HUMAN PAPILLOMAVIRUS PREVALENCE, GENOTYPES, AND FACTORS ASSOCIATED WITH CERVICAL ABNORMALITIES AMONG HIV-POSITIVE AND NEGATIVE WOMEN IN EASTERN AND CENTRAL REGIONS, KENYA

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DECLARATION

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

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This PhD research thesis is dedicated to my family; my daughters Debbie, and Nyla, twin daughters Clara and Claire, my lovely wife Eunice, bro Jones, sis Filiah, mum Idah, and dad John Njue Iguna.

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ABBREVIATION AND ACRONYMS

ANC	Antenatal care
ART	Antiretroviral therapy
ASCUS	Atypical Squamous Cells of Undetermined Significance
CIN	Cervical Intraepithelial Neoplasia
DNA	Deoxyribonucleic acid
E1/2/4/5/6	Early protein 1/2/4/5/6
GAVI	Global Alliance Vaccine Initiative
GOK	Government of Kenya
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
hrHPV	High-risk Human Papillomavirus
HSIL	High-grade Squamous intraepithelial Lesions
ICC	Intraepithelial Cervical Carcinoma
ICC	Invasive Cervical Carcinoma
ISCC	Invasive squamous cell carcinoma
KAIS	Kenya AIDS Indicators Survey
KAPP	Knowledge, Attitude, Perception, and Practice
KEMRI	Kenya Medical Research Institute
KNBS	Kenya National Bureau of Standards
KNCCP	Kenya National Cervical Cancer Program
L1/2	Late protein 1 or 2

LHSIL	Low-grade Squamous intraepithelial Lesions
lrHPV	Low-risk Human Papillomavirus
MoH	Ministry of Health
NACOSTI	National Council for Science, Technology, and Innovations
NRF	National Research Fund
ORF	Open Reading Flame
Pap smear	Papanicolaou smear
PCR	Polymerase Chain Reaction
Rb	Retinoblastoma Tumour Suppressor
RNA	Ribonucleic acid
SCC	Squamous Cell Carcinoma
SERU	Scientific Ethical Review Unit
STI	Sexually Transmitted Infection
UTI	Urinary tract infection
VCT	Voluntary Counselling and Testing
VIA/VILLI	Visual inspection with Acetic Acid/Lugol's Iodine.
WHO	World Health Organization

DEFINITION OF TERMS

Adenocarcinoma – invasive tumour where glandular and squamous cellular layers are merged.

Carcinogenicity: the ability to cause cancer.

Carcinoma in situ (CIS) – pre-invasive malignancy confined to the epithelium and absent in the basement membrane – encompassed by CIN 3.

Cervical Intraepithelial Neoplasia (CIN) – cervical tissue histological analysis by biopsy and graded as CIN1, CIN2, and CIN3 depending on the abnormal epithelium thickness (⅓, ⅔, or entire thickness) following HPV infection within cervical cells. Approximately 60% of CIN1 will regress to normal after 1 year.

Cervicitis: an inflammation of the cervix, the lower, narrow end of the uterus that opens into the vagina. Indications of cervicitis include bleeding between menstrual periods, pain with intercourse or during a cervical exam, and anomalous vaginal discharge.

Cytologically normal cervix – cervix with no abnormal cellular formation observed on the surface upon cytology.

Dysplasia – The term indicates that abnormal cells were found on the surface of the cervix. Cervical dysplasia can range from mild to severe, depending on the appearance of the abnormal cells.

Genital warts: a sexually transmitted infection (STI) by certain low-risk strains of the HPV causing soft growths that appear on the genitals caused and cause pain, discomfort, and itching.

High-grade cervical lesions (HSIL/ CIN-2 / CIN-3 / CIS) - the presence of a high number of precancerous on the cervical surface indicative of being cancerous that invade deeper tissues of the cervix. They are classified as moderate/severe dysplasia, HSIL, CIN-2, CIN-3, or cervical carcinoma in situ (CIS).

HPV vaccination: **a** vaccine that helps protect the body against infection with certain types of oncogenic HPV.

Invasive cervical cancer (ICC) or **cervical cancer** - the presence of high-grade precancerous cells that invade the cervical basement membrane classified from stage I (cancer is in the cervix or uterus only) to stage IV (cancer has spread to multiple organs e.g., liver).

Invasive squamous cell carcinoma – an invasive form of cancer made up of cells that resemble those of squamous epithelium.

Low-grade cervical lesions (LSIL/CIN-1) – initial changes in size, shape, and number of abnormal cells that form on the surface of the cervix and are classified as dysplasia (mild), LSIL, or CIN-1.

Oncogenesis - Carcinogenesis, also called **oncogenesis** or **tumorigenesis**, is the formation of cancer, whereby normal cells **are** transformed into cancer cells.

os – The cervix opening into the uterus is called the internal os while the opening into the vagina is called the external os.

Polymerase Chain Reaction (PCR): a technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Squamous Intraepithelial Lesions (SIL) - abnormal results derived from cervical cytological screening or Papanicolaou smear testing.

Viroporins - short proteins (50 to 120 residues) containing a hydrophobic domain that contains at least one amphipathic alpha-helix responsible for creating a hydrophilic pore/channel that allows the virus to modulate ion homeostasis in infected cells.

ABSTRACT

Human Papillomavirus (HPV) associated cervical cancer caused 3,286 deaths in 2019 in Kenya with a national cervical screening rate of only 3.2%. The overwhelming predominance of distinctive HPV genotypes infection has implications regarding the HPV vaccination efficacy. Approaches to reduce cervical cancer incidence and mortality by either 'screen-and-treat" or "screen, triage and treat" are based on the diagnostic accuracy of available screening methods as well as awareness of the disease and its signs and preventive measures. This comparative study aimed to investigate the Human Papillomavirus prevalence, genotypes, and factors associated with cervical neoplasia among HIV-infected and non-infected women. The diagnostic accuracy of cervical screening methods was also determined. HIV-infected (cases) and non-infected (control) women aged 18-46 years women underwent cervical screening and colposcopy-biopsy confirmatory test alongside filling out a questionnaire on awareness of cervical cancer and its risk factors. A cervical broom was softly rotated 360 degrees five times to exfoliate cells from the region of the transformation zone, squamocolumnar junction, and endocervical canal for HPV genotyping, Pap smear followed by VIA/VILLI test. Laboratory outcome and questionnaire data statistical relationships were computed using the Pearson chi-square test. 161(50.8% cases and 156(49.2%) control, mean age: 34.3, SD ±10:4, range 18-46 years were recruited from Embu (85/317(26.8%)), Isiolo (64/317(20.2%)), Kirinyaga (56/317(17.7%)), Meru (81/317(25.6%)), and Tharaka-Nithi (31/317(9.8%)) Counties. 81/317(25.6%), 84/317(26.5%), 96/317(30.2%) and 78/122(63.9%) participants had VIA, HPV DNA-PCR, Pap smear and cervical histology positive results respectively. A higher primary diagnostic accuracy was established by HPV DNA-PCR (sensitivity: 95.5%; specificity: 92.6%) than Pap smear and VIA test while in triage testing, high sensitivity was obtained by HPV DNA-PCR parallel testing with VIA test (92.6%) and Pap smear test (92.7%). HPV genotypes distribution by cervical dysplasia were CIN1 (cases: HPV81 (12/317(3.8%)) and HPV11 (2/317(0.6%)); control: HPV53 and 66 co-infections (1/317 (0.3%)), CIN2 (cases: HPV11, HPV16, HPV66 ((1/317 (0.3%) each), HPV81 (6/317 (1.9%)); control: HPV81 (2/317(0.6%)) and Invasive Cervical Carcinoma (cases: HPV16 (1/317(0.3%)) and HPV81 (3/317(0.9%)). Most sample isolates had phylogenetic relationship with reference sequences from Iran [22.3% (17/76)], Kenya [22.3% (17/76)], Bangkok [14.4% (11/76] and India [7.9% (6/76)]. Knowledge of HPV screening was significantly influenced by residence, age, education level, married-marital status, religion, and hormonal contraceptive use ($p<0.005$). Fear of embarrassment was the main reason for failing to undergo cervical screening. HPV-PCR is recommended for routine testing and evaluation of Pap test results or during the treatment of cervical lesions. There was a higher frequency of both high-risk and low-risk HPV genotypes and the existence of non-vaccine HPV genotypes associated with cervical dysplasia among HIV-infected than HIV-uninfected women. Increased knowledge, willingness, and perceiving cervical screening as important, as well as personal acceptability to vaccinate against HPV, may reduce the cervical cancer burden.

Keywords: Human Papillomavirus, cervical cancer, screening, genotyping, Knowledge, Attitude, Practice and Perception, HPV vaccination

CHAPTER ONE

INTRODUCTION

1.1 Background of the study.

Human Papillomavirus (HPV) is primarily responsible for 99.7% of cervical cancers globally (Bruni *et al.*, 2021; Mabeya *et al.*, 2018; Manduku *et al.*, 2017). It is sexually transmitted and responsible for cervical neoplastic changes leading to cervical cancer. It was responsible for 311,365 (8.2%) annual global mortality, 37,017 (16.9%) in East Africa, and 5,250 (12.8%) in Kenya in 2020 (Bruni *et al.*, 2021; Mbulawa *et al.*, 2021).

Infections by HPV cause Cervical Intraepithelial Neoplasia (CIN) that lead to carcinoma-*in-situ* (CIS) if it is limited to epithelium. The virus also causes Invasive Cervical Cancer (ICC) if the basement membrane is invaded (Bruni *et al.*, 2021; Park, 2020; Ali *et al.*, 2019). The thickness of abnormal cervical epithelium is used to grade Cervical Intraepithelial Neoplasia as CINI, II, and III. Bethesda System Classification (Sias *et al.*, 2020; Yakub *et al.*, 2019) grades lesions following HPV infection as low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), and invasive cancer (ICC). Low-grade squamous intraepithelial lesions correspond to atypical cells of unknown significance (ASCUS) histologic diagnosis of flat condylomas, and CIN1 whereas HSIL corresponds to CIN 2 and 3 (Bruni *et al.*, 2021; Mabeya *et al.*, 2018; Pineros *et al.*, 2018).

Human Papillomavirus genotypes that affect the cervix are: HPV 6, 11, 16, 18, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 and HPV81 (CP8304). There are eighteen high-risk HPV (hrHPV) that include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 and seven lrHPV 6, 11, 40, 42, 43, 44 and 81

(Mbulawa *et al.,* 2021; Bruni *et al.,* 2021; Ali *et al.,* 2019; Doorslaer *et al.,* 2018; Chen *et al.,* 2018). A study by Sweet *et al* (2020) provided biological evidence of oncogenicity by low-risk HPV (lrHPV) 26, 67 and CP8304.

World Health Organisation (WHO) approaches to reduce cervical cancer mortality are the "screen-and-treat approach", where the decision to treat is based on a positive primary screening test only without triage (i.e. no second screening test and no histopathological diagnosis) and the "screen, triage and treat approach", where the decision to treat is based on a positive primary screening test followed by a positive second test with or without histologically confirmed diagnosis after every 5 years (WHO *Cervical Screening Guidelines*, 2021). It recommends HPV genome detection as the primary screening test rather than visual inspection or cytology preferably with triage in screening and treatment approaches among both the general population of women and women living with HIV. Additionally, the WHO identifies research gaps and further considerations for more data on the specificity and sensitivity of cervical screening tests among women living with HIV (WHO *Cervical Screening Guidelines,* 2021; Elmi *et al.,* 2017).

Risk factors necessary for HPV oncogenicity include having more than one sex partner that increases the woman's probability of acquiring sexually transmitted infections (STIs) that including HPV and Human Immunodeficiency Virus (HIV) (Ermel *et al*., 2019; Yakub *et al.,* 2019). This probability is further increased if the woman is practising unprotected sex. Infection by HIV and the prolonged use of autoimmune disease drugs weakens the immune system responsible for destroying cancer cells in the

cervix. Carcinogenic products in tobacco and steroid hormonal contraceptives have been hypothesized to be mechanisms for HPV tumorigenic effects on cervical tissue (Ermel *et al*., 2019; Elmi *et al*., 2017). Long-term inflammation caused by recurrent Sexually Transmitted Infections (STIs) hormonal changes during pregnancy and the trauma to the cervix during labour, early sex debut, and high parity favour HPV acquisition and persistence which is reduced in women who have undergone caesarean sections (Mbulawa *et al.,* 2021). Increased or decreased transcription of the human genome is regulated following the insertion of specific HPV sequences. Failure to undergo HPV vaccination deprives women of immunity from hrHPV genotypes (Bruni *et al.,* 2021; Yakub *et al.,* 2019; Chan *et al*., 2019; Mabeya *et al.*, 2018).

High HPV prevalence and mixed genotype infections have been reported among HIV-infected women of reproductive age in other parts of Kenya (Bruni *et al.,* 2021). Common HPV genotypes reported are HPV52 in Western Kenya (Sweet *et al*., 2020), HPV81, HPV 16, and HPV52 in the reproductive Health Clinic in Nairobi, Kenya (Omire *et al.,* 2020), the predominance of HPV16 and mixed lrHPV and hrHPV among women with cervical cancer (Bruni *et al.*, 2021). Studies have reported low uptake of cervical screening (Mabeya *et al.*, 2018; Manduku *et al.*, 2017), low of awareness HPV vaccination, and limited knowledge of HPV and cervical cancer (Bruni *et al.,* 2021; Mabeya *et al.,* 2018, Chan *et al*., 2019; Mutambara *et al*., 2017).

This study established the epidemiological data on cervical abnormalities using different cervical diagnostic techniques, HPV prevalence and knowledge,
and acceptability of cervical cancer primary prevention strategies that are required in

determining the disease burden and improving preventive strategies of the disease in Eastern and Central Regions, Kenya.

1.2 Statement of the problem.

According to ICO HPV Information Centre (2021) reports, cervical cancer is the second most common cancer in women aged 15 to 44 years in Kenya. Human Papillomavirus prevalence among women aged 18-46 years with the normal cervix is at 9%, while those with invasive cervical cancer is at 61%. This data only features HPV16 and HPV18 whereas information on other HPV genotypes responsible for cervical dysplasia which is equally important in designing prevention strategies is inadequate. HPV genotypes responsible for cervical dysplasia among HIV-infected women in Eastern and Central Regions, Kenya are unknown since there are no reported HPV genotyping studies done. Current vaccination protects against HPV 6, 11, 16, and 18 (Bruni *et al.,* 2021; Perez *et al.,* 2018) whereas HIV-positive women show diversified spectra of HPV infection with potential cancer risk. The information gap exists on factors associated with cervical neoplasia among HIV-positive women in Eastern and Central Regions, Kenya. Risk factors are important in understanding disease dynamics and designing effective preventive measures. Published observations (Chan *et al.,* 2019; Mabeya *et al.,* 2018) have established the lack of awareness of cervical cancer risk factors, and low uptake of cervical cancer preventive measures such as cervical screening, and HPV vaccination in other regions of Kenya.

1.3 Justification of the study.

Despite the existence of the National Cervical Cancer Control Strategy 2017-2022 in Kenya, the implementation of the national screening program for HIV-positive women

is still low and haphazard (Bruni *et al.,* 2021). The cervical screening rate in Kenya is 3.2% for women aged 18-49 years (Manduku *et al.,* 2017). The overwhelming predominance of wide spectra of HPV genotypes co-infection with HIV among women in Kenya has implications regarding the efficacy of HPV vaccination. Cross protection between HPV genotypes in the current vaccine and predominant HPV genotypes in the population has not been established. Novel HPV genotype sequences responsible for cervical dysplasia among HIV-positive women have been detected. There are no HPV molecular diagnostic techniques in Eastern and Central Regions, Kenya where most health facilities mostly depend on low-sensitive Visual Inspection with Acetic Acid/Lugol's Iodine (VIA/VILLI) (Krug and Varghese, 2019). Oncogenic and possibly-oncogenic HPV genotypes will sensitize cervical cancer and HIV managers to routine patient follow-up and close monitoring for possible cervical neoplasia. Women who show positive oncogenic-HPV and negative cytology have a high risk of cervical neoplasia and management of this must include repeat HPV-genotyping and/or cytology on testing within 12 months (McClymont *et al.,* 2020; Orang'o *et al.,* 2017). Reasons for advanced-stage disease diagnosis when symptoms are apparent and low adherence to treatment due to asthenia, fatigue and disparity need to be determined to reduce cervical cancer burden. This can be achieved by evaluating cervical screening uptake, awareness of factors, common cervical cancer symptoms and prioritization of cervical cancer preventive measures among policymakers (Mbulawa *et al.,* 2021; Omire *et al.,* 2020; Chan *et al*., 2019).

1.4 Research questions.

i) What are the performance characteristics of cervical screening methods based on histopathology of abnormal cytology outcomes among HIV-infected and non-infected women in Eastern and Central Regions, Kenya?

ii) What is the prevalence of HPV genotypes among HIV-infected and non-infected women in Eastern and Central Regions, Kenya in association with social-demographic factors?

iii) What are the phylogenetic relationships of HPV genotypes responsible for cervical abnormalities among HIV-infected and non-infected women in Eastern and Central Regions, Kenya?

iv) What is the level of knowledge, attitude, practice, and perception of factors associated with HPV oncogenesis among HIV-infected and non-infected women in Eastern and Central Regions, Kenya?

1.5 Objective.

1.5.1 Broad objective.

To investigate the Human Papillomavirus prevalence, genotypes, and factors associated with cervical neoplasia among HIV-infected and non-infected women in Eastern and Central Regions, Kenya.

1.5.2 Specific objectives.

1. To evaluate performance characteristics of cervical screening methods based on histopathology of abnormal cytology outcomes among HIV-infected and non-infected women in Eastern and Central Regions, Kenya.

2. To determine the HPV genotypes prevalence among HIV-infected and non-infected women in Eastern and Central Regions, Kenya in association with social-demographic factors.

3. To determine phylogenetic relationships of HPV genotypes responsible for cervical abnormalities among HIV-infected and non-infected women in Eastern and Central Regions, Kenya.

4. To determine the knowledge, attitude, practice, and perception of factors associated with HPV oncogenesis among HIV-infected and non-infected women in Eastern and Central Regions, Kenya.

1.6 Significance of the study.

All participants in this study underwent cervical screening to detect any cervical abnormality which includes the presence of cancerous cells and or cervical infections. The study evaluated the diagnostic value of HPV genotyping, Pap smear and VIA/VILLI cervical screening methods to assist in the development of effective screening strategies for early and accurate cervical abnormality detection.

The study has established a better understanding of high-risk and low-risk HPV genotype distribution among HIV-infected women in Eastern and Central Regions, Kenya which is essential in monitoring possible future cervical dysplasia outcomes. Genotype distribution in the Eastern Kenya region also contributes to a pool of data required in planning and developing a future effective vaccine against HPV.

Human Papillomavirus genotypes detected in association with cervical dysplasia corroborate surveillance data and inform health authorities and scientific audiences of novel HPV sequences and their oncogenicity. The HPV genotypes co-testing with HIV, cervical cytology, and histology in association with KAPP on risk factors associated with cervical cancer has clinical potential to inform future cervical screening strategy through identifying and monitoring vulnerable women. Understanding region-specific factors for HPV oncogenesis, especially in a population with low resources and raised HIV prevalence is critical in accessing disease burden and dynamics and thereby reducing the incidence, morbidity, mortality, and high costs associated. It was advisable to determine KAPP on cervical cancer preventive measures as critical indicators of their acceptability by specific communities before the resourceful intervention. All participants underwent an education on the benefit of cervical screening, adherence to

treatment, HPV vaccination, and factors associated with cervical neoplasia during the study.

1.7 Conceptual framework.

Infection by HPV and/or HIV are the independent variables in this study. HPV clearance or progression into cervical neoplasia is dependent on intervening or risk factors such as high parity, smoking, long-term hormonal contraceptive use and failure to undergo vaccination (Figure1-1).

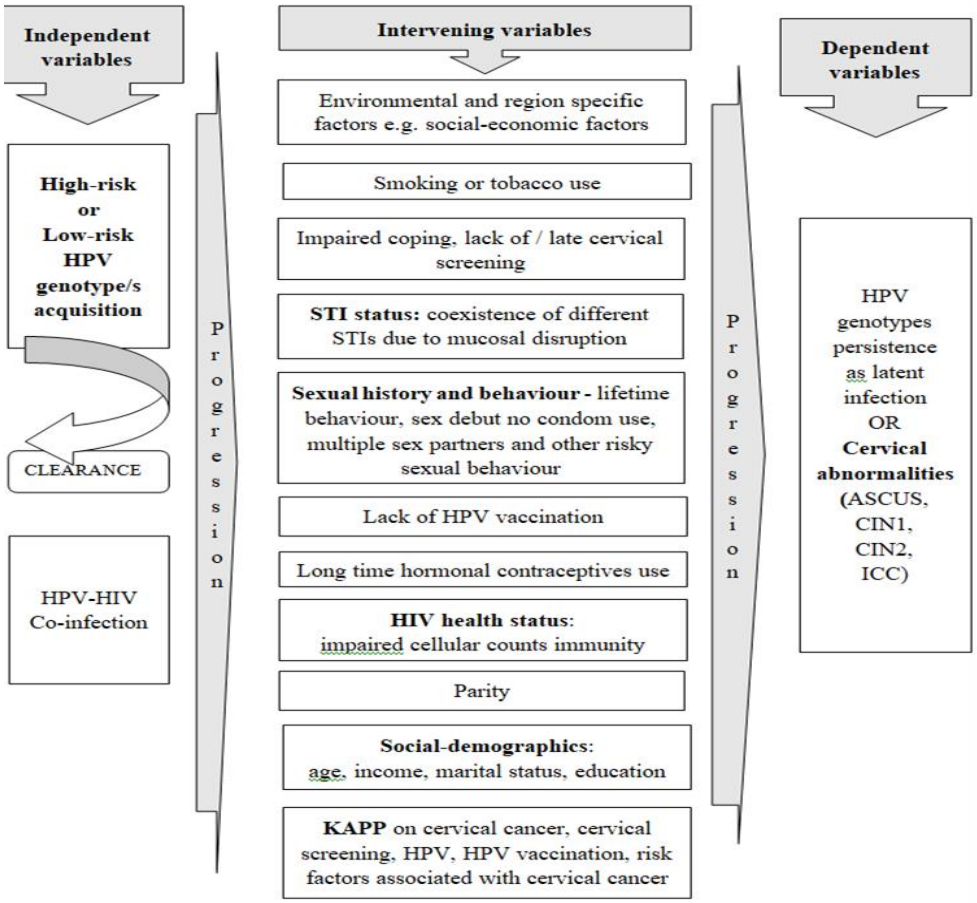

Figure 1-1. Conceptual framework.

