocervical cells (NOS or specify in comments)
- Endometrial cells (NOS or specify in comments)
- Glandular cells (NOS or specify in comments).
- Atypical
- Endocervical cells, favor neoplastic.
- Glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ.
- Adenocarcinoma
- Endocervical
- Endometrial

- Extra uterine
- Not otherwise specified (NOS)

**C. Other Malignant Neoplasm**: (specify)

- Carcinoma
- Sarcomas
- Other tumours

**Ancillary testing**

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

**Automate review**

- If specimen was examined by automated device, specify the device and the result.

**Educational notes and suggestions** (optional)

- Suggestions should be concise and consistent with clinical follow-up guidelines

  by professional organizations (references to relevant publications may be included).

## Appendix 7: HPV DNA testing

### Principle of the procedure

The HPVDNA test using HC2 technology is a signal amplified hybridization antibody capture assays that that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPVRNA probe. The resultant RNA; DNA hybrids are captured on onto the surface of the microplate well coated specific for RNA: DNA hybrids. The immobilized hybrids are reacted with alkaline phosphatase conjugated antibodies specific for RNA: DNA hybrid and are detected with a chemiluminscent substrate. Several alkaline phosphatase molecules are conjugated to each antibody .Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase light is emitted which is measured as relative light units on a luminator. The intensity of the light emitted denotes the presence or absence of the target DNA in the specimen. An RLU measurement equal or greater than the cut off value indicate the presence of HPV DNA sequence in the specimen. An RLU measurement less than the cut off indicate the absence of specific HPV DNA sequence tested or HPVDNA sequence below the detection limit of the assay. High volume samples throughout testing with the Hc2 HPVDNA test can be performed utilizing the (RCS).The instrument processes up to 352 specimens in 8 hours.

To enable high volume sample throughout testing all the procedural steps of the assay are performed by the RCS with the exception of specimen denaturation, chemiluminscent signal detection, and result reporting.

**Appendix 8: Sample collection procedure for Pap smear and HPV DNA**

Pap smear and HPV DNA collection procedure

1) Upon signing of the consent and patients assurance the patient will be placed in a lithotomy position.

2) The speculum will be lubricated and inserted into the vagina until the cervix comes into full view.

3) The cervix broom will be inserted and rotated at 360 degrees at the columnar junction or any suspicious area.

4) It will then be removed and the smear will be made on a slide and the broom will be discarded.

    a. DNAPAP cervical sampler brush will be inserted 1-1.5cm into the *os* of the cervix until the largest outer bristles of the brush touches the ectocervix. It will be rotated three full turns in a counter clockwise direction.

    b. The brush will be inserted into the bottom of the transport tube shaft will be snapped off at the score line and the tube capped securely.

    c. The speculum will be slowly withdrawn and removed.

    d. The patient will be advised to come for the results during the next visit.

**Specimen transport HPV DNA SAMPLES**

The specimen will be kept at -20 degrees for up to 3months prior to testing.

**Procedure for hybrid capture 2**

Thawing samples

Freeze-thaw cycles

**Steps**

- Denaturation
- Hybridization
- Hybrid capture
- Hybrid detection
- Signal generation

**Step 1: Denaturation**

1. Samples will be denatured using heat and a strong base.
2. This step accomplishes three tasks:

- Lyses the cells
- Separates DNA strands
- Eliminates endogenous RNA

**Step 2: Hybridization**

1. RNA probe will be complementary to the target DNA in the denatured patient specimens

and creates RNA: DNA hybrid.

2. The RNA: DNA hybrids serve as the target analyte throughout the assay

Full HPV genome probe ~8000 base pairs length

**Step 3: Hybrid capture**

1. Micro titer plate ("capture plate") coated will be with anti-RNA:DNA antibodies
2. RNA:DNA hybrids present in the solution bind to the antibodies coated on the capture plate.