CHAPTER TWO

LITERATURE REVIEW

2.1 Human Papillomavirus etiology and natural history.

There are over 150 closely related Human Papillomavirus genotypes. They have evolved for over 300 million years within their hosts. They have been detected in multiple mammalian species; however, humans are the only reservoir. (Doorslaer *et al.,* 2018). The twentieth century marked the identification of Papillomavirus after it was established that an infectious agent was responsible for skin warts (Papillomas) in humans (Kim *et al*., 2021; Park, 2020).

The Papillomavirus was first established as a disease-causing agent by Dr Richard Shope and John Bittner, in the 1930s. Shope did not isolate HPV, but he correctly established that the viruses caused warts. Viruses were linked to human cancers by Ludwik Gross in the 1950s. The Discovery of the first human tumour viruses; Epstein Burr virus, Hepatitis B virus, and Papillomaviruses occurred in the 1960s. Then in 1976, German Virologist Harald Zur Hausen pointed out that HPV was responsible for cervical cancer. Originally this theory was rejected by other scientists, but in 1983 and 1984, he identified HPV16 and HPV18 in cervical cancer tissues, which proved his theory. In 2008, he received the Nobel Prize for this research (Kim *et al*., 2021; Chen *et al.,* 2018).

2.2 Classification of Papillomavirus.

Family Papillomaviridae Late protein (L) 1 pair-wise identity across the open reading frame (ORF) forms a basis for the Family's classification. Members of the family

mostly colonize human mucosal and keratinized epithelium. They have been isolated from fish, reptiles, and mammals (Mbulawa *et al*., 2021; Doorslaer *et al*., 2018).

Family Papillomaviridae is divided into Firstpapillomavirinae and Secondpapillomavirinae subfamilies. There are five major genera in the Firstpapillomavirinae subfamily. They include *Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus* and *Nu-papillomavirus* as shown in Table 2.1 (Kim *et al*., 2021; Chen *et al.*, 2020; Van Doorslaer *et al.*, 2018).

Table 2-1. Family Papillomaviridae.

Family:
Papillomaviridae
Sub-family
Firstpapillomavirinae
Major genera:
Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus, Nu-papillomavirus
Minor genera:
Chipapapillomavirus, Deltapapillomavirus, Dyochipapillomavirus, Dyodeltapapillomavirus, Dyepsilonpapillomavirus, Dyoxipapillomavirus, Dyozetapapillomavirus, Epsilonpapillomavirus, Etapapillomavirus, Lotapapillomavirus, Kappapapillomavirus, Lambdapapillomavirus, Omegapapillomavirus, Omikropapillomavirus, Phipapapillomavirus, Pipapapillomavirus, Psipapapillomavirus, Rhopapapillomavirus, Sigmapapillomavirus, Taupapapillomavirus, Thatapapillomavirus, Treisepsilonpapillomavirus, Treisiotapapillomavirus, Treiskappapapillomavirus, Treisthetapapillomavirus, Treiszetapapillomavirus, Upsilonpapillomavirus, Xipapapillomavirus and Zetapapillomavirus
Sub-family
Secondpapillomavirinae *Genus Alefpapillomavirus*

(Source: Thomas *et al.*, 2008; Kim *et al*., 2021).

Species demarcation criteria of >70% nucleotide identity across complete genome sequence of the L1 Open Reading Frame (ORF) of Papillomaviruses within the genus is used to identify and name *Species: Alphapapillomavirus 1,2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13* and *14.* Members of the Alphapapillomavirus genus preferentially colonize the primate's oral and/or anogenital mucosa. Other species e.g., *Alphapapillomavirus 2* and *Alphapapillomavirus 4* cause cutaneous lesions in humans. Members of *Alphapapillomavirus* 7 notably HPV18 and *Alphapapillomavirus 9* dominate malignant tissues hence considered oncogenic and code for a hydrophobic Early protein 5 (E5) found at the 3′-end of the early region (Doorslaer *et al.*, 2018). Alphapapillomavirus HPV-16 is the representative type of species 9, which includes genotypes 31, 33, 35, 52, and 67 (Kim *et al*., 2021; Chen *et al*., 2018).

Human Papillomavirus "type" is distinctively defined when the genome ORF's L1 DNA sequence of the cloned viral genome has at least a 10% difference from that of the wild genotype. In Papillomavirus research, variants are isolates of the same HPV genotype or subtypes when the nucleotide sequences differ by not more than 10%. This criterion for HPV genotype classification has demonstrated amazingly steady and valuable for basic researchers, clinicians, and epidemiologists. Nevertheless, the development of a common nomenclature for HPV variants for the assortment of HPV genotypes is continuously implemented (Kim *et al*., 2021; Park, 2020; Chen *et al.*, 2018).

2.3 Human Papillomavirus transmission and life cycle.

Transmission of mucosa-tropic HPV genotypes is primarily sexual (Chan *et al*., 2019; Yakub *et al.,* 2019; Innes *et al*., 2018) and it's the most transmitted sexual infection. Over 75% of sexually active persons have ever been infected in their lives. The crest age for HPV infection is in the initial years after sexual debut. The disease burden decreases in older age as most genital HPV infections resolve within 12-18 months without symptoms. Other infections persist leading to cervical dysplasia. Invasive cervical carcinoma is a rare outcome after decades of infection (Kim *et al*., 2021; Park, 2020; Omire *et al.,* 2020; Ermel *et al*., 2019; Huang *et al.,* 2018).

The viruses enter through micro-injuries and infect the epithelium's basal cells. HPV life cycle depends on the keratinocyte's separation which is the foremost target cell. As keratinocytes advance to the spinous layer, intensification of viral DNA replication progress leading to the expression of the viral genome. Here, heparin-sulphate-proteoglycan, a basic component of the extracellular lattice, in conjunction with α6-integrin and laminin-5, plays an essential part. The virus is internalized into the cell through clathrin or caveolae-mediated endocytosis and enters the core where the replication starts (Kim *et al*., 2021; Bogani *et al.,* 2020; Pan *et al.,* 2019; Huang *et al.,* 2018).

Following basal cell infection, there is an expression of viral replicates in stem cells creating up to 100 extra-chromosomal duplicates of HPV DNA/cell. Viral early proteins are crucial at this stage. These proteins frame a complex that ties to the viral replication process by incorporating cellular polymerases and embellishment proteins that starts

DNA replication. After entering the suprabasal layers, there is an expression of the late viral genes, self-assembly in the capsid, and discharge to infect new cells (Pan *et al.,* 2019).

2.4 Cell cycle regulation.

The cell cycle is comprised of phases that govern cell proliferation. The first phase is the mitotic segregation phase (M-phase) followed by the mitosis phase where the cell divides. This is followed by the G1 phase consisting of an early stage and a late stage. The two are separated by the restriction (R) point. Advancement of the cell cycle is governed by Cyclin and Cyclin-dependent Kinases (CDK) like retinoblastoma tumor suppressor (RB) protein that determines the phosphorylation of regulatory proteins (Park, 2020).

During progression past R-point, the CDK4 joins Cyclin D to phosphorylate RB while progression to late-stage, Unphosphorylated RB binds to inhibit E2F genes that encode transcription factors in higher eukaryotes. The mitogen-dependent early G1 stage is signalled into the G0 phase (quiescence). In the G0 phase, cells are typically smaller with low metabolic activity and can quit the cell cycle to the G0 phase if no mitogen is present. High phosphorylation of RB by CDK4/cyclin D is crucial for the cell cycle to proceed past the R point. At the late G2 stage mitogens are no longer required for the cycle to proceed to the G1/S checkpoint. This checkpoint is controlled by CDK2 and ensures no damage to the DNA before its replication proceeds. DNA damage may lead to complex repair pathways or apoptosis. In the S (synthesis) phase, DNA replication occurs and then proceeds to the G2 phase; where it is checked before mitosis at the G2/M (Kovacevic *et al.,* 2021; Li *et al.,* 2021; Park, 2020).

2.5 Pathogenesis of Human Papillomavirus.

Almost 90% of invasive cervical cancer is squamous cell carcinomas (SCC) that occur in the cervix transformation zone. Following exposure to the acidic environment of the vagina upon the onset of puberty, columnar epithelial cells transform into the squamous epithelium in a process called metaplasia (Kovacevic *et al.,* 2021; Li *et al.,* 2021; Ali *et al.,* 2019). This physiological process is changed when metaplastic cells are exposed to cancer-inducing factors resulting in the atypical metaplastic epithelium (Ermel *et al*., 2019; Torres-Poveda *et al.,* 2019).

Cervical columnar epithelium metaplasia is responsible for enhancing integration and/or HPV E7 protein expression in a cell that has transformed. This is evident by the fact that the most vulnerable women to cervical cancer are those who acquire HPV during the dynamic periods of metaplasia; early adolescence and first pregnancy (Mbulawa *et al.,* 2021; Sias *et al.,* 2020).

Cervical epithelial differentiation during dysplasia is divided into four categories: mild, moderate, severe dysplasia, and Cervical Intraepithelial Carcinoma (CIS) (Figure 2-1). Further differentiation from CIS to invasive carcinoma is evident by spreading into inner cervical tissues that include the surrounding pelvic region, and finally to more distant parts of the body (Li *et al.,* 2021; Ali *et al.,* 2019; Torres-Poveda *et al.,* 2019; Yakub *et al.,* 2019; Huang *et al.,* 2018). The chance of surviving cervical cancer depends on the diagnosis stage. Women with cancer detected in the pre-invasive and even in the in-situ stages, have a 5-year survival rate of essentially 100% (National Cancer Control Strategy, 2017).

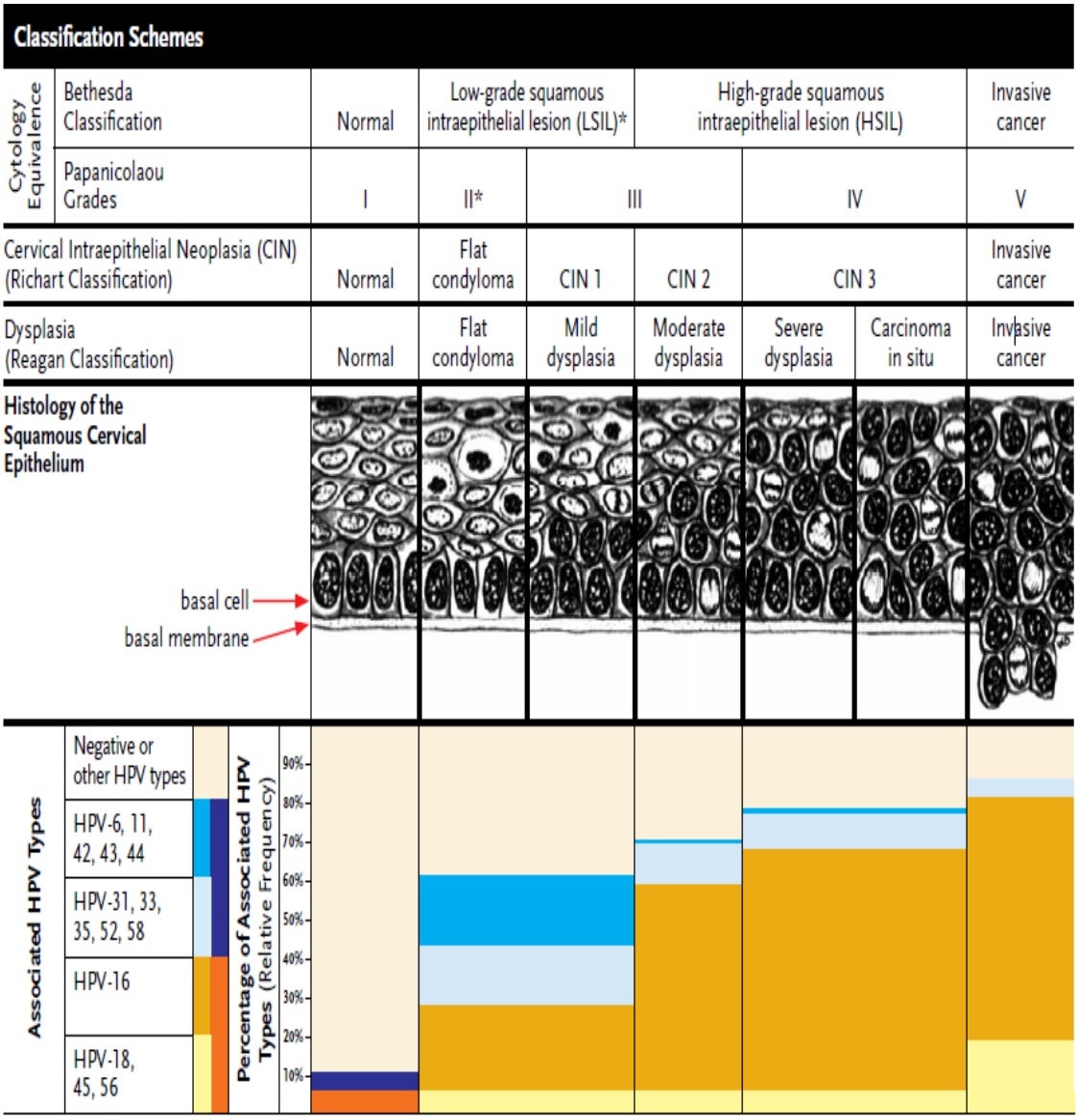

Figure 2-1. Classification scheme of cervical HPV infection.

Source: Atherton *et al.,* 2017.

2.6 Distribution of Human Papillomavirus genotypes

Over 80% of worldwide cervical cancer occur in developing world populations, largely because of failure to establish early and effective screening and follow-up programs (Mbulawa *et al.,* 2021; Menon *et al*., 2016) (Figure 2-2).

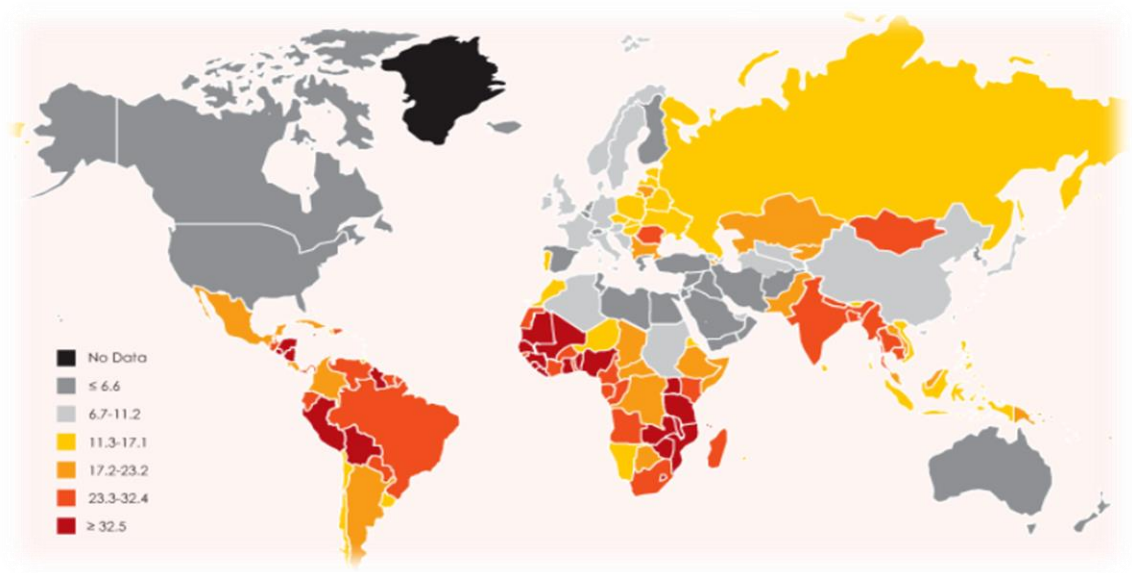

Figure 2-2. Global map showing estimated age-standardized cervical cancer incident rate. (Source: Bruni *et al.*, 2021).

There is little information on HPV genotype prevalence in sub-Saharan Africa (Omire *et al.,* 2020; Pineros *et al.,* 2018). A study by Mbulawa *et al.,* (2021) and Omire *et al.,* (2020) review the existence of a wide spectrum of high risks and low-risk HPV genotypes that have never been associated with cervical dysplasia. Recently published reports review HPV research often covering specific populations in specific geographical areas of a nation. There have been no national or sentinel surveillance programs to assess HPV infection in most countries in Sub-Saharan Africa making it difficult to accurately determine the HPV burden. Few studies on sexually transmitted infection prevalence have been conducted in the region (Bruni *et al.,* 2021; Sias *et al*., 2020).

2.6.1 Human Papillomavirus genotypes distribution in Kenya.

Minimal studies have assessed the complex association between HPV genotypes and HIV infection in Kenya (Omire *et al.,* 2020; Bruni *et al.,* 2021; Torres-Poveda *et al.,*

2019). Data on HPV genotypes is important in vaccine design as well as in evaluating possible cross-protection against non-vaccine genotypes.

A study by Chen *et al.,* (2018) showed a high prevalence of HPV52 than HPV16 and HPV18. Participant age, history of infection by syphilis, anaemia, vaginal bleeding, and parity of five increased the prevalence odds of severe dysplasia and ICC, compared to mild dysplasia. The overwhelming predominance of HPV52 has implications regarding HPV16 and HPV18 vaccine efficacy as a baseline strategy for cervical cancer prevention among HIV-infected women in Western Kenya (Sweet *et al.,* 2020; Chan *et al*., 2019).

In Mombasa located in coastal Kenya, a study showed that 46% of HIV seropositive women harboured multiple HPV genotype infections. Dominant HPV genotypes were HPV 58 (10.5%) while HPV16, HPV53, HPV18, and HPV6 had less than 10% in decreasing order respectively. The prevalence of any high-risk (hrHPV) HPV30, HPV42, HPV45, and HPV56 was 28.8%. Literature shows a higher global HPV prevalence and a wider genotypes spectrum of infection across ages. The data informs on non-vaccine HPV genotypes associated with cervical neoplasia, strengthens the urgency of HPV vaccination in Kenya and highlights the elevated number of women who would have positive results in an HPV-based screening program in the country (Bruni *et al.,* 2021; Perez *et al.,* 2018).

Another study in Mombasa, Kenya, established the most common hrHPV genotypes as HPV 16, followed by HPV 53 and HPV 18, with a combined prevalence of 76 %, and were found in presence of other high-risk HPV infections. Strong associations were

observed between HPV 53 and multiple hrHPV infections with CD4 count <200 cells/µl. In this study, 65 % of the participants had multiple HPV genotype infections (Menon *et al.,* 2016).

In a Kenyatta National Referral Hospital in Nairobi Kenya, hrHPV16 that clustered with HPV from Iran and Africa was the most predominant followed by HPV81, 73, 35, and 52. One HIV-positive woman had cervical cancer and had multiple infections with HPV 26, 35, and 58. HPV 16, 6, and 81 had two variants each. This study provided evidence for the circulation of other HPV 35, 52, 73, 81, 31, 51, 45, 58, and 26 in the Kenyan population that play important roles in cervical dysplasia aetiology but missing in the HPV vaccine (Omire *et al.,* 2020).

A study among 283 HIV-infected discordant women in Kenya established an overall HPV prevalence of 62%, where 47% had hrHPV genotypes. Seven per cent of 268 women with cervical cytology results had high-grade cervical lesions or more severe by cytology. Predominant hrHPV genotype among women with a high-grade lesion or more severe by cytology were HPV-52 (44%), HPV-31 (22%), HPV-35 (22%), HPV-51 (22%) and HPV-58 (22%). High-risk HPV16 and 18 were common among 17% of women with high-grade lesions or more severe. The screening method applied detected 89%of women with a high-grade lesion or more severe and 44% of women with normal or low-grade cytology (Guthrie *et al.,* 2020).

A study by Ermel (2019) showed HIV-infected women were older, with more lifetime sexual partners, less likely to be married, more likely to regularly use condoms, and were more likely to be infected by HPV 16, other oncogenic HPV types, and multiple

oncogenic types. There was a significant association between a higher number of oncogenic HPV types and HIV infection and more lifetime sexual partners. The four most frequently detected HR-HPV types in 105 HIV-uninfected women were HPV 58 ($n = 5$, 4.8%), HPV 45 ($n = 4$, 3.8%), and HPV 52 and 53 (both $n = 4$, 3.8%).

Hereditary neoplasia indicates the host's genetic background contributes to HPV susceptibility. Literature review that hosts genetic factors that polymorphic genes of the major histocompatibility complex (MHC), polymorphism in the p53 gene, and human leukocyte antigen (HLA) are associated with susceptibility and progression of HPV to cervical cancer (Kuguyo *et al.,* 2018).

2.7 Human Papillomavirus genome proteins.

The Human Papillomavirus genome is circular, approximately 5,748 base pairs (bp) to 8,607 bp, enclosed in Late protein 1 and minor L2 capsid proteins resulting in virions approximately 55nm diameter with icosahedral structure (Chen *et al.,* 2020; Van Doorslaer *et al.,* 2018). The virion is non-enveloped. One of the two DNA strands contains all the coding information (Shuling *et al*., 2020). There are seven Open Reading Flames (ORF) encoding viral proteins. The six "early" proteins (E) are E1, E2, E4, E5, E6, and E7. During the early part of the viral replication cycle, transcripts encoding the early proteins are detected in basal and suprabasal epithelial cells and encode proteins that interact with the host cell machinery to aid viral replication and transcription (Chen *et al.,* 2020; Torres-Poveda *et al.,* 2019; Van Doorslaer *et al.,* 2018).

2.7.1 Late protein 1.

Late proteins 1 and 2 are expressed in terminally differentiated keratinocytes to form a viral capsid. Capsid comprises 360 molecules of L1 arranged as 72 capsomers in an icosahedral surface lattice. Late protein 1 transcription starts at Upstream Regulatory Region (URR) where the process fails to incorporate an early polyadenylation signal that terminates early gene mRNAs. Translation of L1 is initiated by the ATG codon which is located immediately at 3′ of a consensus splice acceptor site. N-terminus of L1 carries a consensus MxxWx7YLPP motif. Computational analysis of Papillomavirus genomic sequences suggests that a minority of Papillomavirus species encode an N-terminal extension upstream of the L1 MxxW motif (Zhang *et al*., 2018)

2.7.2 Late protein 2.

Late protein 2 is located at the centre of pentameric capsomers in mature virion. It has a role to encapsulate the HPV genome into infectious virions. In the model, L2 mediated assembly for Papillomavirus virion; L2 localization to sub-nuclear domain causes the subsequent recruitment of E2 with bound viral genome and L1. This L2-L1-E2 genome association confers appropriate concentration for virions assembly. Early protein 2 is catalytically important in the process of DNA encapsulation during viral assembly (Chen *et al.,* 2020; Shuling *et al*., 2020).

2.7.3 Early protein 1.

The HPV E1 protein is the only viral protein with enzymatic activity. The main known function of this protein is the regulation of viral DNA replication. Nevertheless, it has been demonstrated that the ablation of HPV18 E1 mRNA in HeLa cells promotes the

deregulation of several genes, particularly those involved in host defence mechanisms against viral infections; however, the specific contribution of E1 protein in an HPV-independent context is still under study (Chen *et al.,* 2020). Studies have shown that E1 proteins from HPV16 and 18 induce overexpression of different sets of genes associated with proliferation and differentiation processes, as well as down-regulation of immune response genes, including Interferon (IFN) β1and IFNλ1 and Interferon-stimulated gene (ISG), which are important components involved in the antiviral immune response. High-risk HPV E1 proteins play an important role in inhibiting the anti-viral immune response (Shuling *et al*., 2020; Zhang *et al.,* 2018).

2.7.4 Early protein 2.

Early protein 2 is made up of DNA-binding domain and transactivation domains that are connected by a flexible linker sequence often called the "hinge" which varies in length and sequence composition among different genera of Papillomavirus. This is a homodimer that binds E2 Binding Sites (E2BS) in the viral Long Control Region (LCR) (Shuling *et al*., 2020; Zhang *et al.,* 2018).

Mucosa-infective HPVs have two Early Protein Binding Surfaces (E2BS) located close to the viral early promoter. The third E2BS is located at the DNA origin of replication while the fourth E2BS is found in the enhancer region (Zhang *et al*., 2018). The E2 proteins function primarily by recruiting cellular factors to the viral genomes, which activate or repress transcriptional processes (Chen *et al.,* 2020). The E2 proteins bind specifically to sequence motifs in the viral genome and can activate or repress

transcription, depending on the context of these binding sites and the nature of the associated cellular factors (Molina *et al*., 2020; Uniprot, 2019; Zhang *et al.,* 2018).

Human Papillomaviruses have the potential to encode shorter E2 forms that contain the C-terminal domain, the hinge region, and a 10-13 residue peptide from an upstream ORF. These E2 fusion proteins are encoded by spliced messages that link sequences from an alternative reading frame in the E1 region of the genome (designated E8) to the C-terminus of E2. These proteins have been alternatively named E8^E2, E8^E2C, E1^E2, E1M^E2, and E9^E2 (Kuehner & Stubenrauch, 2022). The shorter forms of E2 can act as repressors of E2 function. Repression can be mediated by competition for binding to E2 binding sites (Chen *et al.*, 2020). Early protein 2-interacting proteins have been shown to act in transcription, replication, chromatin remodelling factors, or histones modifying enzymes. Studies have shown the integration of E2 with other proteins for cell apoptosis keratinocytes migration and differentiation, intracellular transport, and RNA processing. It acts as an auxiliary replication factor and loads E1 replication helicase which in turn recruits the cellular DNA replication machinery. It also regulates the replication, transcription, and partitioning of the viral genome during the viral infectious life cycle. E2 also interacts with proteins involved in apoptosis regulation in the cell cycle (Shuling *et al*., 2020).

2.7.5 Early Protein 4.

Human Papillomavirus E4 protein is synthesized as an E1^E4 fusion protein as a result of mRNA splicing. Its first initial sequence of amino acids including the initiation codon contains sequences from E1 open reading frame (ORF) (Molina *et al.,* 2020). The

Papillomavirus E4 ORF is centrally located within E2 ORF in the region that encodes the flexible E2 protein's hinge domain. Early protein 4 gene-product (E1^E4) is translated from a spliced mRNA and includes the E1 initiation codon and its neighbouring sequences. In the HPV lifecycle, the products of the E1^E4 gene become detectable during the onset of viral genome amplification as the late stages of infection begin. E4 is involved in the amplification of the genome, virus synthesis, viral release, and/or viral transmission as evidenced by its high concentration during these processes. E4 is easily detectable in lesions caused by a variety of HPV genotypes by immunostaining biopsy material. It serves as a biomarker of active virus infection, especially by hrHPV genotypes (Shuling *et al*., 2020). Early protein 4 accounts for over 30% of total proteins in cutaneous lesions, higher than virion coat proteins. Generally, E4 is expressed before L2 and L1, with modified structure and function by kinases and proteases at S and G2 cell cycle phases before the cell undergoes true terminal differentiation (Chen *et al.,* 2020). Kinases involved in this transformation also affect other viral proteins and include kinase A, Cyclin-dependent kinase, members of the MAP kinase family, and protein kinase C (Molina *et al*., 2020).

The E4 proteins of the Beta, Gamma, and Mu HPV genotypes assemble into distinctive cytoplasmic, and sometimes nuclear, inclusion granules. In HPV16 these proteins regulate E4 association with the cellular keratin network to reorganize it and cleave protease Calpain which allows assembly into amyloid-like fibres (Shuling *et al*., 2020).

2.7.6 Early Protein 5.

Early protein 5 is made up of 83 amino acids, highly hydrophobic, associated with internal cellular membranes of the Endoplasmic Reticulum, Golgi apparatus, and perinuclear region. It can induce anchorage-independent growth in human keratinocytes and murine fibroblasts; as demonstrated with oncogenic HPV 16 (Chen *et al.,* 2020; Shuling *et al*., 2020). The HPV-induced lesions have abundant E5 which is expressed in the early stages of malignant transformation representing a target for early intervention before the progression of premalignant lesions to cervical cancer (Shuling *et al*., 2020).

Early protein 5 hyperactivates ligand-dependent epidermal growth factor (EGF) receptor (EGFR) signalling pathways, resulting in enhanced extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) activity as shown in Figure 2-3. This is accomplished by impaired endosome maturation resulting in delayed EGFR degradation. Reduced apoptosis favors the expression of E5 and immune evasion (Chen *et al.,* 2020). Early protein shares a similarity to the viral membrane called viroporins that modulate ion homeostasis by creating channels for small molecules and ions to passage into the infected cells (Shuling *et al*., 2020). Viroporins-mediated membrane permeability occurs at various stages during infection, often controlling viral entry and release, although they are not usually essential for viral genome replication (Zhang *et al.,* 2018).

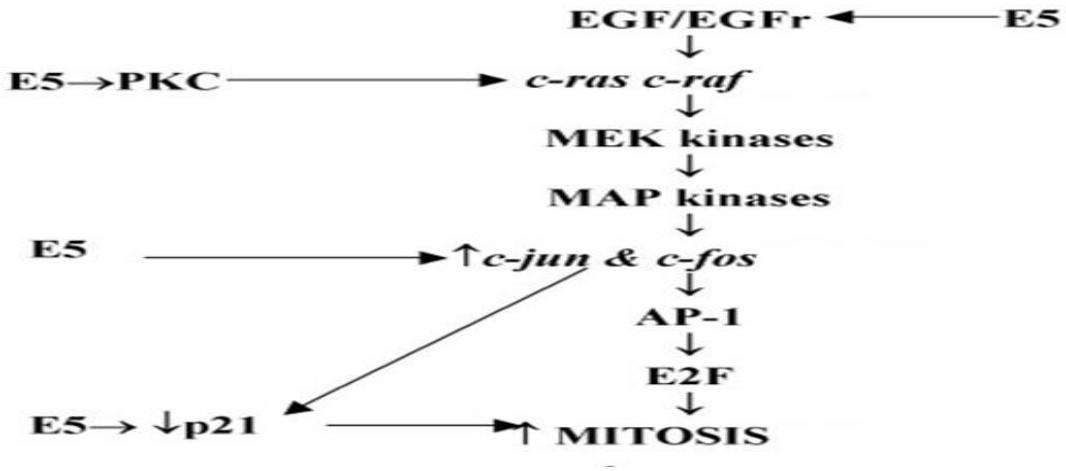

Figure 2-3. Points at which HPV-16 E5 affects the epidermal growth factor signal transduction pathway.

HPV-16 E5 stimulates c-ras, causing c-RAF to attach to plasma membranes, activating enzyme cascades through the MEK and MAP kinases, which in turn migrate to the nucleus to phosphorylate c-fos transcription factors. E5 can interdict this pathway via:

(i) Induction of protein kinase C which activates c-RAF.

(ii) Initiation of c-jun and c-fos and junB transcription.

(iii) Repression of p21 expression: a cyclin-dependent kinase inhibitor which causes pocket protein phosphorylation, the release of E2F and cyclins A, B, and E synthesis.

(Source: Cancer Cell International 2006, 6:19 http://www.cancerci.com).

2.7.7 Early Protein 6.

The major immortalizing proteins of the oncogenic hrHPV are oncoproteins E6 and E7. The transforming and immortalizing capacity of hrHPV and not HPV genotypes to cervical carcinoma is correlated with the activity of E6 and E7. Early protein 6 is a polypeptide of approximately 150 amino acids with a conserved C-X-X-C motif to form two zinc fingers (Chen *et al.,* 2020; Shuling *et al.,* 2020). It has been demonstrated to degrade with Tumor Suppressor Gene (TSG) p53 in-vivo via the APT-dependent ubiquitin pathway. These explain the fact that low levels of wild-type p53 are detected in hrHPV positive cancer-cell lines and E6-immortalised-mammalian cells. There's a low affinity of E6 interaction with HPV hence no degradation of P53 as compared to hrHPV. E6 also interacts similarly with other proteins in the cell cycle to cause

disruption resulting in the arrest of apoptosis and carcinogenic progression. These proteins are ERC-55, paxillin, HDLG, MCM7, and Interferon Regulatory Factor (IRF). E6 is involved in the transformation and immortalization of mammary epithelial cells that have an aberrant retinoblastoma tumor suppressor (RB) pathway by down-regulation of CDK/cyclin inhibitor p16-3) (Shuling *et al*., 2020).

2.7.8 Early protein 7.

Early protein 7 (E7) is a phosphoprotein of approximately 100 amino acids and is detected at a variety of locations within the cell; cytoplasm, nucleus, and nucleoli. The N-terminal region of E7 comprises two regions known as conserved domains 1 and 2 (CD1 and CD2). Conserved Domain 2 contains protein binding L-X-C-X-E motif. It is a short motif that mediates the interaction with the retinoblastoma tumor suppressor protein (pRB) and its related proteins p107 and p130 (Chen *et al.,* 2020). These proteins are linked to cell cycle control and are degraded by the HR-HPV E7 oncoprotein (Zhang *et al.,* 2018). Protein RB normally binds and inactivates E2F1-3 transcription factors, maintaining the cell in a quiescent state in the G0/G1 cell cycle phase. Stimulation of progression from the G1 to S phase allows the virus to efficiently use the cellular DNA replicating machinery to achieve viral genome replication. The HR-HPV E7 proteins target the active dephosphorylated form of pRB for proteasomal degradation. This activates E2F-regulated transcription, which includes the transcription of cyclin A and cyclin E. These cyclines are positive regulators of cyclin-dependent kinases (CDKs) inducing cell cycle progression and sustained proliferative signalling (Shuling *et al*., 2020).

Figure 2-4 shows a productive HPV life cycle to the development of cervical cancer. The left image shows the proposed route of HPV infection via micro-injuries in the mucosal epithelium. There's an exceedingly controlled expression of a few viral gene products, where early oncogenes, by focusing on their substrates p53/pRb, advance ceaseless cell proliferation. There's a profoundly controlled expression of a few viral quality items, where E6/E7 oncogenes, by focusing on their particular substrates p53/pRb, advance persistent cell expansion. This permits the infection to open up its genome, complete its life cycle, and eventually deliver new virions where E6 and E7 proteins are exceedingly over-expressed (Chen *et al.*, 2020).

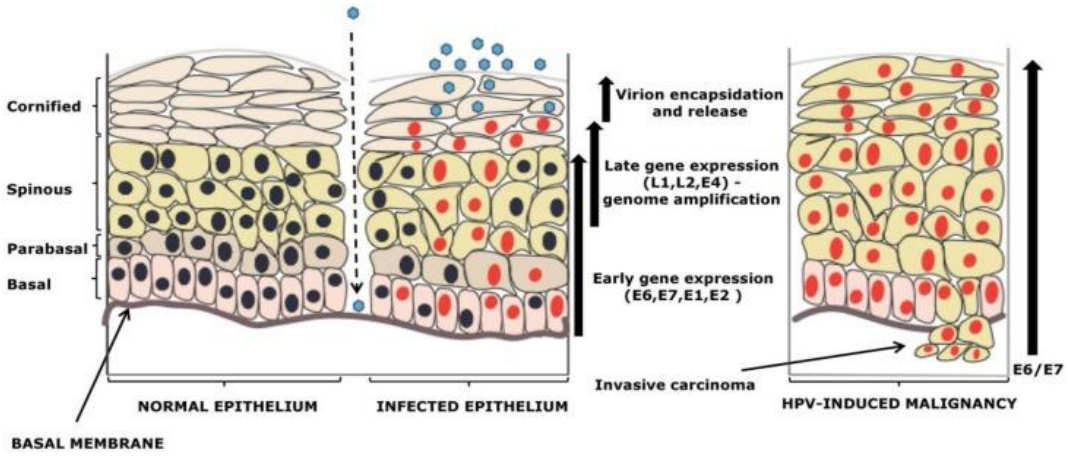

Figure 2-4. Productive Human Papillomavirus life cycle to cancer development.

Viral genome maintenance is facilitated by the expression of E6 and E7 together with E1 and E2. Increased levels of the viral replication proteins facilitate viral genome amplification. The expression of L1 and L2 allows for the formation of infectious virions (virus assembly) (Source: Thomas *et al.*, 2008; Kim *et al*., 2021).

2.8 Cervical screening.

Cervical cancer incidence and mortality can be reduced by screening although most eligible women of reproductive age have either never been screened or are screened late (Bruni *et al.*, 2021; National Cancer Screening Guidelines, 2019). Only 5% of women

in low-income countries undergo cervical screening while in economically advanced countries the screening rate is above 70% (Bruni *et al.*, 2021; Chan *et al.*, 2019; Pineros *et al.*, 2018) (Figure 2-5).

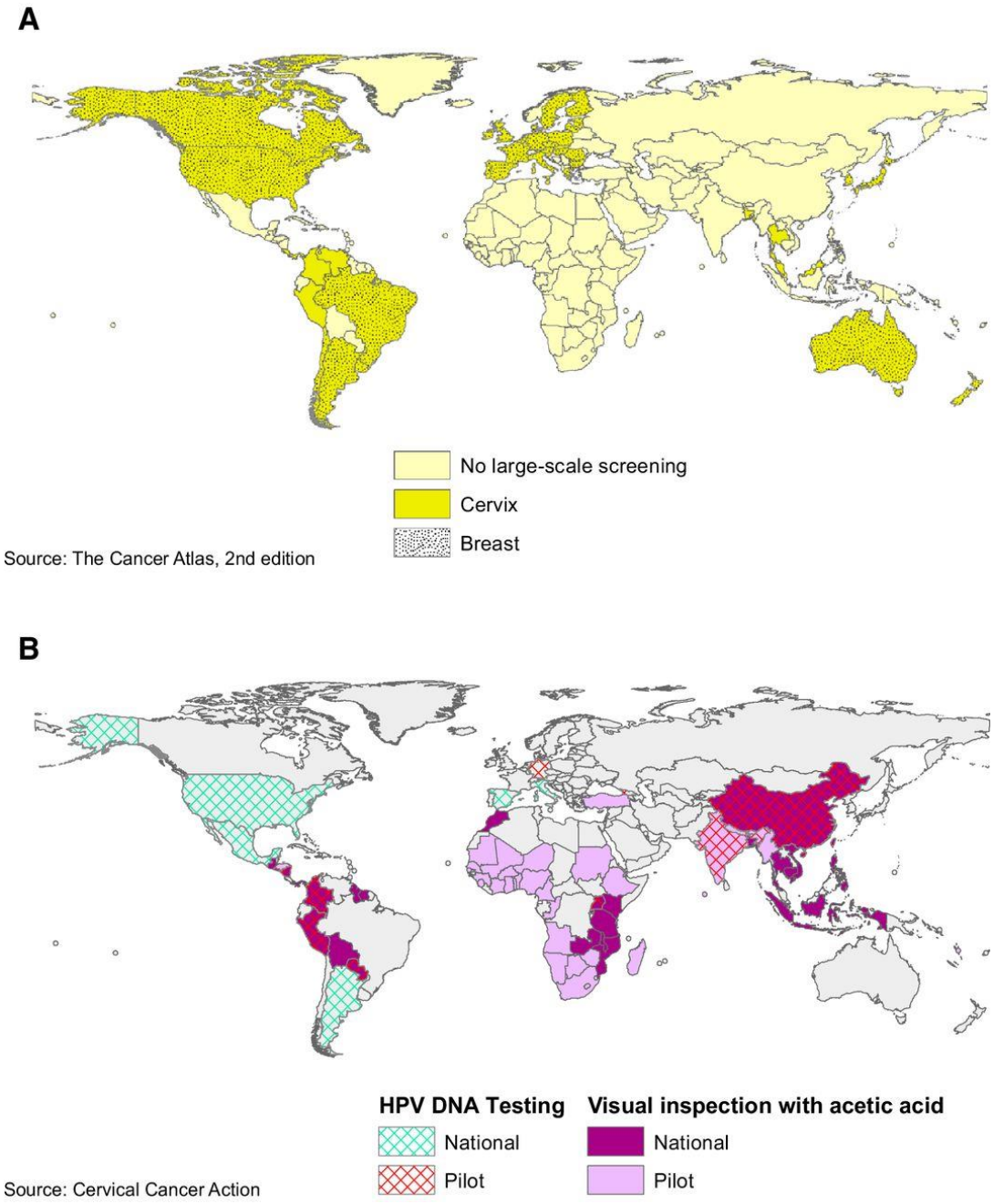

Figure 2-5. Global HPV testing.

Part A shows Global cervical and breast screening coverage while Part B shows cervical screening by DNA detection and Visual inspection by Acetic acid initiatives at the national level (Source: Bruni *et al.*, 2021).

A great screening test is satisfactory to women and suppliers, precise, reproducible, secure, and accessible. Currently, MOH, Kenya prioritizes cervical screening for women aged 25–49 years (Akanda *et al.,* 2020; Manduku *et al.,* 2017). The age range and screening intervals depend on resources. Countries with effective screening programs have significantly reduced the cervical cancer burden by 80% over the last four decades (Bruni *et al.,* 2021). The screening program includes the testing of an asymptomatic "health" and symptomatic populace. Cervical screening points to identify pre-invasive cancers and anticipate mortality from cervical cancer by treating pre-cancerous lesions (McClymont *et al.,* 2020; Chan *et al*., 2019). In the Kenyan rustic setting, an approach that meets the expressed characteristics and guarantees at the slightest 70% scope of the qualified populace is set with the respective algorithm (Akanda *et al.,* 2020; Manduku *et al.,* 2017).

2.8.1 Human Papillomavirus DNA assay.

Tests for HPV detection may not continuously give genotype-specific information. The HPV algorithm (Figure 2-6) is provided by MOH Kenya and WHO for HPV detection and typing and requires careful standardization from sample collection, DNA extraction, and testing. Two HPV molecular testing procedures are available; those that depend upon the use of signal amplification to detect the targets and those that depend on target amplification itself (WHO *Cervical Screening Guidelines*, 2021).