**Step 4: Hybrid Detection**

1. Antibodies will be conjugated to multiple alkaline phosphatase enzymes and bind to multiple sites on the captured RNA: DNA hybrids.
2. Unwanted RNA is destroyed in this step.
3. Plate will be incubated at room temperature for 30 minutes.
4. A wash step is done.

**Step 5: Signal Generation**

The alkaline phosphatase enzymes will react with the detection substrate, resulting in a chemiluminescence signal.

The plate will incubated for 15 minutes at room temperature and read in the illuminometer.

**Digene HC 2 DNA test summary procedure**

| Assay step | Time | Equipment |
|---|---|---|
| Denaturation | 45min | Vortexer/Waterbath |
| Hybridization | 1hour | Microplate |
| Capture | 1hour | Rotary shaker |
| Signal amplification | 30min | No equipment |
| Wash | 10min | Automated |
| Signal generation | 15min | No equipment |
| Read signal | 30min | DML 2000 or DML 3000 |

Total time approximately 4-5hours 88 STM sample

Negative calibrator

Equals or greater than10 and equals or less than 250 RLU s

% CV equal or less than25 %

Positive calibrator

% CV equals or less than 15%

Pos. Cal. / Neg Cal ratio

2.0 – 15

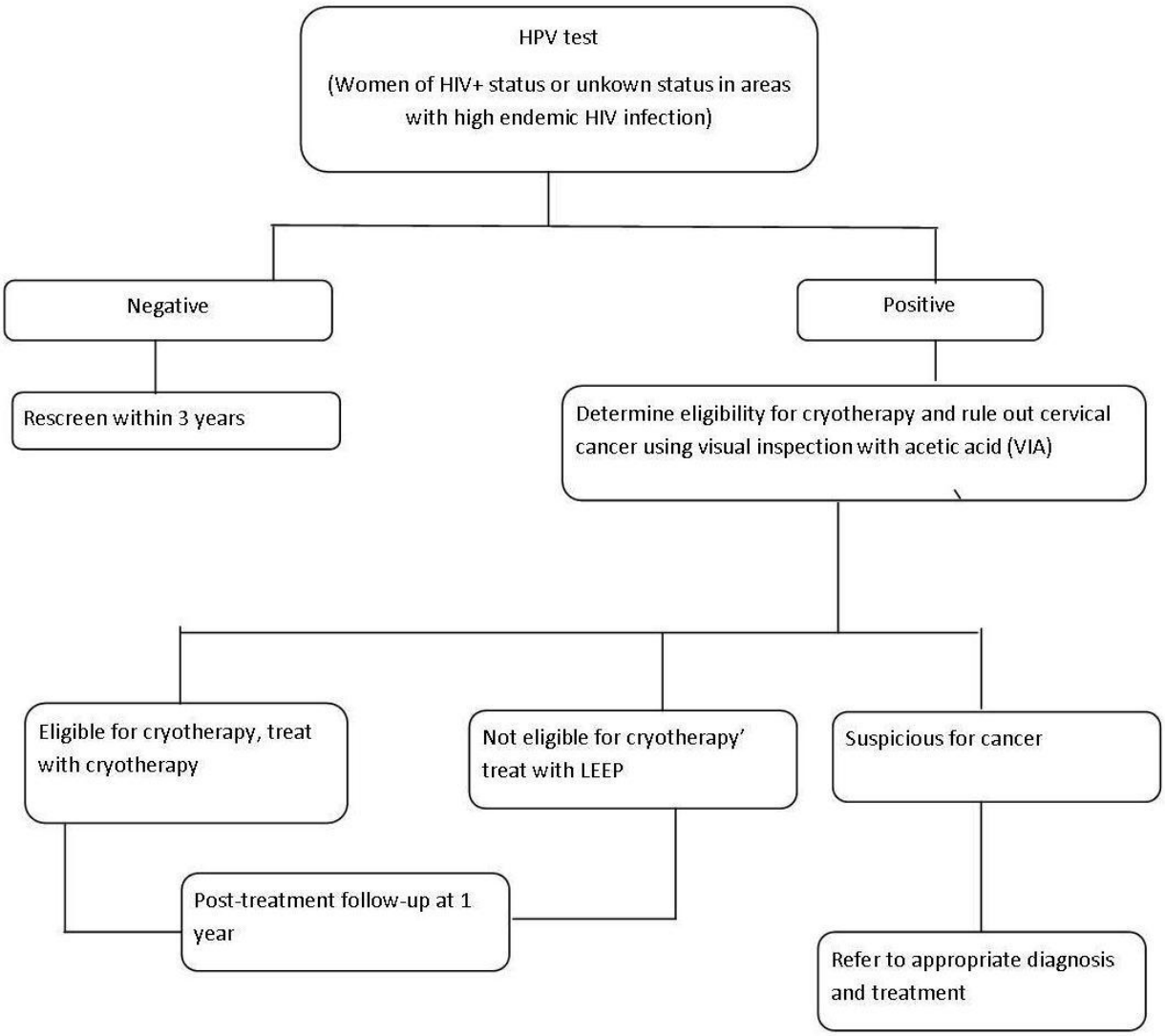
2 QCs required per assay

QC Validation criteria High Risk HPV Test

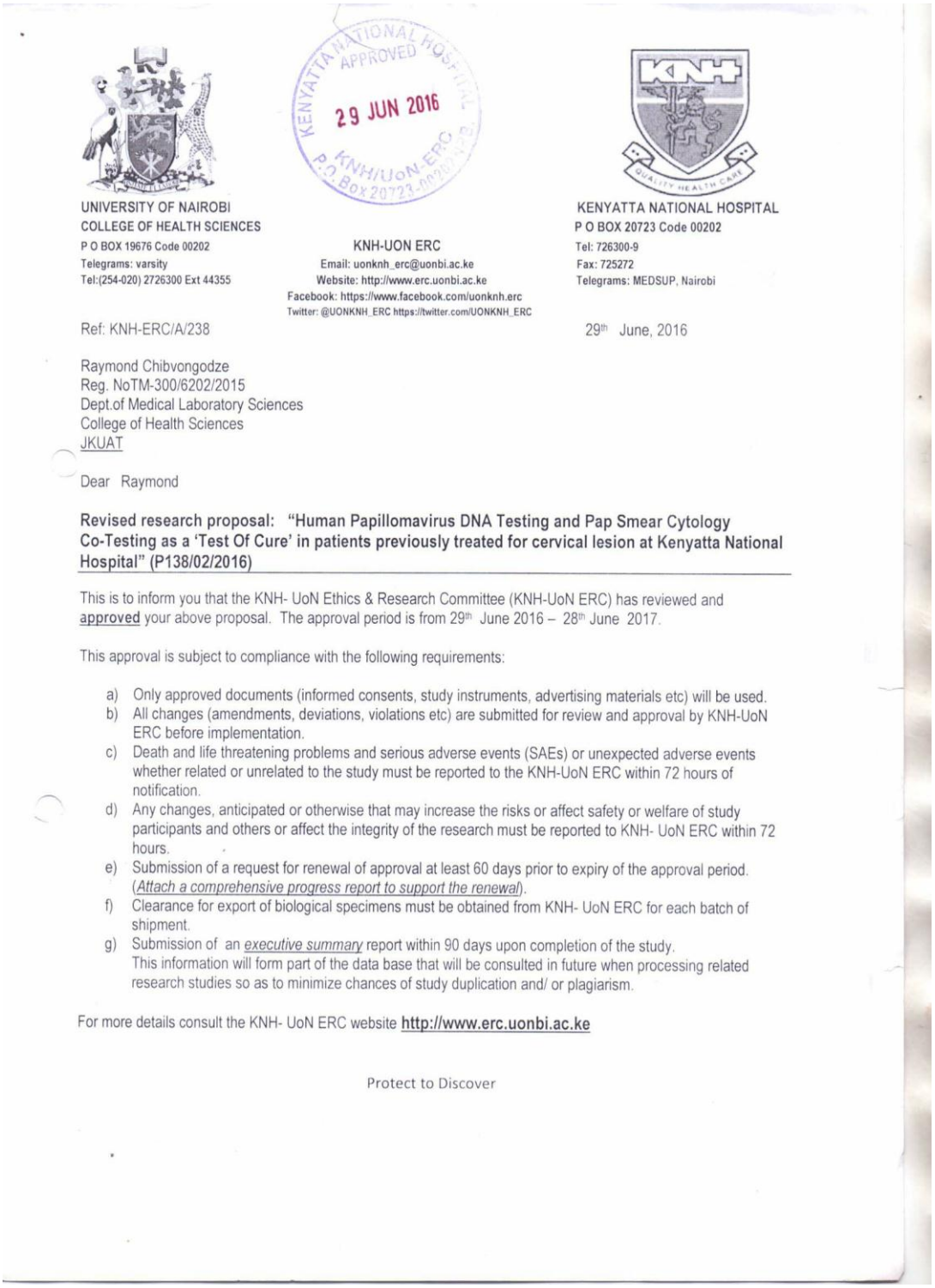
QC1-LR: less than 1.0 RLU/Cutoff Ratio (-)

QC2-HR**:** 2-8 RLU/Cutoff Ratio (+)

**Appendix 9: WHO Flow chart showing who recommendations of HIV infected women or unknown status in areas of high endemic HIV infection.**



## Appendix 10: Ethical approval



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Ref: KNH-ERC/A/238

29th June, 2016

Raymond Chibvongodze
Reg. NoTM-300/6202/2015
Dept.of Medical Laboratory Sciences
College of Health Sciences
JKUAT

Dear Raymond

**Revised research proposal: "Human Papillomavirus DNA Testing and Pap Smear Cytology Co-Testing as a 'Test Of Cure' in patients previously treated for cervical lesion at Kenyatta National Hospital" (P138/02/2016)**

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and approved your above proposal. The approval period is from 29th June 2016 – 28th June 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to Discover

Yours sincerely,

PROF M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH- UoN ERC
Supervisors: Dr. M. Kyama, J.K.U.A.T
Dr. J. Ndung'u, University of Nairobi

# Appendix 11: Publication



East African Medical Journal Vol. 94 No. 3 March 2017
HPV DNA TESTING AND PAP SMEAR CYTOLOGY CO-TESTING AS A 'TEST OF CURE' IN PATIENTS PREVIOUSLY TREATED FOR CERVICAL LESIONS BY LEEP AT KENYATTA NATIONAL HOSPITAL

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# HPV DNA TESTING AND PAP SMEAR CYTOLOGY CO-TESTING AS A 'TEST OF CURE' IN PATIENTS PREVIOUSLY TREATED FOR CERVICAL LESIONS BY LEEP AT KENYATTA NATIONAL HOSPITAL

R. CHIBVONGODZE, C. NYIRAKANANI, J.A OJWANG, O.M. MUTUKU, J.R. NDUNG'U and C.M. KYAMA,

## ABSTRACT

*Background:* **HPV infection is a pre-requisite for the development of the majority (99.7%) of precancerous cervical lesions. Treatment of cervical precancerous lesions reduces the risk of invasive cervical cancer by 90%; however treated women still have five times risk of invasive cancer compared to women who have always had a normal Pap smear, thus special follow-up measures are critical to reduce these risks.**

*Objective:* **To determine the utility of co-testing by conventional Pap smear and HPV testing as a 'test of cure' in patients previously treated for cervical lesions by LEEP at KNH.**