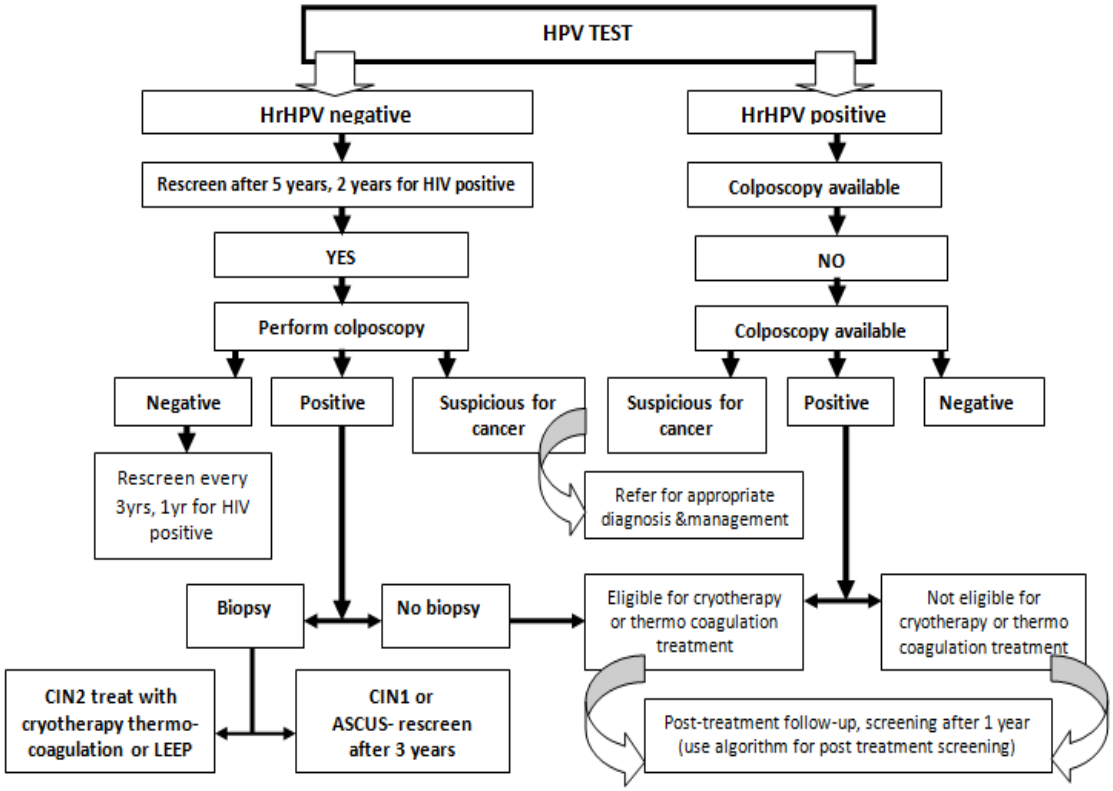

Figure 2-6. Human Papillomavirus test algorithm.

(Source: WHO *Cervical Screening Guidelines*, 2021).

i) Signal amplification.

The foremost widely-used signal amplification method for HPV is the Digene® Hybrid Capture® 2 (HC2) HPV Test (Qiagen, USA). Here, alkaline lysis is used to release sample DNA for hybridization to the Ribonucleic acid (RNA) probes complementary to 13 hrHPV genotypes; 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (Van Doorslaer *et al.*, 2018).

The presence of RNA-DNA hybrids indicates the presence of HPV DNA since the complex does not occur naturally. The complex is captured by a specific monoclonal antibody bound to microtitre plates. Non-specifically bound materials are removed by washing then the DNA-RNA hybrids are detected with the same monoclonal antibody

conjugated to alkaline phosphatase. The chemiluminescence detection system is used to detect and quantitate localized enzymes. The assay does not distinguish specific HPV genotypes and it is commonly used in clinical diagnosis (WHO *Cervical Screening Guidelines,* 2021).

ii) Target amplification.

Target amplification systems most commonly use a Polymerase Chain Reaction (PCR). Type-specific PCR assays have been developed not only to detect but also to quantitate the presence of a single HPV genotype for epidemiological studies of different HPV genotypes' infections. This is achieved by amplifying a portion of the HPV genome that is relatively conserved. The L1 region is most conserved among all HPV genotypes and it's mostly targeted in the assay. The assay uses a set of degenerate primers, MY09/MY11, and results in a 450 bp amplicon. This enables typing-determination by subsequent molecular techniques like Restriction Fragment Length Polymorphism (RFLP), DNA sequencing, and Reverse Blotting Hybridization (RBH) with genotype-specific oligonucleotide probes. The degenerate MY09/MY11 primers were subsequently replaced by primers, PGMY09/11, to improve specificity and sensitivity (WHO *Cervical Screening Guidelines*, 2021). Additional primer sets targeting the same region of L1 are widely used. These include GP5+/6+ producing an amplicon of ~160 bp and SPF10, producing an amplicon of 65 bp. The PGMY09/11 system is marketed as the Research Use Only (RUO) Linear Array HPV Genotyping Test (Roche™), the SPF system is commercially available as INNO-LiPA HPV Genotyping v2 (Innogenetics™), and GP5+/6+ systems are marketed as RUO HPV Genotyping LQ

Test (Qiagen™) and Multiplex HPV Genotyping Kit - Multimetrix, Heidelberg (WHO *Cervical Screening Guidelines,* 2021; Labnet, 2009™).

iii) Human Papillomavirus culture.

The Organotypic Raft Culture Model System (ORCMS) has been used to demonstrate the full product life-cycle of HPVs in vitro. The culture involves spontaneously immortalized keratinocytes cell line Normal Immortal Keratinocytes (NIKS) or Human Foreskin Keratinocytes (HFCs) immortalized by telomerase to recapitulate the replicative viral life cycle. The NIKS are excellent in supporting the life cycle of several hrHPV α-genotypes such as HPV16, 18, 31, 45, and 58, while the immortalized HFKs have been utilized for studies on the life cycle of a few of the lrHPV genotypes such as HPV-11 (Jug *et al.,* 2021; Akanda *et al.,* 2020; Krug *et al*., 2019).

2.8.2 Papanicolaou smear.

The Pap smear was introduced in the 1940s by George Papanicolaou and his associate Herbert Traut in a study on vaginal cancer detection (WHO *Cervical Screening Guidelines,* 2021). As a screening test, the smears were used to detect both precancerous and cancerous changes in the endocervical canal of the uterus. The test has also been used to reveal cancers in the endometrium, vagina, and fallopian tubes. Pap smears identify atypical squamous cells or intraepithelial neoplastic lesions in the cervix (Jug *et al.,* 2021; Akanda *et al.,* 2020).

Cervical pathology is categorized as Atypical Squamous Cells of Undetermined Significance (ASCUS), atypical squamous cells that cannot exclude high-grade lesions (ASC-H), low-grade squamous intraepithelial lesions (LSIL), high-grade Squamous

Intraepithelial Lesions (HSIL), or cellular changes associated with inflammation, radiation or intrauterine contraceptive device (IUD) use. Infections and presence of microbial organisms are reported as *Chlamydia trachomatis* infection, fungal organisms morphologically consistent with *Candida*, the shift in flora suggestive of bacterial vaginosis, bacteria morphologically consistent *Actinomyces spp* or cellular changes consistent with *Herpes simplex virus* and/or *Cytomegalovirus* (Jug *et al.,* 2021; Krug *et al*., 2019; Onywera *et al*., 2019).

The test is highly specific, especially concerning high-grade SIL and cancers, with reported specificities ranging from 86 to 100%, even in low-resource settings (Manduku *et al.,* 2017. However, this sensitivity is hampered by the need for a cytologist to interpret the results, sample collection procedures, transport, and storage of the smears before the examination (Hawkes *et al*., 2020). Other errors may occur in reagent preparation, storage, and the competency of the cytologist. The high false-negative rate reported ranges from 13 to 70% as shown in a meta-analysis of well-controlled studies (Jug *et al.,* 2021; National Cancer Screening Guidelines, 2019) (Figure 2-7).

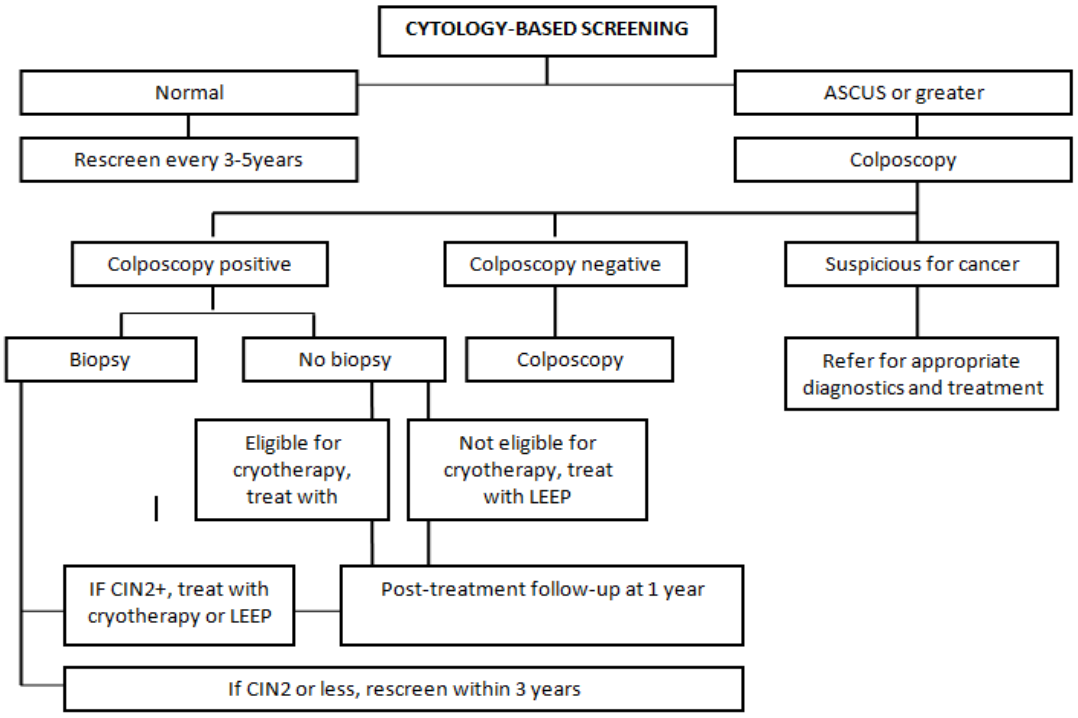

Figure 2-7. Cytology-based screening algorithm.

(Source: WHO *Cervical Screening Guidelines*, 2021).

2.8.3 Visual inspection with acetic acid / Lugol's iodine solution.

This is a two-step approach where Visual Inspection with an Acetic Acid solution (VIA) is performed followed by Lugol's Iodine solution (VILLI) which is based on the colour change in the transformation zone of the cervix. Squamous epithelium contains glycogen, while precancerous lesions and invasive cancer cells contain small or little glycogen. Squamous epithelium, being glycophilic, colours with Iodine to brown or dark mahogany. If the epithelium contains a lot of cellular proteins, acetic acid coagulates these proteins, which may obliterate the colour of the stroma. The resulting acetowhitening is seen distinctly against the normal pinkish colour of the surrounding normal squamous epithelium of the cervix (WHO *Cervical Screening Guidelines*, 2021). Immature metaplasia and anti-inflammatory lesions are partially glycogenated

and when decoloured, they appeared as scattered, ill-defined areas. Precancerous lesions and invasive cancer cells do not decolour with iodine since they are not glycogenated and appear as well-defined, thick, mustard saffron yellow areas. The advantages of VIA/VILLI are simplicity and the low cost of the test. The tests do not require specialized types of equipment or a cytologist (Jug *et al.*, 2021; Akanda *et al.*, 2020; Manduku *et al.*, 2017) (Figure 2-8).

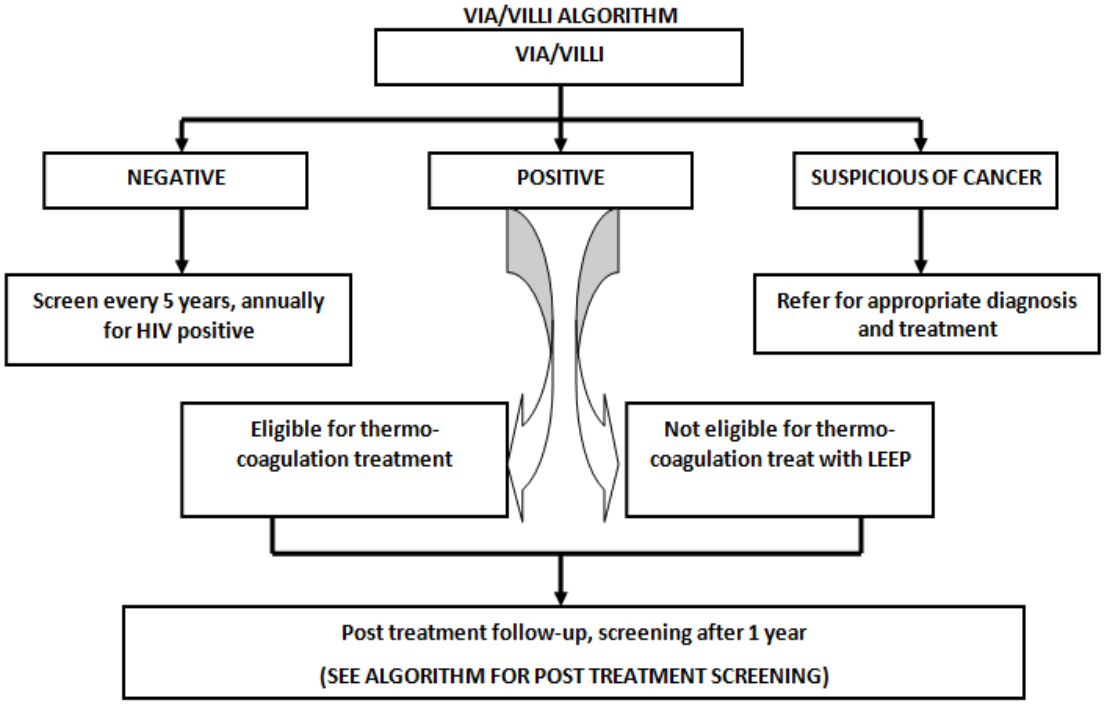

Figure 2-8. VIA/VILLI algorithm.

(Source: WHO *Cervical Screening Guidelines*, 2021).

2.8.4 Colposcopy.

Colposcopy involves the identification of precancerous and cancerous lesions through illuminated and magnified views of the cervix, vagina, vulva, or anus by the use of a colposcope (WHO *Cervical Screening Guidelines*, 2021).

Colposcopy of the cervix is utilized as an assessment of unusual cervical cancer screening tests after anomalous cytology and/or HPV testing. Colposcopic assessment is based on the finding malignant and premalignant epithelium have particular visual characteristics in terms of form, colour, and vascular patterns that are recognizable. The improved visualization of epithelial surfaces with colposcopy compared with gross visual examination improves the colposcopist's capacity to recognize normal and abnormal zones and to get coordinated biopsies (Jug *et al.,* 2021; WHO *Cervical Screening Guidelines,* 2021; Karagu *et al.,* 2017).

2.8.5 Cervicography.

This is a strategy where high-quality Colposcopic-type photos of the cervix are taken by paraprofessional medical personnel within the field and reported by specialists. Cervicography is restricted by its reliance on an expert, trained evaluator to translate the cervigram. The capacity to identify pre-malignant cervical outcomes is profoundly dependent on the assessors. Additionally, Cervicography has a high sensitivity for low-grade lesions, but sensitivity and specificity for high-grade intraepithelial lesions are lower than with ordinary Pap smears. It can be considered a complementary test to cytology, but its utility as an essential screening device is constrained (National Cancer Screening Guidelines, 2019).

2.8.6 Triage tests.

These include HPV DNA genotyping, cytology, VIA and colposcopy that may or may not include a biopsy for histological diagnosis. Some of these triage tests may be conducted sequentially e.g. cytology followed by colposcopy with biopsy (WHO *Cervical Screening Guidelines,* 2021).

2.8.7 Recommendations for screening special populations.

Kenya Ministry of Health guidelines for screening special populations like immunosuppressed especially HIV-positive women, pregnant, post-partum women, those with a history of total abdominal hysterectomies, vaccinated and those aged over 50 years are provided to all health facilities in the country. For immunosuppressed and/or HIV-positive women, cervical screening should begin immediately after diagnosis or at an age of twenty-five years or either way. This should be a lifetime engagement with a screening frequency of one year by VIA/VILLI or cytology technique or two years by HPV DNA screening technique. In pregnant women, cervical screening can be done in the first trimester but treatment for precancerous lesions should be avoided. A gynaecologist or obstetrician can undertake a biopsy in any trimester. In post-partum women, cervical screening can be done six weeks after delivery (Orang'o *et al.,* 2020).

Cervical screening ought to be suspended for those who had a total hysterectomy for benign causes without a history of gynaecological malignancy. Those with an intact cervix and a history of subtotal hysterectomy should proceed with scheduled screening as per the national algorithm. Women aged between 50-64 years ought to be screened for five years interim on an individualized premise utilizing HPV DNA and cytology methods (WHO *Cervical Screening Guidelines,* 2021). Cervical screening is not recommended for women aged over this age group. This is because it is not possible to reach the squamocolumnar junction since it is taken up into the endocervical canal in postmenopausal women (Orang'o *et al.,* 2020; Innes *et al.,* 2018). Women who had been immunized ought to get scheduled screening as per the national algorithm.

Human Papillomavirus DNA PCR co-testing with cervical Pap smear cytology is usually reflex testing in response to ASCUS results. HPV DNA PCR-positive women with negative Pap smear results have a high risk of cervical neoplasia and management of this may include repeat HPV and cytology testing at 12 months or HPV genotyping (Jug *et al*., 2021).

2.9 Human Papillomavirus vaccination.

Vaccine introduction has been one of the foremost effective public interventions for combating infectious diseases. Vaccines against HPV are now available. Cervarix™, a bivalent vaccine that provides immunity against HPV16 and HPV18, and Gardasil 4™, a quadrivalent vaccine against HPV genotypes 16, 18, 6, and 11 (Kann *et al.,* 2021; Wakwoya *et al*., 2020). The two vaccines are used in a three-dose schedule (Bogani *et al.,* 2020). Both vaccines have shown more than 90% efficacy (Chan *et al*., 2019; Perez *et al.,* 2018; Weyn *et al.,* 2016). The WHO recommends bivalent or quadrivalent immunization within the two-dose plan with a six-month interval between dosages for females less than 15 years including females 15 years or older during the second dose sexual orientation. The third dose is scheduled after the primary dosage in case the interim between the two dosages is shorter than five months and for fifteen years old or more for sexually active immunocompromised persons (Bogani *et al.,* 2020; Wakwoya *et al*., 2020; Perez *et al.,* 2018). The targets for vaccination in Kenya are young juvenile girls and boys before first coitus aged 9-13 years in upper primary school education. Gardasil 9™, a nanovalent HPV vaccine that provides immunity against HPV genotypes 16, 18, 6, 11, 31, 33, 45, 52, and 58 was affirmed by Food and Drug

Administration (FDA) in 2018 in the USA for persons aged 27 to 45 years (Bogani *et al.*, 2020; Mabeya *et al.*, 2018; Perez *et al.*, 2018) (Figure 2-9).

Characteristics	Bivalent 2vVPH	Quadrivalent 4vVPH	9-valent 9vVPH
Commercial Name producer	Cervarix™, GSK	Gardasil™, Merck	Gardasil 9™, Merck
Types of virus like particles (VLP)	16 18	6 11 16 18	6 11 16 18 31 33 45 52 58
Dose of L1 protein	20/20 µg	20/40/40/20 µg	30/40/60/40 µg 20/20/20/20/20 µg
Adjuvant	ASO4 (500 µg aluminum hydroxide, 50 µg 3-O-deacylated-4'-monophosphoryl lipid A)	AAHS (225 µg amorphous aluminum hydroxyphosphate sulfate)	500 µg AAHS
Licensed schedules	0, 1, 6 month 0, 6 month	0, 2, 6 month 0, 6 month	0, 2, 6 month 0, 6 month

Figure 2-9 Human Papillomavirus vaccine characteristics.

(Source: WHO *Cervical Screening Guidelines*, 2021).

The WHO urges countries to introduce cost-effectiveness strategic programs for HPV vaccination in consideration of their cervical cancer burden. By the end of 2019, more than 35% of countries had introduced a national-wide HPV vaccination schedule (Bogani *et al.*, 2020). Australia, the first country in the world to introduce the HPV vaccine in National Immunization Program in 2007, has consistently recorded HPV three-dose vaccine coverage among 70% of young girls aged 15 years and above since 2009. In the United States in 2014 recorded only 44% of girls and 25% of boys recommended three-dose vaccine coverage (Perez *et al.*, 2018; Weyn *et al.*, 2016).

Logistical difficulties and costs in resource-constraint countries have significantly delayed the implementation of the three-dose vaccine regimen. Where HPV vaccination

programs have been rolled out effectively, the benefits are already very apparent (Bruni *et al.,* 2021). Efforts are being made to roll out the HPV vaccine in other countries with budgetary help from the Global Alliance for Vaccine and Immunization (GAVI) (Perez *et al.,* 2018).

The government of Kenya, through the Ministry of Health, initiated a successful HPV vaccine pilot study in Eldoret located in western and Kitui located in the Eastern and Central regions, Kenya of the country. These studies have revealed low to full vaccine uptake among Kenyans due to distance to health facilities for the three doses and lack of knowledge on the vaccine. The Government then introduced a national HPV vaccine program in November 2019. This school-based vaccine roll-out is similar to HPV vaccination programs initiated in Lesotho, South Africa, and across sub-Saharan African countries like Rwanda, Tanzania, and Uganda (Mabeya *et al*., 2018).

2.9.1 Global vaccination programs challenges.

Global vaccination programs are faced with great challenges. Firstly, the HPV vaccines are prophylactic and therefore, those already infected who are the expansive larger parts of the populace remain at risk of cervical cancer (Kann *et al.,* 2021; Bogani *et al.,* 2020; Perez *et al.,* 2018). Secondly, resource-constrained nations have monetary challenges in introducing the vaccine and maintaining vaccination programs. Thirdly, HPV vaccines developed over a decade ago are highly likely to be less effective against the ever-mutating HPV genome. Novel helpful approaches to combat HPV infections are needed and an increased understanding of the HPV life cycle will help in distinguishing such approaches as viral replication (McClymont *et al*., 2020; Perez *et al.*, 2018).

2.10 Human Papillomavirus co-infection with Human Immunodeficiency virus.

Human Immunodeficiency virus causes cervical epithelial tight junction disruptions that favour HPV penetration into basal epithelial cells. Basal epithelial cells are HPV targets by the adjunction of HIV proteins (tat and gp120) with cytokines in cervical *ex vivo* models. HPV transcription and expression of HPV E-oncogenes and L capsid proteins are facilitated by HIV tat protein in cell cultures. HPV can escape the immune response by inducing a shift to T helper type (Th) polarization from Th1 to Th2, with a parallel move into production of cytokine and vice versa in later stages of HIV infection hence allowing persistence of HPV (Kann *et al.,* 2021; Stelzle *et al.,* 2020).

At the clinical level, HPV acquisition through mucosal surfaces is increased among HIV-infected persons than uninfected. HIV infection represses immunity hence increasing HPV viral load, concomitant diverse HPV genotypes, decreased HPV clearance and thus increased HPV persistence (Stelzle *et al.,* 2020; Chan *et al*., 2019). HIV-positive patients with lower immunity levels show frequent reactivation of latent HPV type/s infection. Lack of HPV clearance may lead to cervical cancer, in 15 to 20 years and less among immunocompromised individuals. HIV viremia is a predictor value for more opportunistic infections by activating immune defects instead of depletion of $CD4^+$ T-cell count, such as cytokine production alterations (Sias *et al.,* 2020; Stelzle *et al.*, 2020).

2.11 Knowledge, attitude, and perception of cervical screening and HPV vaccination.

Studies have reported that knowledge of cervical screening and HPV vaccination in less developed countries is low (Okunowo *et al.,* 2018). HPV can be prevented, controlled,

and monitored through an increase in awareness, practice, and change in attitude and perception of risky behaviour, screening, vaccination, and treatment (Bruni *et al.*, 2021; Mabeya *et al.*, 2018; Mutambara *et al*., 2017). However, most eligible women have either never been screened or are screened late and have low knowledge of HPV screening and vaccination. There is a lack of data on knowledge of HPV vaccination and acceptability among women in Kenya (Bruni *et al.,* 2021; Mabeya *et al.*, 2018) and across different populations in the world (Bruni *et al.,* 2021) before and after initiation into national programs (McClymont *et al.*, 2020; Perez *et al.*, 2018). Despite high acceptability, doubts surround the efficacy and safety of the HPV vaccine. Willingness to vaccinate against HPV in Kenya was reported to be high in Eldoret despite the limited knowledge of HPV and cervical cancer (Mabeya *et al.,* 2018).

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Research design.

This was a comparative cross-sectional study involving HPV infections in Human Immunodeficiency Virus (HIV) infected and non-infected women in Eastern and Central Regions, Kenya.

3.2 Study variables.

The dependent variables in this study were HIV serostatus and cervical HPV infection. Independent variables were residence, age, marital status, education attainment, and wealth status. Intervening variables were income level, the proximity of health facilities concerning the participant's residence, awareness of cervical screening and HPV vaccination, and exposure to cervical cancer risk factors. Risk factors focused in the study were multiple sex partners, current smoking habits, high parity (>3 children), early sex debut, hormonal contraceptives use, HIV positive status, recurrent warts and urethral tract infections (UTI).

3.3 Location of the Study.

The study was done in the Eastern part of Kenya; Isiolo, Kirinyaga, Meru, Tharaka-Nithi, and Embu Counties (Appendix 2). Study sites were Reproductive health clinics and Voluntary Counselling and Testing (VCT) centres in county referral hospitals in each county namely; Isiolo Referral Hospital in Isiolo County, Kirinyaga Level Five Hospital in Kirinyaga County, Meru Teaching and Referral Hospital in Meru County, Chuka Level Five Hospital in Tharaka-Nithi County and Embu Teaching and Referral Hospital in Embu County. All reproductive health clinics in these hospitals offer family

planning services, well-baby clinics, Pap smear tests, HIV tests, VIA/VILLI tests, and colposcopy. Patients requiring specialized treatment are referred to Kenyatta National Hospital, Nairobi-Kenya.

3.4 Study Population.

Participants were women aged 18-46 years from Eastern Kenya attending HIV Voluntary Counselling and Testing (VCT), Family Planning (FP), and Antenatal Clinic (ANC) centres.

i) Inclusion criteria.

Included were:

- women aged 18-46 years; are the most sexually active age group with a higher risk of contracting HIV and HPV (Karagu *et al.,* 2017).
- those who were willing to consent to the study,
- residents of respective counties; Meru, Isiolo, Kirinyaga, Embu, and Tharaka-Nithi,
- women due for routine HPV screening as part of the Kenya National Cervical Cancer, Program (KNCCP) for HIV-positive women.

ii) Exclusion criteria.

Excluded were:

- women with an eroded cervix,
- women who were pregnant,
- those with a history of ablative procedures for cervical disease in the last six months,
- women with a history of hysterectomy,

- mentally incompetent participants.

Women who were excluded from the study were referred for specialized cervical screening programs provided by MOH, Kenya, or non-governmental organizations.

3.5 Sampling techniques.

A stratified sampling technique was used to obtain five strata based on county of residence; Embu, Meru, Isiolo, Kirinyaga, and Tharaka-Nithi. Simple random sampling was then used to recruit the required number of participants in each stratum presenting with cervical abnormalities.

3.6 Sample size determination.

The sample size was obtained through a calculation using Chow *et al.,* (2017) equation using cervical abnormalities cases reported in Eastern and Central Regions, Kenya in 2014.

$n = (Z_{\alpha/2}+Z_{\beta})^2 * (p_1(1-p_1) + p_2(1-p_2)) / (p_1-p_2)^2$

$n = (1.96)^2 * (4(1-4.0) + 9.25(1-9.25)) / (4.0-9.25)^2$

n = 350 +/- 5% confidence interval for HIV-infected and non-infected women.

Where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g., for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_{β} is the critical value of the Normal distribution at β (e.g., for a power of 80%, β is 0.2 and the critical value is 0.84), p^1 is the proportion (4%) of women diagnosed cervical abnormalities in Eastern and Central Regions, Kenya by VIA/VILLI method from January to December 2015 and p^2 are the expected HPV prevalence (9.25%) among HIV-infected and non-infected women.

3.6.1 Sample size distribution.

Sample distribution was dependent on the reproductive health clinic's report of 27 cases of cervical abnormalities in Eastern and Central Regions, Kenya counties for 6 months; starting in December 2017 by VIA/VILLI method. Embu County reported 30.0% of the cases, Meru 22.2%, Isiolo and Kirinyaga 18.5%, and Tharaka-Nithi 10.8%. Based on this report, cases and an equal number of control participants were distributed as follows: Embu 41, Meru 30, 1siolo 26, Kirinyaga 26, and Tharaka Nithi 15 participants.

3.7 Questionnaire.

A questionnaire was developed in English (Appendix 10) and translated into the Kiswahili language (Appendix 11). Certificates of translation and back translation were obtained (Appendix 4-5) before the questionnaire was reviewed at KEMRI Scientific Ethical Review Unit (Appendix 12), NACOSTI (Appendix 14), Kenyatta University Graduate School as well as respective County Health Boards and hospital administration of all study sites (Appendix 15-20). It was pre-tested in a group of 10 women in Embu (5/10) and Isiolo (5/10) to affirm clarity.

Four sections of the questionnaire were: 1) Section A, focuses on social demographic, and economic factors. 2) Section B focuses on factors associated with HPV oncogenesis that include steroid hormonal contraceptive use, multiple sex partners, sex debut, smoking habits, history of infection by warts, HIV, recurrent urinary tract infection (UTI), and Herpes. 3) Section C had KAPP questions on HPV screening, vaccination, and cervical screening techniques; HPV DNA PCR test, Pap smear test, and VIA/VILLI test. Cervical screening and vaccination knowledge were reported as yes/no, attitude as

willing/not willing, practice as done/not done, and perception as important/not important. 4) Section D had questions on knowledge of cervical cancer, its signs, and symptoms, frequency of cervical screening, and relative history of a cervical cancer diagnosis. After filling out the questionnaire, participants underwent an HIV test to confirm their status.

3.8 Human Immunodeficiency virus determination.

Kenya National HIV testing algorithm was applied as baseline and confirmation of HIV serostatus of case and control groups of participants. Three millilitres of venous whole blood was taken for serological HIV testing. Alere Determine®HIV-1/2 test by Abbort Co. was used as baseline screening while the First Response® HIV 1-2-0 card test by Premier Medical Corporation® was used as a confirmatory test. The tie-breaker test was done using the Uni-Gold™ Recombigen® HIV-1/2 by Trinity Biotech. Confirmed HIV-negative blood samples were included as a negative control (Musyoki *et al.,* 2018). HIV test was then followed by an examination of the cervix and a collection of cervical samples.

3.9 Collection and storage of cervical exfoliated cell samples.

External genitalia was examined while the participant lay in a lithotomic position. A speculum was rinsed in warm water, lubricated, and used to locate the cervical opening (os) under direct light. The mucus plug-in os was removed and wiped to ensure sufficient cells were collected. A cervical broom (Dacron cervical broom; Digene Corporation, Silver Spring, Maryland STM™) was softly rotated five times at 360 degrees to exfoliate cells from the region of the transformation zone, squamocolumnar

junction, and endocervical canal. Exfoliated cells were spread evenly and fixed immediately on a clean glass slide. The broom bristles were then dipped into aqueous Minimum Essential Media (MEM) and the broom handle snapped so that it remained in the tightly closed vial and stored at 1-4° C (WHO *Cervical Screening Guidelines,* 2021; Sias *et al*., 2020; Elmi *et al.*, 2017; Orang'o *et al.,* 2017;).

3.9.1 Cervix visual inspection.

A two-step approach was applied where visual inspection with acetic acid and Lugol's iodine solution (VIA/VILLI) was performed as per the National cervical VIA/VILLI test algorithm (Karagu *et al.,* 2017; WHO *Cervical Screening Guidelines,* 2021). VIA results are based on the colours taken up by the cervical transformation zone where acetowhitening is distinct when compared to the pinkish squamous epithelium of the cervix. Results were reported as either positive or negative.

3.9.2 Cytology.

A standardized protocol available in the laboratories for cytopathology requested Pap smear staining, and examination was followed for detection of nuclei and cytoplasm cytological changes following HPV infection (Jug *et al.,* 2021; Klug *et al.,* 2018). Cytopathologists supervised by a pathologist at Embu and Meru Hospitals were required to fill a pathology synoptic reporting form using the Bethesda 2001 guidelines for reporting slides using a binocular microscope. Slides were later transferred to Kenya Medical Research Institute (KEMRI) for examination by a pathologist to ensure the quality of cytology results. Pap smear results were classified as normal or abnormal (ASCUS, CIN1, CIN2, CIN3, or ICC). Results on cervical infections such as

candidiasis, cervicitis, trachomatis, and bacterial vaginitis were obtained from the pathologist's reports.

3.9.3 Histology.

Cervical biopsies were collected within 1-2 weeks following abnormal Pap smear results (LSIL, HSIL, ASC-H, and AGC). Samples for histological analysis were collected in formalin. Macroscopic description of the specimen and the tissue components present, i.e. ectocervix, endocervix, transformation zone, and isthmus were recorded. A cytology report accompanied the samples to allow the pathologist to determine the correlation between cytological and histological findings. Reports on all grades of squamous and/or glandular intra-epithelial neoplasia were made. Pathologist report included graded invasive lesions, koilocytosis and koilocytosis-associated changes. Grading included Carcinoma-in-Situ (CIS), Invasive cervical cancer (ICC), or Cervical Intraepithelial Neoplasia (CIN); CIN1, CIN2 or CIN3 depending on the abnormal-epithelium thickness of ⅓, ⅔, or entire thickness respectively) (Wilbur and Nayar, 2015). The cytological report was used as a reference in the histology report and included all pathological lesions (neoplastic and non-neoplastic). The pathologist's report included a biopsy that failed to reveal the source of the abnormal cells in a smear. Consideration was made to differentiate between a biopsy that was technically adequate but failed to identify a lesion and a technically inadequate biopsy. (Park, 2020; Orang'o *et al.,* 2020; Elmi *et al.,* 2017).

3.9.4 Human Papillomavirus DNA detection by Polymerase Chain Reaction.

All samples stored for HPV DNA detection were analysed in a 20-sample batch by the following procedure:

i) Preparation of lysis master mix.

Mastermix was prepared by mixing 240µl of VDR Lysis Buffer, 8µl of Carrier RNA, and 280µl of Isopropanol per sample volume in 96-well format HighPrep™ Viral DNA/RNA Lysis kit, MagBio Genomics, Inc. US/Canada.

ii) Human Papillomavirus DNA Extraction.

Cervical brushes stored in universal bottles at 1-4ºC were vortexed for five minutes then centrifuged at 100r/min for five minutes. HighPrep™ Viral DNA/RNA Lysis kit, MagBio Genomics, Inc. US/Canada was used where 528µl of the lysis-master mix was transferred to each sample well. The sample was allowed to attain room temperature, and then 200µl of the sample deposit was transferred into each sample well and mixed by vortexing for one minute.

Ten microlitres of MAG-S1 particles and 10µl Pro K Solution were added to each well and mixed by shaking for five minutes. The sample plate was put on the magnetic separation device for ten minutes to magnetize the HighPrep™ MAG-S1 particles. With the plate on the magnetic separation device, the supernatant was separated and discarded by pipetting then the plate was removed from the magnetic separation device. 400 µl HSW Buffer was added to each sample and pipette-mixed 15 times to resuspend the HighPrep™ MAG-S1 particles. The sample plate was placed back on the magnetic separation device for five minutes or until the magnetic beads cleared from the solution. With the plate on the magnetic separation device, the supernatant was removed and

discarded by pipetting. The plate was then removed from the magnetic separation device then 500µl of 70% ethanol was added to the sample, and pipette-mixed 15 times to resuspend the MAG-S1 particles then placed back on the magnetic separation device and for five minutes to clear the magnetic beads from the solution. The supernatant was then removed by pipetting. This step was repeated twice to obtain excellent results.

At room temperature, beads were dried by incubating for ten minutes with the plate still on the magnetic separation device. The plate was then removed from the magnetic separation device and 50-100µl nuclease-free water was added to each well and pipette-mixed 25 times to completely resuspend the MAG-S1 magnetic particles and later incubated at room temperature for ten minutes. The sample plate was placed back on the magnetic separation device for five minutes until the magnetic beads cleared from the solution. The eluate (cleared supernatant containing the DNA) was transferred to a new microplate for storage at -20°C.

iii) Polymerase Chain reaction.

HPV detection was achieved by amplification of HPV L1 portion of the genome that is relatively conserved through L1 consensus nested PCR in the ABI thermocycler Model 9600 supplied by Applied Biosystems™. HPV consensus primary forward primer PGMY09 (GCACAGGGACATAACAATGG) and reverse primer PGMY11 (CGTCCCAAAGGAAACTGATC) that target 450 base pair (bp) region in the L1 ORF in were used. Secondary primer sets targeting the same region of L1, forward MGP5+ (ACGTTGGATGTTTGTTACTGTGGTGGATACTAC) and reverses, MGP6+ (ACGTTGGATGGAAAAATAAACTGTAAATCATATTCCT) were used to produce

shorter amplicon of ~160 bp (WHO *Cervical Screening Guidelines*, 2021; Jug *et al.*, 2021; Krug *et al*., 2019). Primer sets were obtained from Iqaba Biotech™.

Five micro–Molar PGMY09 primer working stock was prepared by adding 50µL PGMY09 100µM primers to 350µL of nano-pure water (1ml total volume). 5µM working stock PGMY11 primer was then prepared by adding 50 µL each 5 biotinylated PGMY11 100 µM primers to 750µL molecular biology-grade water (1mL total volume). They were later distributed each 5µM working stock in 45–90µL aliquots and stored at -20°C (Jug *et al.*, 2021).

In the primary PCR, 5µl of the extracted DNA was amplified in a reaction mix containing 1X PCR buffer 2.0 mM MgCl2, 500nM forward primer MY09, 500nM reverse primer MY11, and 100µM of each dNTPs and 0.13µl of *Taq* polymerase enzyme. After an initial denaturation at 95ºC for four minutes, the reaction was cycled 30 times at 95ºC for twenty seconds, 56ºC for forty seconds, and 72ºC for two minutes. The final extension was done at 72ºC for seven minutes (Wai *et al.*, 2020). In the nested PCR, 5µl of the primary PCR product, 2.0mM MgCl2, 500nM of MGP5+, 500nM of MGP6+, and 400µM of dNTPs and 0.13µl of *Taq* polymerase enzyme was used. The cycling conditions were as follows; an initial denaturation at 95ºC for four minutes, then 95ºC for 20 seconds, annealing at 60ºC for 40 seconds, and extension at 72ºC for seven seconds. The final extension was at 72ºC for seven minutes. After the nested PCR, a 5µl aliquot of the PCR product was mixed with 1µl of 6X loading dye and loaded onto a 2% agarose with 2µl ethidium bromide gel alongside a 100 bp ladder for electrophoresis. The presence of the expected 160bp amplicon was considered positive

for HPV DNA PCR. Positive control of known CIN2 samples and negative control of distilled water were incorporated in all primary and secondary primer PCR runs (Hawkes *et al.,* 2020; Wai *et al.,* 2020).

3.9.5 Gel electrophoresis.

Tris-Borate-EDTA (TBE) 10X was prepared by dissolving 162 g Tris base, 50 g boric acid, and 9.5 g EDTA in sterile, molecular biology-grade water to a volume slightly less than one litre. The pH of the solution was adjusted to 8.8 by adding Hydrochloric acid. The final volume of one litre was obtained by adding molecular biology-grade water. The solution was stored at room temperature (WHO *Cervical Screening Guidelines,* 2021; Surriabre *et al.,* 2020).

Tris-Borate-EDTA 5X was later prepared by diluting 10X TBE in molecular biology-grade water (100 mL of 10 X TBE to a final volume of 2 L) where 200mL was used to set an agarose gel and the other volume to fill the electrophoresis tank. Four grams of agarose powder were added to 200ml of 0.5X TBE and then microwaved to boil. It was then cooled for fifteen minutes at 60°C in a thermostatic water bath after which 2µL Ethidium bromide was added. The mixture was poured into a 14-well combs gel electrophoresis apparatus. When the gel cooled and solidified, electrophoresis buffer (0.5 X TBE) was added to the electrophoresis chamber to cover the gel by one centimetre. The combs were then carefully removed (WHO *Cervical Screening Guidelines,* 2021; Omire *et al.,* 2020).

The workspace was prepared by covering it with a clean absorbent disposable laboratory pad and PCR equipment setup was ensured. Following PCR, the microplate in the cool rack was transferred to the workplace where 4µl of loading buffer was put in the reagent reservoir and 10µL was transferred into each sample using a 12-well multi pipette, mixed evenly by up and down pipetting. 10µl were then deposited into the 12 central wells of the Agarose gel. Three microlitres of the ladder were placed into each first column whereas positive and negative controls were included in the second and last column of the agarose gel respectively. The cover was placed on the electrophoresis apparatus, and electrodes were connected appropriately before turning on the power. Electrophoresis was allowed to run at 105watts constant power until bromophenol blue was one centimetre to the edge (Omire *et al.,* 2020; Wai *et al.,* 2020 Surriabre *et al*., 2019).

3.9.6 Gel Ultra-violet visualization.

The gel in a support tray was removed from the electrophoresis apparatus and placed on a UV transilluminator. Under UV light, a picture was taken using the electronic camera, medium aperture (5.6–8), ~600ms, and time adjusted to give a faint clear contrast. Visualization of a 450bp PCR product band in the MY09/MY11 lane and/or a 160 bp band in the nested PCR lane on the agarose gel provided evidence of HPV DNA in the sample, pending genotyping with direct DNA sequencing as a means of final validation (Jug *et al.,* 2021; Omire *et al.,* 2020). Digital pictures were labelled, saved as a .jpg file, and printed.

3.9.7 Human Papillomavirus DNA sequencing.

DNA product for Circle sequencing was amplified in ABI-thermocycler Model 9600 (Applied Biosystems) by 20 reaction cycles of 1µL positive PCR-product, 1µL of 5µM MGP6+ primer, 1µLBigDye® Terminator, 3.5µL buffer (5x), 13.5ml nanopore water according to the protocol. Sep column (Princeton Separations, Adelphia, NJ) was used for dye-terminator clean-up followed by sequencing in ABI3130 four-capillary Genetic Analyzer.