*Design:* **Cross sectional descriptive study.**

*Setting:* **Kenyatta National Hospital and KAVI molecular laboratory.**

*Subjects:* **Women on follow for cervical lesions post LEEP treatment.**

*Results:* **Out of the 25 participants, 22(88%) had a report of NILM while 3(12%) had a report of ≥ASCUS). 16 (64%) were positive for HPV. HPV 56 was the commonest HPV subtype detected in 11 patients (41%). The Cohen's Kappa correlation between Pap smear and HPV DNA test not statistically significant,k=0.143, 95% CI: -0.17 to 0.46, p=0.166.There was no statistically significant association between HIV status and pap smear findings post LEEP, $X^2$=0.711, p=0.399**

*Conclusions:* **Co-testing with HPV DNA testing and Pap smear is a useful approach to stratify women with no cytological abnormalities according to their risk of residual disease**

## INTRODUCTION

Cervical cancer is the second commonest cancer among women worldwide (1). Approximately 80% of cervical cancer related deaths occur in developing countries(1). This is attributable to the general unavailability of sound and effective screening programs in these settings. Approximately 68% of the estimated 33.3 million individuals living with HIV/ AIDS in the world live in sub- Saharan Africa (2). These areas with high HIV prevalence rates are also burdened with high cervical cancer rates(3).

HIV infected women are at an increased risk of infection with HPV, in addition, studies have shown that HIV infected women have higher prevalence of HPV, higher incidence of HPV, higher HPV viral load, longer persistence of HPV, higher likelihood of multiple HPV subtypes and greater prevalence of oncogenic subtypes compared to HIV negative women(4,5). There is also increased persistence and recurrence after treatment in HIV positive patients with certain studies documenting a recurrence >50% (6). Treatment of cervical precancerous lesions reduces the risk of invasive cervical cancer by 90%; however treated women still have five times risk of invasive cancer compared to women who have always had a normal Pap smear(7,8). Thus it is essential to have special follow-up strategies to reduce these risks.

Currently follow- up protocols in KNH involves screening with Pap smears only; which have their own limitations. Pap smear has low sensitivity because detectable cytological changes are not always present after infection with HPV. In addition, the low sensitivity could be due to other reasons such as inadequate sampling, poor quality of smears due to obscuration by inflammation and subjectivity in the interpretation of the Pap smears. The incorporation of high risk HPV genotype testing in addition to Pap smear cytology testing is the approach with the most potential to increase the efficiency and effectiveness of screening in this group of women.

## MATERIALS AND METHODS

***Study Site:*** KNH Clinic 66, Kenyatta National Hospital Cytology laboratory and KAVI molecular laboratory.

***Study period:*** July- November 2016

***Study design:*** Cross sectional descriptive study.

***Ethical considerations:*** Ethical approval was sou from KNH/UoN ethics committee (Protocol num P138/02/2016).

***Study population:*** Women on follow for cervical les post LEEP treatment.

***Inclusion criteria***

Women previously treated by LEEP

Women who gave consent to participate in the study

***Exclusion criteria***

Women who have had treatment with 6 months of the st

Women with cervical lesions without history of treatm

Patients treated by other methods e.g. cryotherapy or t abdominal hysterectomy

Those who decline to give consent.

***Sampling methods:*** Consecutive and snowballing

Specimen processing: Samples for convention Pap smears and HPV were collected using respective brushes and preserved immediately. The alcohol fixed Pap smears were stained using the H&E protocol and reported using the 2014 Bethesda system for reporting cervical smears (9). HPV DNA testing was done using the PCR, REAL TIME. The Pap smears were stained using the Papanicolaou staining procedure in the cytology laboratory. The HPV DNA testing was done in KAVI molecular laboratory by a technologist and the principal investigator.

***Statistical analysis:*** Cohen's kappa test was done to determine the agreement between Pap smear results and HPV results. Chi square test was used to determine associations between HIV status with Pap smear results post LEEP. Chi square test was used to determine associations between HIV status and HPV positivity post LEEP A p-value below 0.05 was regarded as statistically significant.

## RESULTS

Table 1 shows the Pap smear findings. A minority of the women, 3 cases (12%) had abnormal Pap smear cytological findings (ASCUS and above). High grade squamous intraepithelial lesion (HSIL), Low grade squamous intraepithelial lesion (LSIL) and atypical squamous cells cannot exclude High grade squamous intraepithelial lesi (ASC-H) contributed a single case (4%) each.

**Table 1:**
**Pap smear findings**

| Result | n=25 | % |
|---|---|---|
| NILM | 22 | 88 |
| LSIL | 1 | 4 |
| ASC-H | 1 | 4 |
| HSIL | 1 | 4 |

Figure 1 shows the HIV status of the study participants. Out of the 25 participants, eleven (44%) were HIV positive and fourteen (56%) were HIV negative.

**Figure 1**
**HIV status of study participants**

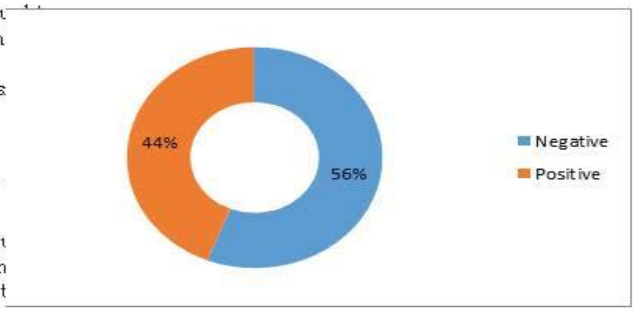


Table 2 shows High risk HPV test results. Most of the women were positive for high risk HPV on follow up (16 cases) (64%) and a few were negative (9 cases) (36%). Single and multiple HPV infections equally distributed each with 8 cases (50%).

**Table 2**
**High risk HPV test results**

| **HPV genotypes** | **n=27** | % |
|---|---|---|
| 16 | 1 | 3.7 |
| 18 | 2 | 7.4 |
| 31 | 1 | 3.7 |
| 33 | 0 | 0 |
| 35 | 1 | 3.7 |
| 39 | 1 | 3.7 |
| 45 | 0 | 0 |
| 51 | 6 | 22.2 |
| 52 | 1 | 3.7 |
| 56 | 11 | 40.7 |
| 58 | 0 | 0 |
| 59 | 0 | 0 |
| 68 | 3 | 11.1 |
| **Single vs. multiple** | **n=16** | % |
| Single infection | 8 | 50 |
| Multiple infections | 8 | 50 |

Table 3 shows the cross tabulation of HPV and Pap smear results. The majority of the women thirteen (52%) were positive for HPV but without any a detectable cytological abnormality. All women with abnormal Pap smear findings (three) (12%) were also positive for high risk HPV. Cohen Kappa test was run to determine if there was an agreement between Pap smear and HPV results. There was poor (non to slight) agreement between the tests, k=0.143, 95% CI: -0.17 to 0.46, p=0.166 (p>0.005).

**Table 3**
**Cross tabulation of HPV results and Pap smear**

| Pap smear results | HR-HPV pos | HR-HPV neg | Total | Cohen's Kappa(k) | p- value |
|---|---|---|---|---|---|
| NILM | 13 | 9 | **22** | 0.143 | 0.166 |
| Abnormal(≥ ASCUS) | 3 | 0 | **3** | | |
| **Total** | **16** | **9** | **25** | | |

Table 4 shows the cross tabulation of HIV status and Pap smear findings post LEEP. Cervical lesions were more common in HIV positive patients 2 cases (66.6%) compared to HIV negative patients 1 case (33.3%). A Chi- square test was done to determine if there is an association between HIV status and Pap smear findings. There was no statistically significant association HIV status and pap smear findings post LEEP, $X^2$=0.711, d.f= 1, p=0.399 (p>0.005).