Sequences were edited with CHROMAS software Version 2.4.3 and then blasted in NCBI http://blast.ncbi.nlm.gov/blast.cgi. HPV genotype sequences with unique divergence were phylogenetically analysed and referenced from GenBank. Representative sequences and their references in the input file underwent multiple alignments with CLUSTAL W in MEGA X software. The Tamura-Nei model was used to infer evolutionary history. Neighbor-Joining and BioNJ algorithms were used to construct initial trees for the heuristic search of the matrix of pair-wise distances by the Maximum Composite Likelihood (MCL) method by selecting the topology with a superior log-likelihood value. Eighty-six nucleotide sequences were involved while codon positions were 1st+2nd+3rd (Hawke *et al.,* 2020; Elmi *et al.,* 2020; Surriabre *et al*., 2019; Menon *et al.,* 2016).

3.9.8 Disposal of the laboratory samples and used consumables.

Electrophoresis gel was discarded in a jar with distilled water and activated charcoal for later date incineration together with serum, and plasma, using HIV test kits pipette tips, laboratory pad, and used gloves. Used electrophoresis buffer was discarded in a drain

while the gel tray and combs were washed in tap water with detergent, rinsed with distilled water air-dried, and returned to storage (WHO *Cervical Screening Guidelines,* 2021).

3.10 Statistical analysis.

Statistical Package for the Social Sciences (SPSS) 17.0 for the windows package program was utilized for data load and computation. Categorical variables such as location, age, education, marital status, religion, income level, sex debut, parity, number of sexual partners, HPV prevalence, KAPP on risk factors, and infections associated with cervical cancer were presented as numbers and percentages. Pearson chi-square test was used to compute association in the distribution of social demographic and risk factors data with HPV prevalence, sequences, and HIV serostatus. Logistic regression analysis was used to compute the association between cervical screening results and HPV vaccination, KAPP, and social-demographic factors by Odds ratios (OR) and 95% confidence intervals (CI).

Income status was categorized according to World Bank's (2021) global aggregation and comparison. World Bank poverty lines are set at $1 and $2 per. day (more precisely $1.08 and $2.15 in 1993 Purchasing Power Parity (PPP) terms). The current poverty line is US$1.90 per day in 2011 PPP). Individual income status was determined as low (US$1.90 per day), middle (US$1.90 PPP - US$5.50 PPP per day), or high (over US$5.50 PPP per day). Religion was categorized as Christians and Muslims since they were the only religious affiliations reported (Stover *et al.,* 2021).

Confirmed CIN1+ biopsy samples were used as the gold standard to determine the diagnostic value of cervical screening methods used. Diagnostic accuracy of the VIA/VILI, Pap smear test, and HPV genotyping methods of cervical screening were determined using the formula:

$$\text{Diagnostic accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

Where TP: true positive, TN: true negative, FP: false positive, FN: false negative.

3.11 Ethical considerations.

The study was reviewed and approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (KEMRI-SERU) for ethical compliance (Appendix 12). Since humans were involved in this study, the following was observed:

3.11.1 Consent.

Participants were requested to consent by signing an informed consent form before recruitment (Appendix 7). The aim of the study and the procedures involved were explained to the participants during the recruitment stage (Appendix 6). Those who refused to participate were allowed to leave the research at any point they decide to do so

3.11.2 Confidentiality.

Participants' names were translated into codes to ensure confidentiality. Participants were informed of their results in privacy while the confidentiality of the subject's identity, test results, filled questionnaire forms, and samples was upheld. All these data

were stored under passwords and usernames. Names of participants, their contact addresses, and results remained in the custody of the Principal Investigator (PI) with restricted access only by him and co-investigators. The contact address of the PI was given in case participants had questions or concerns about the research.

3.11.3 Risks.

There was no harm, injury, or bleeding associated with cervical screening except slight discomfort that lasted for a few minutes. Slight pain was experienced during venepuncture where controlled bleeding was expected to collect blood for HIV testing.

3.11.4 Benefits of the study to participants.

Participants benefited from the study by undergoing cervical screening as primary preventive care against cervical cancer. They also benefitted by knowing their HIV status. Participants with abnormal cervical laboratory outcomes were referred for colposcopy at the respective referral hospitals while HIV-positive women were referred for antiretroviral therapy.

CHAPTER FOUR

RESULTS

4.1 Performance characteristics of cervical screening methods based on histopathology outcome among HIV-infected and non-infected women in Eastern and Central Regions, Kenya.

In total, 350 participants were eligible and approached to join the study where 317 agreed to fill out the questionnaire and undergo HIV test and cervical screening. Overall HPV prevalence by HPV L1 gene Polymerase Chain Reaction method was 26.5 % (84/317). Visual Inspection by Acetic acid and Lugol's Iodine (VIA/VILLI) method detected 25.6% (81/317) positive cytology following HPV infection while the Pap smear method detected 30.3% (96/317).

4.1.1 Overall Human Immunodeficiency virus serostatus of women participants aged 18-46 years attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

A total of 156 (49.2%) women participants aged 18-46 years were HIV negative and 161 (50.8%) were HIV positive as shown in Figure 4-1.

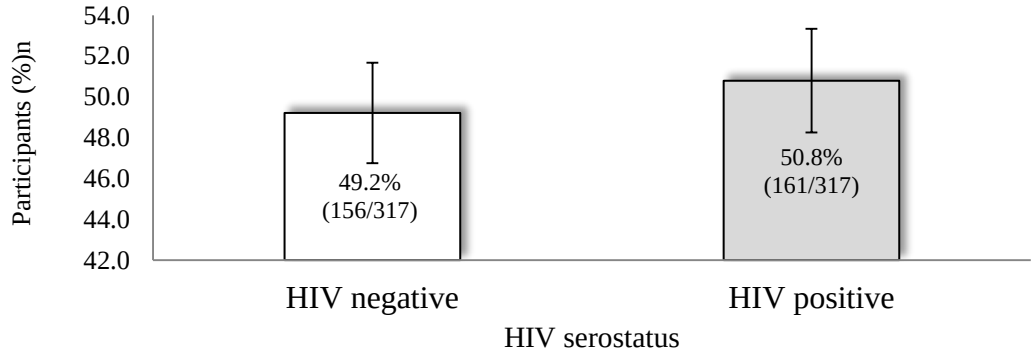

Figure 4-1. HIV serostatus of women participants aged 18-46 years from Kirinyaga, Embu, Meru, Isiolo, and Tharaka-Nithi Counties (N=317).

The graph shows the total number of HIV-infected and non-infected women participants. It does not represent HIV prevalence in the region since most HIV-positive participants were recruited purposively from HIV Voluntary and Testing Centers (VCT) and Reproductive Health Clinics. This enabled recruiting a target population of HIV-infected participants. The 5% Confidence

Interval overlap between HIV-positive and negative participants gives equal statistical representation in subsequent statistical analysis.

4.1.2 Association between cervical visual inspection outcome and Human Immunodeficiency Virus (HIV) status of participants.

A total of 81/317 (25.6%) participants had abnormal Visual Inspection by Acetic acid and Lugol's Iodine (VIA/VILLI) outcome while 236/317 (74.4%) had normal VIA/VILLI results. VIA/VILLI test results were significantly associated with HIV infection where the majority of those who showed abnormal cervical outcomes were HIV-infected [62/317 (19.6%)] ($p<0.001$) (Table 4-1).

Table 4-1. VIA/VILLI test results of HIV-infected and non-infected women seeking Reproductive-health care in Eastern and Central Regions, Kenya.

Diagnostic method		HIV serostatus		Total	p-value
		Negative	Positive	(n=317)	
VIAVILLI results†	Normal	137	99	236	<0.001**
		43.2%	31.2%	74.4%	
	Abnormal	19	62	81	
		6.0%	19.6%	25.6%	
	Total	**156**	**161**	**317**	
		49.2%	**50.8%**	**100.0%**	

***: the probability at the 0.001 level; †: Visual Inspection with Acetic acid/Lugol's iodine

4.1.3 Association between Pap smear cytology findings and Human Immunodeficiency Virus (HIV) status.

The majority of HIV-non-infected women had normal Pap smear test results [42.9% (136/317)] compared with HIV-infected women [26.8% (85/317)]. There was a significant association between abnormal cytology outcomes and HIV infection where the majority of women with LSIL [17/317 (5.4%)], HSIL [22/317 (6.9%)] and invasive cancer [5/317 (1.6%)] were HIV-infected ($p<0.001$) as shown in Figure 4-2. Increased cases of cervicitis [45/317 (14.2%] and candidiasis 47/317 (14.8%) were reported

among HIV-infected than HIV-non infected women. Therefore, HIV was a predictor of cervical dysplasia, infection, and inflammation.

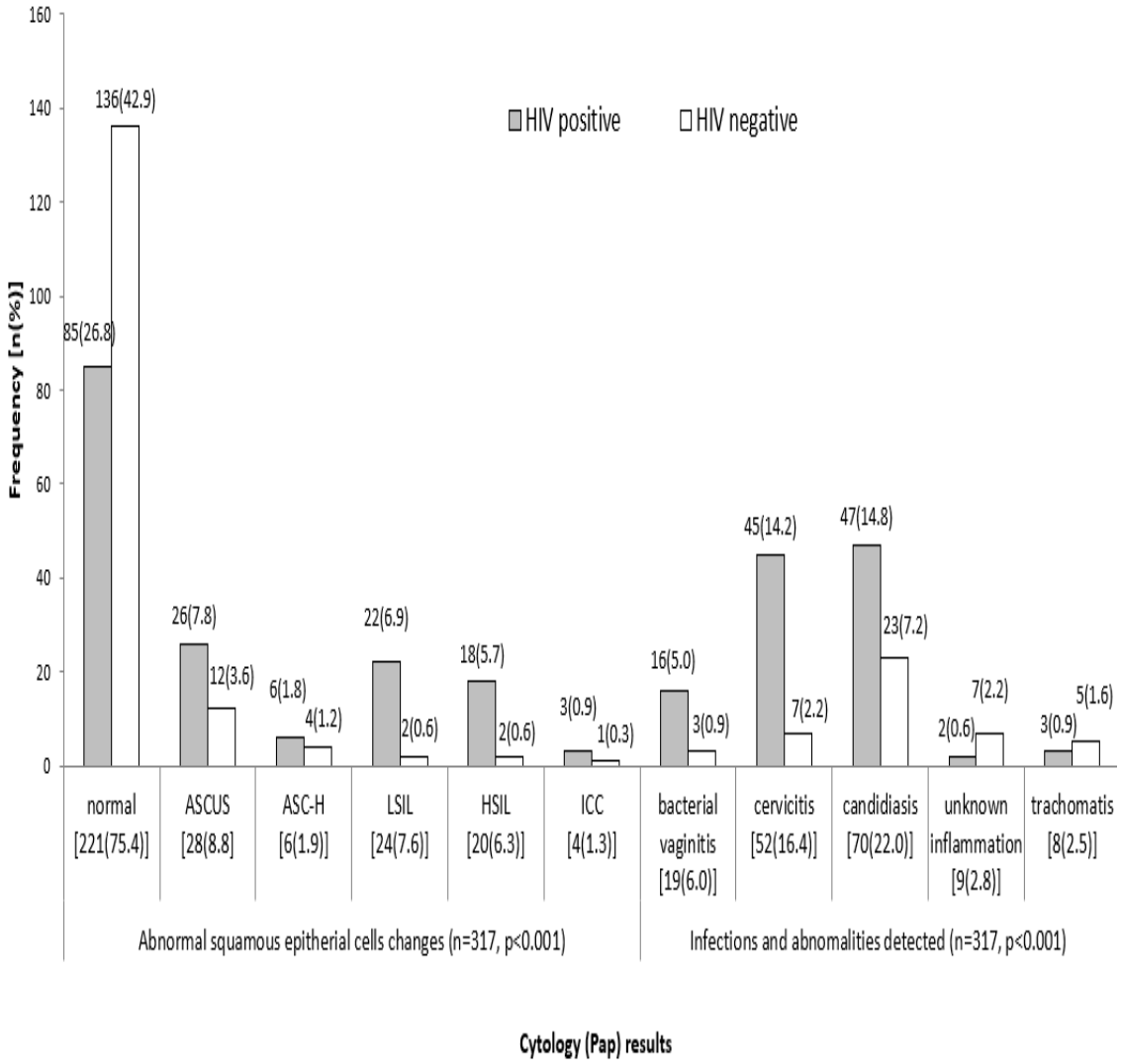

Figure 4-2. Pap smear cytological results of HIV-infected and non-infected women attending Reproductive-health Clinics in Eastern and Central Regions, Kenya.

The graph shows the Cytology Pap smear outcome of all HIV-infected and non-infected participants presented in two categories: Abnormal squamous epithelial changes (n=317), and infections and cervical abnormalities detected (n=317). In the first category, 221 participants had normal cytology while others had abnormal cytology reported as Atypical Squamous Cells of Undetermined Significance (ASCUS) and cannot exclude HSIL (ASC-H), Low-grade Intraepithelial Lesions (LSIL), High-grade Intraepithelial Lesions (HSIL), and Invasive Cervical Cancer (ICC). In the other category, cervical infections (bacterial vaginitis, candidiasis caused by *Candida albicans*, and trachomatis caused by *Chlamydia trachomatis*) are shown alongside unknown cervical inflammation.

4.1.4 Human Papillomavirus detection by Polymerase Chain Reaction in Eastern and Central Regions, Kenya.

Figure 4-3a shows the arrangement of electrophoresis gel with samples E09, E07, E04, E10, and E34 from Embu County (E). Positive samples for HPV DNA L1 protein and positive-control (+VE) sample produced visible electrophoresis bands at 160bp in secondary PCR (Figure 4-3b) and 450bp in Primary PCR whereas negative control (+VE), and HPV DNA L1 protein negative samples were produced no band. Positive and negative samples for HPV L1 protein are presented in Figures 4-3c-f.

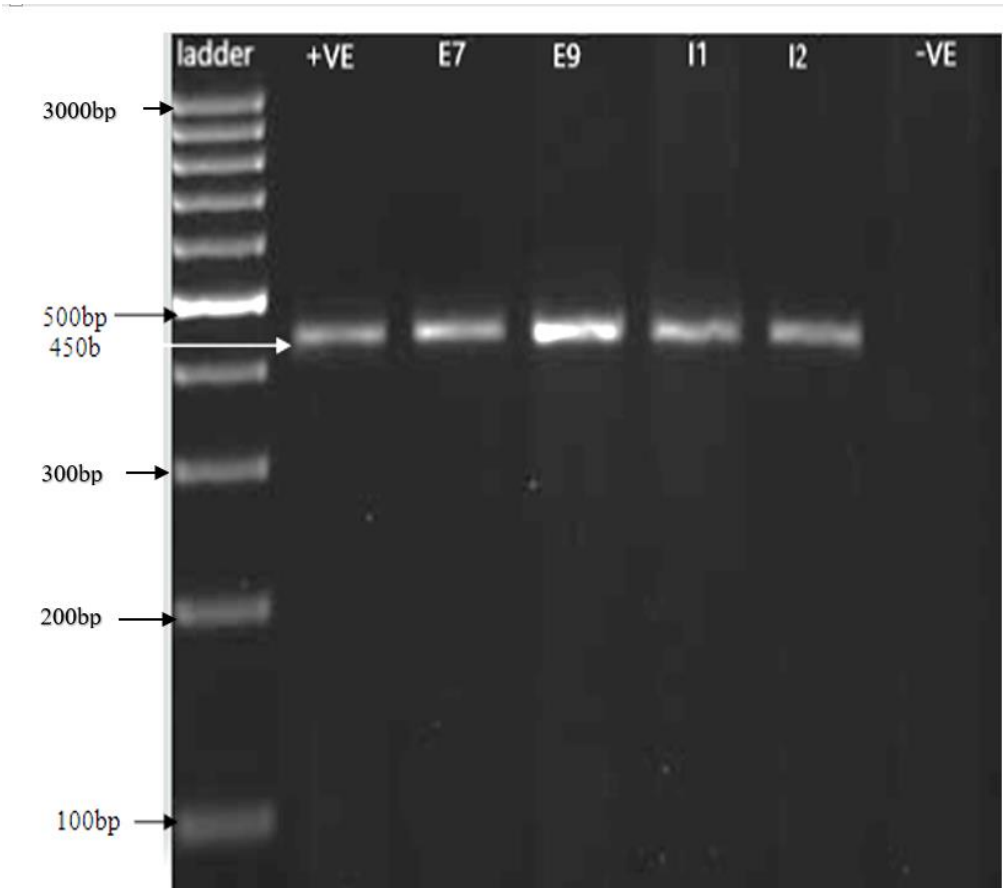

Figure 4-3a Primary PCR gel electrophoresis image

The image shows the Primary PCR electrophoresis gel. Four per cent agarose gel stained with ethidium bromide showing 450bp amplified product of primer set PGMY09/PGMY11. Lane arrangement (from left): ladder (5000bb) used as a marker, +VE (positive control), HPV positive samples E07, E09, I1, and I2 showing 450bp PCR product amplified (between 400bp and 500bp ladder) and -VE (negative control) with no band.

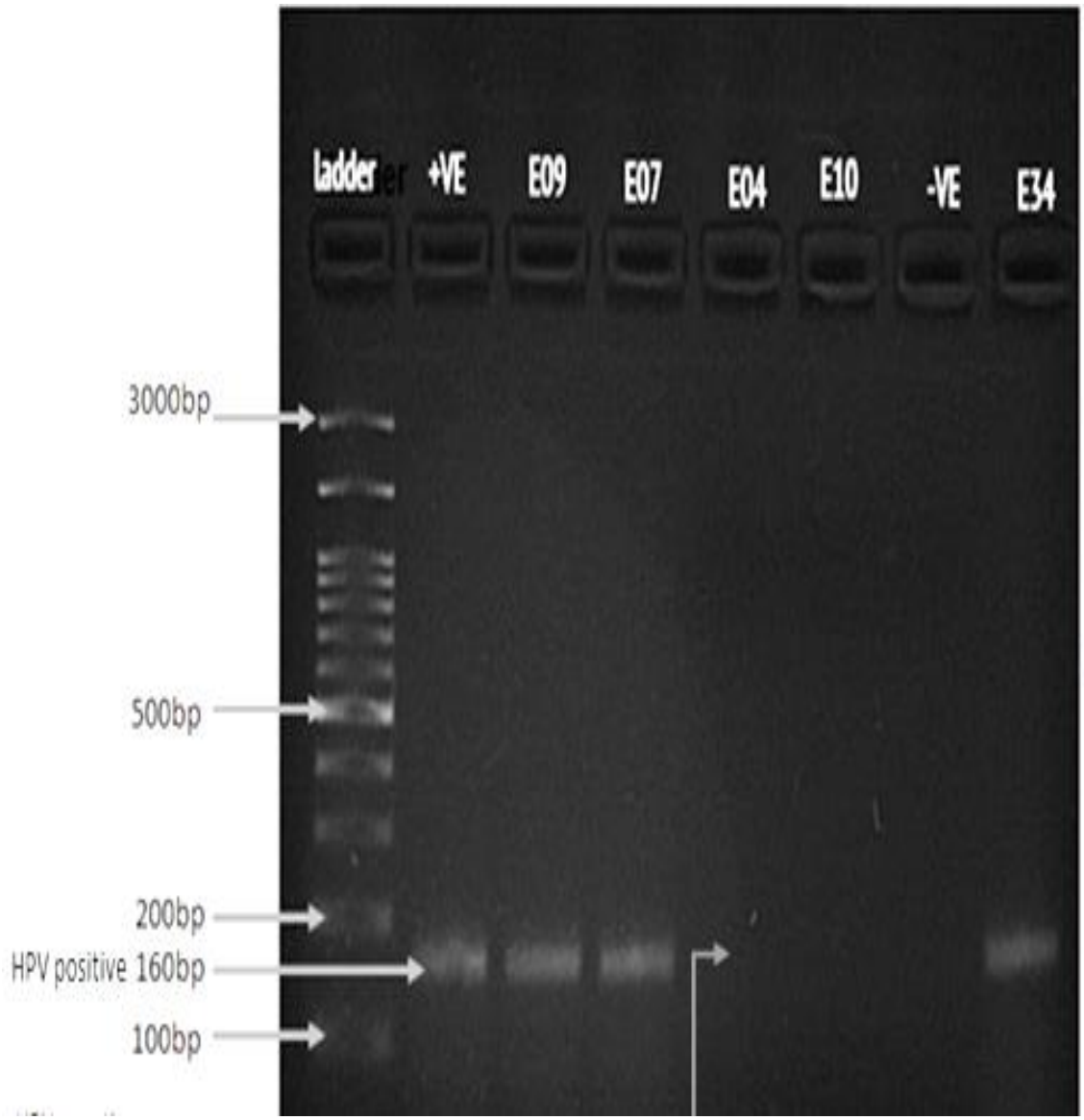

Figure 4-3b. Nested PCR agarose gel electrophoresis image.

The image shows Nested PCR agarose gel electrophoresis. Four per cent agarose gel stained with ethidium bromide showing 160bp amplified product of primer set MGP05+/MGP06+. Lane arrangement (from left): ladder (5000bp) used as marker, +VE (positive control), E09, E07, E04, E10 (samples from Embu (E) County), -VE (negative control) and E34 (sample 34 from Embu County. Positive samples (E09, E07, and E34) show 160bp PCR product amplified (between 200bp and 100bp) unlike negative samples (E04 and E10).

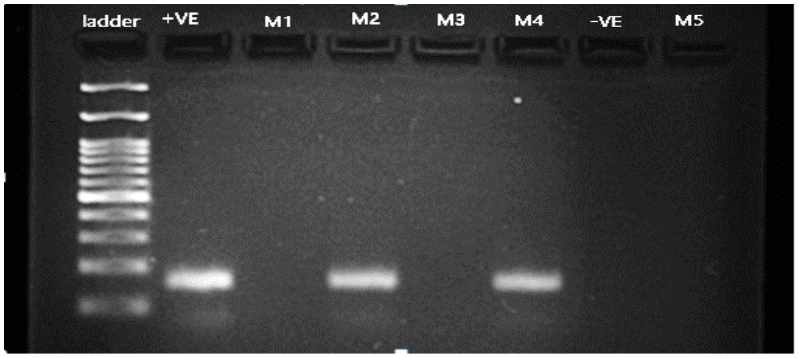

Figure 4-3c: Gel electrophoresis image of secondary PCR product of samples collected from Meru County

Ladder (5000bp), positive control (+VE), Meru (M) HPV positive samples (M2 and M4) and HPV negative samples M1, M3, M5), and negative control (-VE).

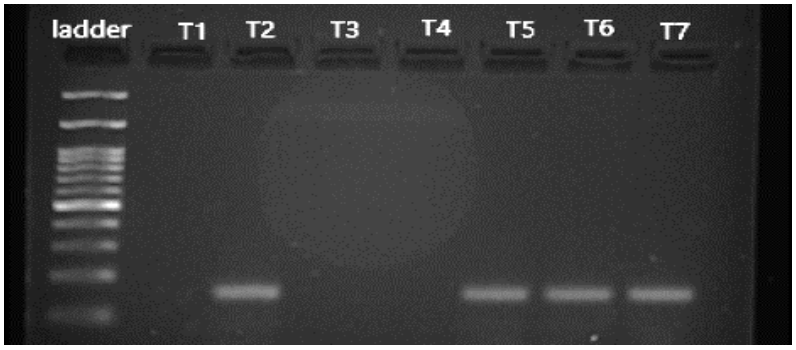

Figure 4-3e: Gel electrophoresis image of secondary PCR product of samples collected from Tharaka-Nithi County

Ladder (5000bp), Tharaka-Nithi (T) HPV positive samples (T2, T5, T6, T6, and T7), and HPV negative samples (T1, T3, and T4).

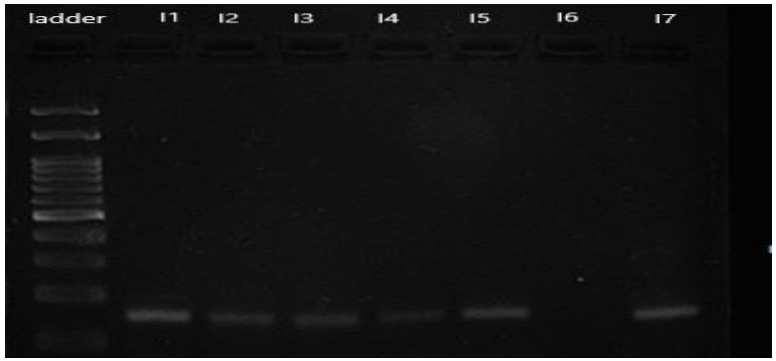

Figure 4-3d: Gel electrophoresis image of secondary PCR product of samples collected from samples Isiolo County

Ladder (5000bp), Isiolo (I) HPV positive samples (I1, I2, I3, I4, I5, and I7), and HPV negative samples (I6).

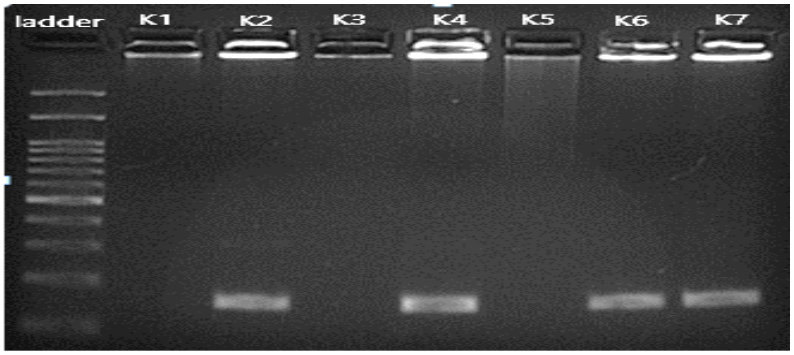

Figure 4-3f: Gel electrophoresis image of secondary PCR product of samples collected from Kirinyaga County

Ladder (5000bp), Kirinyaga (K) HPV positive samples (K2, K4, K6, and K7), and HPV negative samples (K1, K3, and K5).

4.1.5 Human Papillomavirus Polymerase Chain Reaction results and Human Immunodeficiency virus status.

In total, 84/317 (26.5%) participants were HPV-infected [HIV negative 17/317 (20.2%) and HIV positive 67/317 (21.1%)] while 233/317 (73.5%) were not infected. A significantly higher HPV infection rate was established among HIV-infected than non-infected women ($p<0.001$) (Table 4-2).

Table 4-2. The HPV infection rate among HIV-infected and non-infected women attending Reproductive Health Clinics in Eastern and Central Regions, Kenya.

HIV serostatus of participants		HPV PCR results†		Total (n=317)	p-value
		Negative	Positive		
HIV	Negative	139	17	156	<0.001***
		43.8%	5.4%	49.2%	
	Positive	94	67	161	
		29.7%	21.1%	50.8%	
	Total	**233**	**84**	**317**	
		73.5%	**26.5%**	**100.0%**	

***: the probability at the 0.001 level. †HPV PCR results: Human Papillomavirus results are described as Positive (presence of HPV L1 genome in sample) or Negative (absence of HPV L1 genome in sample).

4.1.6 Comparison of cervical screening results with histology outcome.

Overall, 78/317 (24.6%) participants had positive histology tests [ASCUS: 34/317 (10.7%) CIN1: 17/317 (5.3%), CIN2: 16/317 (5.0%), CIN3: 6/317 (1.9%) and ICC: 5/317 (1.6%)] and were significantly associated with other cervical diagnostic methods test outcome. Several CIN1+ cases were reported as normal by VIA (CIN1: 1/78), HPV DNA-PCR (CIN2, 3 and ICC: 1/78) and Pap smear test (CIN1: 2/78, CIN3: 3/78 and CIN3: 1/78). However, the number of these cases was reduced upon triage testing by VIA -Pap smear test (CIN1: 1/78), Pap smear-HPV DNA-PCR (CIN1: 2/78) (Table 4-3).

Table 4-3. Comparison of cervical screening methods results with histology outcome.

Cervical screening methods and results			Result category	Categories of Histological analysis (N=78) ASCUS	CIN1	CIN2	CIN3	ICC	Positive (ASCUS, CIN+)	Negative	Total	p-value
Primary screening approach												
Positive VIA test		Normal	FP	24(7.6)	2(0.6)				26(8.2)	194(61.2)	220(69.4)	0.001*
		Abnormal	TP	10(3.2)	15(4.7)	16(5.0)	6(1.9)	5(1.6)	52(16.4)	45(14.2)	97(30.6)	
Positive HPV DNA-PCR		Negative	FP	23(7.3)		1(0.3)	1(0.3)	1(0.3)	26(8.2)	207(65.3)	233(73.5)	0.001*
		Positive	TP	11(3.5)	17(5.4)	15(4.7)	5(1.6)	4(1.3)	52(16.4)	32(10.1)	84(26.5)	
Positive Pap smear test		Normal	FP	22(6.9)	2(0.6)	3(0.9)	1(0.3)		28(8.8)	208(65.6)	236(74.4)	0.001*
		Abnormal	TP	12(3.8)	15(4.7)	13(4.1)	5(1.6)	5(1.6)	50(15.8)	31(9.8)	81(25.6)	
Triage screening approach with positive primary test												
Positive VIA test	Pap smear	Normal	FP	3(0.9)	1(0.3)				4(1.3)	11(3.5)	3(0.9)	0.001*
		Abnormal	TP	9(2.8)	14(4.4)	13(4.1)	5(1.6)	5(1.6)	46(14.5)	20(6.3)	46(14.5)	
		Total		12(3.8)	15(4.7)	13(4.1)	5(1.6)	5(1.6)	50(15.8)	31(9.8)	50(15.8)	
Positive DNA-PCR	VIA test	Normal	FP	2(0.6)	2(0.6)	3(0.9)	1(0.3)		8(2.5)	13(4.1)	21(6.6)	0.001*
		Positive	TP	7(2.2)	14(4.4)	12(3.8)	5(1.6)	5(1.6)	43(13.6)	20(6.3)	63(19.9)	
		Total		9(2.8)	16(5.0)	15(4.7)	6(1.9)	5(1.6)	51(16.1)	33(10.4)	84(26.5)	
Abnormal Pap smear	DNA-PCR	Negative	FP	3(0.9)	1(0.3)	1(0.3)			5(1.6)	21(6.6)	3(0.9)	0.001*
		Positive	TP	7(2.2)	14(4.4)	15(4.7)	6(1.9)	5(1.6)	47(14.8)	24(7.6)	47(14.8)	
		Total		10(3.2)	15(4.7)	16(5.0)	6(1.9)	5(1.6)	52(16.4)	45(14.2)	52(16.4)	
Total				34(10.7)	17(5.3)	16(5.0)	6(1.9)	5(1.6)	78(24.6)	239(75.4)	317(100.0)	0.001*

VIA test: Visual Inspection with Acetic Acid test; HPV DNA-PCR: Human Papillomavirus Deoxyribonucleic Polymerase Chain Reaction; Abnormal histology: Atypical Squamous Cells of Unknown Significant (ASCUS), Cervical Intraepithelial Neoplasia (CIN2+) and Intraepithelial Cervical Carcinoma (ICC); *: the probability at the 0.001 level; FP: False Positive; TP: True Positive.

Table 4.3 shows the Primary cervical screening approach where results obtained by VIA, HPV-PCR and Pap smear are categorised as positive, negative, normal or abnormal by histology reports of ASCUS, CIN+ including ICC. In the Triage screening approach, positive primary test results are combined with triage test results and categorised by histology report. Total positive results of ACSUS, CIN1, 2,3 and ICC are also shown alongside Negative samples and Total (N=317).

4.1.7 Sensitivity, Specificity, Diagnostic Accuracy, Positive and Negative Predictive Value of cervical screening methods in comparison with histopathology of abnormal cytology outcome.

Human Papillomavirus DNA-PCR had the highest sensitivity (61.9%), Positive Predictive value (61.9%), and Diagnostic accuracy (81.7%) while Pap smear had the highest Specificity value (87.0) in the Primary testing approach. HPV DNA-PCR co-testing with Pap smear showed the highest Specificity (95.4%), Diagnostic accuracy (94.3%) and highest Negative Predictive Value while co-tested with VIA test (99.9%) in triage testing. All screening method results were significantly associated with histological analysis ($p<0.001$) (Table 4-4).

Table 4-4 Diagnostic accuracy of cervical screening approaches in comparison with histology outcome.

Cervical screening methods and approaches	Abnormal histology (%)										p-value
	ASCUS and CIN +					CIN+					
	Sensitivity	Specificity	PPV	NPV	D/A	Sensitivity	Specificity	PPV	NPV	D/A	
The primary cervical screening approach											
VIA test	53.6	81.2	53.6	81.2	77.6	95.5	79.9	43.3	99.1	82.0	0.001
HPV DNA-PCR	61.9	86.6	61.9	86.6	81.7	93.2	84.2	48.8	98.7	85.5	0.001
Pap smear	61.7	87.0	61.7	87.0	81.4	86.4	84.2	46.9	97.5	84.5	0.001
Triage cervical screening approach											
VIA and Pap smear	92.0	64.8	69.7	67.6	90.0	83.3	72.0	16.1	98.5	72.7	0.001
HPV-PCR and VIA test	84.3	94.7	68.3	90.7	84.2	99.9	93.1	11.1	99.9	93.1	0.001
Pap and HPV DNA-PCR	90.4	95.4	66.2	89.4	94.3	99.0	95.0	15.4	99.9	95.0	0.001

VIA test: Visual Inspection with Acetic Acid test; HPV DNA-PCR: Human Papillomavirus Deoxyribonucleic Polymerase Chain Reaction; Abnormal histology: Atypical Squamous Cells of Unknown Significant (ASCUS), Cervical Intraepithelial Neoplasia (CIN2+) and Intraepithelial Cervical Carcinoma (ICC); Sensitivity =TP/(TP+FN); Specificity = TN/(TN+FP); Positive Predictive Value (PPV) = TP/(TP+FP); Negative Predictive Value (PPV) NPV=TN/(FN+TN), Diagnostic accuracy = TP+TN/TP+TN+FP+FN where TP=True-Positive, FP= False Positive, TN=True-Negative; FP= False-Positive and p-value: probability at the 0.001 level.

4.1.8 Diagnostic value of cervical screening methods and approaches in comparison with Histology results by HIV serostatus.

There was reduced sensitivity and increased specificity among HIV-infected than non-infected women in the primary and triage cervical screening approach. There was no difference in NPV by primary or triage screening approach but the diagnostic accuracy increased by HIV-negativity status in all approaches (Table 4-5).

Table 4-5 Diagnostic value of cervical screening methods and approaches in comparison with Histology results by HIV serostatus.

Cervical screening approach and methods	HIV Status	Diagnostic values of cervical screening methods					p-value
		Sensitivity	Specificity	PPV	NPV	D. accuracy	
The primary cervical screening approach							
VIA	Negative	75.0	91.2	31.6	98.5	92.5	0.001
	Positive	88.9	76.0	51.6	96.0	91.0	
Pap smear	Negative	87.5	91.2	35.0	99.3	93.5	0.001
	Positive	97.2	66.4	45.5	98.8	86.6	
HPV DNA-PCR	Negative	87.5	94.6	46.7	99.3	96.7	0.001
	Positive	97.2	72.8	50.7	98.9	91.5	
Triage screening approach							
VIA test – Pap smear	Negative	75.0	95.3	46.2	98.6	96.4	0.001
	Positive	86.1	82.3	58.5	95.3	94.8	
Pap smear – DNA-PCR	Negative	75.0	98.0	66.7	98.6	98.9	0.001
	Positive	94.4	80.0	57.6	98.0	96.1	
HPV DNA-PCR – VIA	Negative	62.5	95.9	45.5	97.9	96.0	0.001
	Positive	86.1	83.2	59.6	95.4	95.6	

VIA test: Visual Inspection with Acetic Acid test; HPV DNA-PCR: Human Papillomavirus Deoxyribonucleic Polymerase Chain Reaction; Abnormal histology: Atypical Squamous Cells of Unknown Significant (ASCUS), Cervical Intraepithelial Neoplasia (CIN2+) and Intraepithelial Cervical Carcinoma (ICC); Sensitivity =TP/(TP+FN); Specificity = TN/(TN+FP); Positive Predictive Value (PPV) = TP/(TP+FP); Negative Predictive Value (PPV) NPV=TN/(FN+TN), Diagnostic accuracy = TP+TN/TP+TN+FP+FN where TP=True-Positive, FP= False Positive, TN=True-Negative; FP= False-Positive and p-value: probability at the 0.001 level.

4.2 Human Papillomavirus (HPV) infection rate and cervical dysplasia in association with social-demographic factors and Human Immunodeficiency Virus (HIV) status.

The HPV infection rate and cervical dysplasia among HIV-infected and non-infected participants varied by specific social demographic factors. HIV-infected women had a higher HPV prevalence, higher rate of positive VIA/VILLI test results, abnormal cervical cytology, and histology results than HIV-non-infected women.

4.2.1 Social demographic factors and Human Immunodeficiency Virus (HIV) status of women attending Reproductive Health Clinics Eastern and Central Region, Kenya.

Most participants recruited into the study were residents from Embu County [85/317 (26.8%)], aged $\leq$35 years [219/317 (69.1%)], were educated up to secondary school level [135(42.6%)], married [226/317 (71.3%)] and those with low-income status earning less than $1.90 per day [208/317 (65.6%)]. Age was significantly associated ($p=0.016$) with HIV status: more women aged below 35years had a higher HIV infection rate than those aged over 35 years as seen in Table 4-6.

Table 4-6. Association between social-demographic characteristics and HIV status of women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

Characteristics	Category	HIV serostatus [N (%)]		Total (N=317)	p-value
		HIV negative	HIV positive		
Residence	Embu	41(12.9)	44(13.9)	85(26.8)	**0.359**
	Isiolo	38(12.0)	26(8.2)	64(20.2)	
	Kirinyaga	23(7.3)	33(10.4)	56(17.7)	
	Meru	40(12.6)	41(12.9)	81(25.6)	
	T. Nithi	14(4.4)	17(5.4)	31(9.8)	
Age (years)	≤35 years	69(21.8)	93(29.3)	162(51.1)	**0.016****
	>35 years	87(27.4)	68(21.5)	155(48.9)	
Education level	Primary	43(13.6)	53(16.7)	96(30.3)	**0.216**
	Secondary	70(22.1)	65(20.5)	135(42.6)	
	College	37(11.7)	30(9.5)	67(21.1)	
	University	6(1.9)	13(4.1)	19(6)	
Marital status	Married	117(36.9)	109(34.4)	226(71.3)	**0.416**
	Separated	14(4.4)	18(5.7)	32(10.1)	
	Single	19(6)	22(6.9)	41(12.9)	
	Divorced	6(1.8)	12(3.7)	18(5.7)	
Income status§	Low	106(33.4)	102(32.2)	208(65.6)	**0.495**
	Middle	45(14.5)	50(15.8)	95(30.0)	
	High	5(1.6)	9(2.8)	14(4.4)	
Total		**156(49.2)**	**161(50.8)**	**317(100)**	

T. Nithi: Tharaka Nithi County, §Income: low (1.90), middle (1.9-5.5) and high (> 5.50) US$ PPP/day), **: the probability at the 0.05

4.2.2 Social demographic factors, cervical visual inspection outcome, and Human Immunodeficiency Virus (HIV) status of women participants.

Participants with the highest VIA/VILLI positivity rate were residents of Kirinyaga County, women aged over 35 years, university/college level educated, divorced, Muslims, and those with middle income. Women with increased risk of having VIA/VILLI positive outcomes were residents of Meru County, (OR: 13.46; CI: 2.85-63.52), those aged over 35 years (OD: 5.68; CI: 2.68-12.061), college and/or university educated (OR: 11.33; CI: 2.8-45.07), single (OR: 31.50CI: 3.51-282.31) and those in low-income status (OR: 4.80: CI: 2.21-10.37) (Table 4-7).

Table 4-7. Association between cervical visual inspection outcome and HIV status among women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

Social-demographic factors		HIV Status	VIA/VILLI results		Total (N=317)	Odds (Crude)	CI (95%)	p-value
			Negative	Positive				
Residence	Embu	Negative	34(40.0)	7(8.2)	41(48.2)	2.267	0.808-6.36	0.021*
		Positive	30(35.3)	14(16.5)	44(51.8)			
		Total	64(75.3)	21(24.7)	85(100.0)			
	Isiolo	Negative	33(51.6)	5(7.8)	38(59.4)	4.125	1.21-14.09	
		Positive	16(25.0)	10(15.6)	26(40.6)			
		Total	49(76.6)	15(23.4)	64(100.0)			
	Kirinyaga	Negative	18(32.1)	5(8.9)	23(41.0)	3.388	1.07-11.28	
		Positive	17(30.4)	16(28.6)	33(58.9)			
		Total	35(62.5)	21(37.5)	56(100.0)			
	Meru	Negative	38(46.9)	2(2.5)	40(49.4)	13.46	2.85-63.52	
		Positive	24(29.6)	17(21.0)	41(50.6)			
		Total	62(76.5)	19(23.5)	81(100.0)			
	T. Nithi	Negative	14(45.2)	0(0.0)	14(45.2)	1.41	1.04-1.92	
		Positive	12(38.7)	5(16.1)	17(54.8)			
		Total	26(83.9)	5(16.1)	31(100.0)			
Age (years)	≤35	Negative	64(39.5)	5(3.1)	69(42.6)	5.68	2.68-12.061	<0.001**
		Positive	60(37.0)	33(20.4)	93(57.4)			
		Total	124(76.5)	38(23.5)	162(100.0)			
	>35	Negative	73(47.1)	14(9.0)	8756.1)	3.26	1.28-8.28	
		Positive	39(25.2)	29(18.7)	68(43.9)			
		Total	112(72.3)	43(27.7)	155(100.0)			
Education	Primary	Negative	38(39.6)	5(5.2)	43(44.8)	4.247	1.43-12.61	0.009*
		Positive	34(35.4)	19.19.8)	53(55.2)			
		Total	72(75.0)	24(25.0)	96(100.0)			
	Secondary	Negative	61(45.2)	9(6.7)	70(51.9)	3.71	1.56-8.81	
		Positive	42(31.1)	23(17.0)	65(48.1)			
		Total	103(76.3)	32(23.7)	135(100.0)			
	College/ university	Negative	38(71.8)	5(15.0)	43(50.0)	11.33	2.8-45.07	
		Positive	23(64.5)	20(48.7)	43(50.0)			
		Total	61(136.3)	18(63.7)	86(100.0)			
Marital status	Married	Negative	106(46.9)	11(4.9)	117(51.8)	5.369	2.57-11.186	<0.001**
		Positive	70(31.0)	39(17.3)	109(48.2)			
		Total	176(77.9)	50(22.6)	226(100.0)			
	Single	Negative	18(41.5)	1(2.4)	19(46.3)	31.50	3.51-282.31	
		Positive	8(19.5)	14(34.1)	22(53.7)			
		Total	26(63.4)	15(36.6)	41 (100.0)			
	Divorced	Negative	2(33.3)	1(16.7)	3(50.0)	4.00	0.134-119.23	
		Positive	1(16.7)	2(33.3)	3(50.0)			
		Total	3(50.0)	3(50.0)	6(100.0)			
	Widowed	Negative	3(25.0)	0(0.0)	3(25.0)	1.50	0.945-2.38	
		Positive	6(50.0)	3(25.0)	9(75.0)			
		Total	9(75.0)	3(25.0)	12(100.0)			
Income level	Low (<US$1.9)	Negative	96(46.2)	10(4.8)	106(51.0)	4.80	2.21-10.37	<0.001**
		Positive	68(32.7)	34(16.3)	102(49.0)			
		Total	164(78.8)	44(21.1)	208(100.0)			
	Middle (>US$1.9)	Negative	41(37.6)	9(8.3)	50(54.1)	0.75	0.78-7.21	
		Positive	31(28.4)	28(25.7)	59(54.1)			
		Total	72(66.1)	37(33.9)	109 (100.0)			

**: the probability at the 0.001 level, *: the probability at the 0.05 level, -: negative, +: positive, T.Nithi: Tharaka Nithi County; CI: Confidence Interval; Odds: Odd Ratio.