**Table 4**
**Cross tabulation of HIV status and Pap smear findings post LEEP**

| Pap smear results | HIV neg | HIV pos | Total | Pearson value | p-value |
|---|---|---|---|---|---|
| NILM | 13 | 9 | **22** | 0.711 | 0.399 |
| Abnormal (≥ ASCUS) | 1 | 2 | **3** | | |
| **Total** | **14** | **11** | **25** | | |

Table 5 shows the cross tabulation ofHPV positivity between HIV positive and HIV negative women was comparable each accounting for 63.6% and 64.3% respectively.

**Table 5**
**Cross tabulation of HIV status and HPV positivity**

| HPV results | HIV neg | HIV pos | Total | Pearson value | p-value |
|---|---|---|---|---|---|
| Negative | 5 | 4 | **9** | 0.001 | 0.97 |
| Positive | 9 | 7 | **16** | | |
| **Total** | **14** | **11** | **25** | | |

A Chi- square test was done to determine if there is an association between HIV status and HPV positivity. There was no statistically significant association HIV status and HPV positivity post LEEP, $X^2$=0.001, d.f= 1, p=0.973 (p>0.005

Table 6 shows the comparison of diagnosis pre and post treatment. The majority of patients with abnormal results (88%) had successful treatments

| | | Post treatment | | |
|---|---|---|---|---|
| | | Neg | Pos | **Total** |
| **Pre-treatment** | normal | 1 | 0 | **1** |
| | abnormal | 21 | 3 | **24** |
| | **Total** | **24** | **3** | **25** |

## DISCUSSION

Co-testing with HPV DNA testing and Pap smear has proved to be a useful approach to stratify women with no cytological abnormalities according to their risk of residual disease which improves the efficacy of screening programs in this group of women. A Cohen Kappa correlation was done to ascertain the agreement of HPV results and Pap smear results. However, the test was not statistically significant (k=0.143, p=0.166).This can possibly be because most HPV infections detected were not yet associated with any cytological changes. This makes HPV testing in addition to Pap smear testing a useful and valuable combination as it helps to stratify women according to their risk for future recurrent disease.

In this study, follow up of women post LEEP treatment

revealed that 64% of women were HPV positive and 12% had ASCUS or more severe cytological abnormalities. The prevalence of cytological abnormalities was comparable to those reported by Gosvig CF et al in Denmark in which they reported 17% of women with cytological abnormalities post LEEP treatment(10). However HPV positivity in these two studies differs significantly with 64% in our study against 48%. This difference can partly be explained by earlier follow in that study (mean=3.4 months) compared to this study (mean=18 months). The delay in follow up in this study could have accounted for the higher HPV positivity due to new infections.

This study showed that cervical lesions after treatment are more common in HIV positive women (1.8%) compared to HIV negative women (0.7 %). This is consistent to the one reported by Chirenje M.Z et al in Zimbabwe which showed cervical lesions in 3.8% and 1.8% respectively(11). These results are also supported by results of a study byHeard et al who reported that 54% of HIV positive women previously treated for cervical lesions had residual or recurrent disease at 36 months(12).However, a Chi- square test revealed no statistically significant association between HIV infection and presence or absence of cervical lesions (p=0.399).

HPV positivity after LEEP in HIV positive patients was 63.6 %. This value was comparable to findings by Gingelmaier et al which showed a positivity rate of 57 %(13). HPV positivity in HIV negative patients was 64.3% in our study. This figure is higher compared to figures reported by Kreimer AR et al and Kocken M et al which ranged from 10-37% (5,6). This may be attributable to the detection of HPV at a fixed period post treatment in those studies unlike ours which had various periods with a mean of 18 months post treatment. This could explain the higher rates of HPV positivity possibly due to new infections rather than persistent infections.

## ACKNOWLEDGEMENTS

To the University of Nairobi, KAVI institute for permitting the study to be done within their premises. I am also grateful to staff in clinic 66, Kenyatta National Hospital for their support.

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