4.2.3 Association between social demographic factors and cytology results in cervical infections and other abnormalities by Human Immunodeficiency Virus (HIV) status.

Among the 317 (100%) women participants, 19(6.0%) had vaginitis, 52(16.4%) had bacterial cervicitis, 70(22.0%) had candidiasis, and 9(2.8%) had unexplained cervical inflammation while 8(2.5%) were diagnosed with trachomatis caused by *Chlamydia trachomatis*. These results were obtained from Pap smear pathological report. A significant association between HIV infection and abnormal cytological outcomes across all social demographic categories was established where the majority of HIV-infected women were diagnosed with vaginitis, bacterial cervicitis, candidiasis, and trachomatis than HIV non-infected ($p<0.005$) by the County of residence, age, religion, marital status and number of sex partners (Table 4-8).

Table 4-8. Association between social demographic factors and cytology results in cervical infections and other abnormalities among women attending Reproductive-health Clinics in Eastern and Central Regions, Kenya.

Category	HIV Status	Cytology results in infections and other abnormalities (N=317)					Total (N=317)	Negative (N=317)	p-value
		Vaginitis	Bacterial cervicitis	Candidiasis	Unexplained inflammation	Trachomatis			
Residence									
Embu	-		1(0.3)	9(2.7)		2(0.6)	12(3.8)	29(9.1)	0.001**
	+	2(0.6)	8(2.4)	11(3.4	3(0.9)	2(0.6)	26(8.2)	18(5.7)	
	Total	**2(0.6)**	**9(2.7)**	**20(6.2)**	**3(0.9)**	**4(1.2)**	**38(12)**	**47(14.8)**	
Isiolo	-		5(1.6)	15(4.7)	1(0.3)	1(0.3)	22(6.9)	16(5.0)	
	+		9(2.7)	1(0.3)	2(0.6)	1(0.3)	13(4.1)	13(4.1)	
	Total		**14(4.4)**	**16(5.0)**	**3(0.9)**	**2(0.6)**	**35(11.0)**	**29(9.1)**	
Kirinyaga	-	8(2.4)	1(0.3)	4(1.2)	1(0.3)		14(4.4)	9(2.8)	
	+		10(3.1)	6(1.8)	2(0.6)	1(0.3)	19(6.0)	14(4.4)	
	Total	**8(2.4)**	**11(3.4)**	**10(3.1)**	**3(0.9)**	**1(0.3)**	**33(10.4)**	**23(7.3)**	
Meru	-	8(2.4)		12(3.8)			20(6.3)	20(6.3)	
	+	1(0.3)	11(3.4)	4(1.2)		1(0.3)	17(5.4)	24(7.6)	
	Total	**9(2.7)**	**11(3.4)**	**16(5.0)**		**1(0.3)**	**37(11.7)**	**44(13.9)**	
T. Nithi	-			7(2.2)			7(2.2)	7(2.2)	
	+		7(2.2)	1(0.3)			8(2.5)	9(2.8)	
	Total		**7(2.2)**	**8(2.4)**			**15(4.7**	**16(5.0)**	
Age (years)									
≤35	-	7(2.2)	4(1.2)	18(6.0)	2(0.6)		31(9.8)	38(12.0)	0.001***
	+	2(0.6)	23(7.2)	16(5.4)	5(1.6)	4(1.2)	50(15.8)	43(13.6)	
	Total	**9(2.7)**	**27(8.5)**	**34(10.7)**	**7(2.2)**	**4(1.2)**	**81(25.6)**	**81(25.6)**	
>35	-	9(2.7)	3(0.9)	29(9.1)		3(0.9)	44(13.9)	43(13,6)	
	+	1(0.3)	22(6.9)	7(2.2)	2(0.6)	1(0.3)	33(10.4)	35(11.0)	
	Total	**10(3.1)**	**25(7.9)**	**36(11.3)**	**2(0.6)**	**4(1.2)**	**77(24.3)**	**78(24.6)**	
Marital status									
Married	-	9(2.7)	7(2.2)	33(10.4)	2(0.6)	3(0.9)	54(17.0)	63(19.9)	0.005
	+	3(0.9)	34(10.7)	16(5.4)	5(1.6)	4(1.2)	62(19.6)	47(14.8)	
	Total	**12(3.8)**	**41(12.9)**	**49(15.4)**	**7(2.2)**	**7(2.2)**	**116(36.6)**	**110(34.7)**	
Separated	-	5(1.6)		3(0.9)			8(2.5)	6(1.9)	
	+		6(1.8)	4(1.2)			10(3.2)	8(2.5)	
	Total	**5(1.6)**	**6(1.8)**	**7(2.2)**			**18(5.7)**	**14(4.4)**	
Single	-	1(0.3)		10(3.1)			11(3.5)	8(2.5)	
	+		4(1.2)	1(0.3)	1(0.3)		6(1.9)	16(5.0)	
	Total	**1(0.3)**	**4(1.2)**	**11(4.1)**	**1(0.3)**		**17(5.4)**	**24(7.6)**	
Divorced	-			1(0.3)			1(0.3)	2(0.6)	
	+				1(0.3)	1(0.3)	2(0.6)	1(0.3)	
	Total			**1(0.3)**	**1(0.3)**	**1(0.3)**	**3(0.9)**	**3(0.9)**	
Widowed	-	1(0.3)					1(03)	2(0.6)	
	+		1(0.3)	2(0.6)			3(0.9)	6(1.9)	
	Total	**1(0.3)**	**1(0.3)**	**2(0.6)**			**4(1.3)**	**8(2.5)**	
Number of sex partners									
1		7(2.2)	3(0.9)	28(8.8)	1(0.3)		39(12.3)	59(18.6)	0.001***
	+	3(0.9)	27(8.5)	13(4.1)	4(1.2)	3(0.9)	50(15.8)	38(12.0)	
	Total	**10(3.1)**	**30(9.5)**	**41(12.9)**	**5(1.6)**	**3(0.9)**	**89(28.1)**	**97(30.6)**	
>1	-	9(2.7)	4(1.2)	19(6.0)	1(0.3)	3(0.9)	36(11.4)	22(6.9)	
	+		18(5.7)	10(3.1)	3(0.9)	2(0.6)	33(10.4)	40(12.6)	
	Total	**9(2.7)**	**22(6.9)**	**29(9.1)**	**4(1.2)**	**5(1.6)**	**69(21.8)**	**62(19.6)**	
Total		**19(6.0)**	**52(16.4)**	**70(22.1)**	**9(2.8)**	**8(2.5)**	**158(49.8)**	**169(50.2)**	

***: the probability at the 0.001 level, **: the probability at the 0.05 level, -: negative, +: positive, T.Nithi: Tharaka Nithi County

4.2.4 Cervical histology results by Human Immunodeficiency Virus (HIV) status of women attending Reproductive Health Clinics in Eastern and Central Regions, Kenya.

Two hundred and thirty-nine women (64.6%) had normal histology results [HIV positive: 96(30.0%); HIV negative: 143/317 (45.1%)]. Total abnormal histology results obtained were ASCUS [34/317 (10.7%)], CIN1 [17(5.4%)], CIN2 [16(5.0%)], CIN3 [6(1.9%)] and ICC [5(1.6%)]. There was a significant association between HIV infection and cervical histological results ($p<0.001$). A statistically significant relationship was also established between cervical histology outcome and all age categories ($p<0.001$), number of sex partners [one ($p=0.05$ and more than one ($p<0.001$), parity [$\leq$3($p=0.043$) and >3($p=0.001$)] and hormonal contraceptive use ($p<0.001$). The positivity rate for abnormal cytology results by either HIV serostatus was high across all age groups, the number of sex partners, parity, and choice of family planning method. HIV infection was seen to be a predictor for cervical dysplasia across all age, parity, and choice of family planning method categories. HIV status was also seen to associate with cervical dysplasia among women residents from Meru and Isiolo Counties and married where the majority of women in these categories were HIV-infected (Table 4-9).

Table 4-9. Cervical histology results by HIV serostatus among women attending Reproductive-health Clinics in Eastern and Central Regions, Kenya.

Characteristics	Category	HIV Status	Cervical histology results						Total (N=317)	p-value
			Normal	ASC	CIN1	CIN2	CIN3	ICC		
Residence	Embu	-	35(11.0)	3(0.9)	2(0.6)	1(0.3)			41(12.9)	**0.248**
		+	29(9.1)	4(1.3)	6(1.8)	4(1.3)	1(0.3)		44(13.9)	
		Total	**64(20.2)**	**7(2.2)**	**8(2.4)**	**5(1.6)**	**1(0.3)**		**85(26.8)**	
	Isiolo	-	35(11.0)	1(0.3)			1(0.3)	1(0.3)	38(12.0)	**0.011***
		+	15(4.7)	5(1.6)	1(0.3)	4(1.3)	1(0.3)		26(8.1)	
		Total	**50(15.8)**	**6(1.8)**	**1(0.3)**	**4(1.3)**	**2(0.6)**	**1(0.3)**	**64(20.2)**	
	Kirinyaga	-	22(6.9)	1(0.3)					23(7.3)	**0.070**
		+	19(6.0)	8(2.4)	2(0.6)	2(0.6)	1(0.3)	1(0.3)	33(10.4)	
		Total	**41(12.9)**	**9(2.7)**	**2(0.6)**	**2(0.6)**	**1(0.3)**	**1(0.3)**	**56(17.7)**	
	Meru	-	38(12.0)	2(0.6)					40(12.6)	**0.006***
		+	25(7.9)	7(2.2)	4(1.3)	3(0.9)		2(0.6)	41(12.9)	
		Total	**63(19.9)**	**9(2.7)**	**4(1.3)**	**3(0.9)**		**2(0.6)**	**81(25.6)**	
	T. Nithi	-	13(4.1)			1(0.3)			14(4.4)	**0.110**
		+	8(2.5)	3(0.9)	2(0.6)	1(0.3)	2(0.6)	1(0.3)	17(5.4)	
		Total	**21(6.6)**	**3(0.9)**	**2(0.6)**	**2(0.6)**	**2(0.6)**	**1(0.3)**	**31(9.8)**	
Age in years	≤35	-	61(19.2)	4(1.3)	1(0.3)	1(0.3)	1(0.3)	1(0.3)	69(21.8)	**<0.001*** **
		+	51(16.1)	19(5.9)	11(3.5)	8(2.4)	2(0.6)	2(0.6)	93(29.3)	
		Total	**112(35.3)**	**23(7.1)**	**12(1.9)**	**9(2.7)**	**3(0.9)**	**3(0.9)**	**162(51.1)**	
	>35	-	82(25.9)	3(0.9)	1(0.3)	1(0.3)			87(27.4)	**<0.001*** **
		+	45(14.2)	8(2.4)	4(1.3)	6(1.8)	3(0.9)	2(0.6)	68(21.5)	
		Total	**127(40.1)**	**11(3.5)**	**5(1.6)**	**7(2.2)**	**3(0.9)**	**2(0.6)**	**155(48.9)**	
Marital status	Married	-	109(34.4)	5(1.6)		1(0.3)	1(0.3)	1(0.3)	117(36.9)	**0.001***
		+	71(22.4)	15(4.7)	9(2.7)	10(3.1)	3(0.9)	1(0.3)	109(34.4)	
		Total	**180(56.8)**	**20(6.2)**	**9(2.7)**	**11(3.5)**	**4(1.3)**	**2(0.6)**	**226(71.3)**	
	separated	-	12(3.8)	1(0.3)		1(0.3)			14(4.4)	**0.325**
		+	10(3.2)	2(0.6)	3(0.9)	2(0.6)	1(0.3)		18(5.7)	
		Total	**22(6.9)**	**3(0.9)**	**3(0.9)**	**3(0.9)**	**1(0.3)**		**32(10.1)**	
	Single	-	17(5.4)	1(0.3)	1(0.3)				19(6.0)	**0.126**
		+	11(3.5)	7(2.2)	1(0.3)	1(0.3)	1(0.3)	1(0.3)	22(6.9)	
		Total	**28(8.8)**	**8(2.4)**	**2(0.6)**	**1(0.3)**	**1(0.3)**	**1(0.3)**	**41(12.9)**	
	divorced	-	4(1.3)	1(0.3)	1(0.3)				6(1.8)	**0.273**
		+	4(1.2)	2(0.6)	2(0.6)	1(0.3)		2(0.6)	11(3.5)	
		Total	**8(2.5)**	**3(0.9)**	**3(0.9)**	**1(0.3)**		**2(0.6)**	**17(5.4)**	
Number of sex partners	1	-	93(29.3)	1(0.3)		2(0.6)	1(0.3)	1(0.3)	98(30.7)	**0.001*** **
		+	55(17.3)	13(4.1)	9(2.7)	6(1.8)	3(0.9)	2(0.6)	88(27.8)	
		Total	**148(46.7)**	**14(4.4)**	**9(2.7)**	**8(2.4)**	**4(1.3)**	**3(0.9)**	**186(58.7)**	
	>1	-	50(15.8)	6(1.8)	2(0.6)				58(18.3)	**0.005** **
		+	41(12.9)	14(4.4)	6(1.8)	8(2.4)	2(0.6)	2(0.6)	73(23.0)	
		Total	**91(28.7)**	**20(6.2)**	**8(2.4)**	**8(2.4)**	**2(0.6)**	**2(0.6)**	**131(41.3)**	
Parity	≤3	-	33(10.4)	1(0.3)	1(0.3)	1(0.3)		1(0.3)	37(11.7)	**0.043** **
		+	18(5.7)	5(1.6)	4(1.3)	3(0.9)	1(0.3)		31(9.8)	
		Total	**51(16.1)**	**6(1.8)**	**5(1.6)**	**4(1.3)**	**1(0.3)**	**1(0.3)**	**68(21.5)**	
	>3	-	110(34.7)	6(1.8)	1(0.3)	1(0.3)	1(0.3)		119(37.5)	**0.001*** **
		+	78(24.6)	22(6.9)	11(3.5)	11(3.5)	4(1.3)	4(1.3)	130(41.0)	
		Total	**188(59.3)**	**28(8.8)**	**12(1.9)**	**12(1.9)**	**5(1.6)**	**4(1.3)**	**249(78.5)**	
FP	None	-	92(29.0)	5(1.6)	2(0.6)	2(0.6)	1(0.3)	1(0.3)	103(32.5)	**<0.001*** **
		+	76(24.0)	16(5.0)	13(4.1)	8(2.4)	4(1.3)	3(0.9)	120(37.9)	
		Total	**168(53.0)**	**21(6.6)**	**15(4.7)**	**10(3.1)**	**5(1.6)**	**4(1.3)**	**223(70.3)**	
	hormonal	-	51(16.1)	2(0.6)					53(16.7)	**<0.001*** **
		+	20(6.3)	11(3.5)	2(0.6)	6(1.8)	1(0.3)	1(0.3)	41(12.9)	
		Total	**71(22.4)**	**13(4.1)**	**2(0.6)**	**6(1.8)**	**1(0.3)**	**1(0.3)**	**94(29.7)**	
Total		-	143(45.1)	7(2.2)	2(0.6)	2(0.6)	1(0.3)	1(0.3)	156(49.2	**0.001**
		+	96(30.0)	27(8.5)	15(4.7))	14(4.4)	5(1.6)	4(1.3)	161(50.8)	
			239(64.6)	**34(10.7)**	**17(5.4)**	**16(5.0)**	**6(1.9)**	**5(1.6)**	**317(100)**	

***: the probability at the 0.001 level; ASC: comprises of ASCUS: Atypical squamous cells of undetermined significance/that cannot exclude HSIL (ASCUS/H), FP: family planning; ICC: invasive cervical cancer; CIN: cervical intraepithelial neoplasia – graded as 1, 2 or 3.

4.2.5 Human Papillomavirus (HPV) prevalence by County of residence.

Most participants who tested HPV positive by DNA PCR were HIV-infected {Embu [15(17.6%)], Isiolo [12(18.8%)], Kirinyaga [17(30.3%)], Meru [18(22.2%)] and Tharaka-Nithi [5(16.1%)]}. HIV-infected women residing in any Eastern Kenya County were at significant risk of acquiring HPV infection as demonstrated in (Table 4-10).

Table 4-10. HPV molecular positivity and HIV serostatus by county of residence and age of women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

Category			HPV PCR RESULTS		Total	p-value
			negative	positive		
County of residence						
Embu	HIV status	Negative	36(42.4)	5(5.9)	41(48.2)	0.017***
		Positive	29(34.1)	15(17.6)	44(51.8)	
	Total		**65(76.5)**	**20(23.5)**	**85(100.0)**	
Isiolo	HIV status	Negative	33(51.6)	5(7.8)	38(59.4)	0.003**
		Positive	14(21.9)	12(18.8)	26(40.6)	
	Total		**47(73.4)**	**17(26.6)**	**64(100.0)**	
Kirinyaga	HIV status	Negative	19(33.9)	4(7.1)	23(41.1)	0.009**
		Positive	16(28.6)	17(30.4)	33(58.9)	
	Total		**35(62.5)**	**21(37.5)**	**56(100.0)**	
Meru	HIV status	Negative	37(45.7)	3(3.7)	40(49.4)	0.001***
		Positive	23(28.4)	18(22.2)	41(50.6)	
	Total		**60(74.1)**	**21(25.9)**	**81(100.0)**	
T. Nithi	HIV status	Negative	14(45.2)	0(0.0)	14(45.2)	0.027**
		Positive	12(38.7)	5(16.1)	17(54.8)	
	Total		**26(83.9)**	**5(16.1)**	**31(100.0)**	

: the probability at the 0.05 level; *: the probability at the 0.001 level; PCR: Polymerase Chain Reaction

4.2.6 Human Papillomavirus prevalence and risk of infection by social-demographic factors.

Age was significantly associated with HPV infection (p=0.04) where women aged below 30 years were at an increased risk (OR: 1.16, CI: 0.68-1.98) of HPV infection than those aged above 30 years (OR: 1.04, CI: 0.89-1.21). Other categories of women at significant risk ($p<0.05$) of HPV infection were those with more than one sex partner those using hormonal contraceptives, HIV-infected with abnormal VIA/VILLI (OR: 18.38 CI: 9.86-34.27), and cytology tests (OR: 273.60 CI: 93.24-802.76), (Table 4-11).

Table 4-11 HPV molecular positivity and risk of infection by social-demographic factors.

Characteristic	Category	HPV infection			Crude ODD	95%CI		p-value
		Negative	positive	Total		Lower	Upper	
Age in years	≤35	163(74.4)	56(25.6)	219(100)	1.16	0.68	1.98	0.04*
	>35	70(71.4)	28(28.6)	98(100)	1.04	0.89	1.21	
Religion	Cristian	190(74.5)	65(25.5)	255(100)	1.29	0.73	2.37	0.409
	Muslim	43(69.4)	19(30.6)	62(100)	1.07	0,83	0.54	
Parity	3	49(72.1)	19(27.9)	68(100)	0.91	0.50	1.66	0.001*
	>3	184(73.9)	65(26.1)	249(100)	0.97	0.82	1.15	
Sex partners	One	145(78.0)	41(22.0)	186(100)	1.73	1.04	2.86	0.032*
	More	88(67.2)	43(32.8)	131(100)	1.16	1.01	1.34	
FP	None	172(77.1)	51(22.9)	223(100)	1.1	1.00	1.40	0.024*
	hormonal	61(64.9)	33(35.1)	94(100)	1.8	1.07	3.08	
Sex debut	<20	24(75.0)	8(25).0)	32(100)	1.09	0.47	2.53	0.041*
	>20	209(73.3)	76(26.7)	285(100)	1.02	0.83	1.26	
Smoking	No	230(73.7)	82(26.3)	312(100)	1.88	0.31	11.39	0.490
	Yes	3(60.0)	2(40.0)	5(100)	1.01	0.97	1.04	
Total		**233(73.5)**	**84(6.5)**	**317(100)**				

OD: Odd Ratio; CI: 95% Confidence Interval; **: the probability at the 0.05 level; ***: the probability at the 0.001

4.3 Phylogenetic relationships of Human Papillomavirus (HPV) genotypes obtained in the sampled population.

Human Papillomavirus L1 gene sequences obtained in Eastern and Central Regions, Kenya showed diversified HPV genotypes prevalence by all categories of HIV serostatus, social-demographic factors, oncogenic risk, and cervical cytology and histology laboratory outcome.

4.3.1 HPV DNA sequence alignment.

Alignment of DNA sequences of 160bp fragment of L1 gene of overall 25.6% (81/317) samples from Eastern Kenya and 76 reference DNA sequences obtained from GenBank were used to construct a phylogenetic tree using MEGA. A section of MEGA alignment of sample isolates and reference sequences is shown in Figure 4-3. The alignment shown is a section of the complete 160bp sequence.

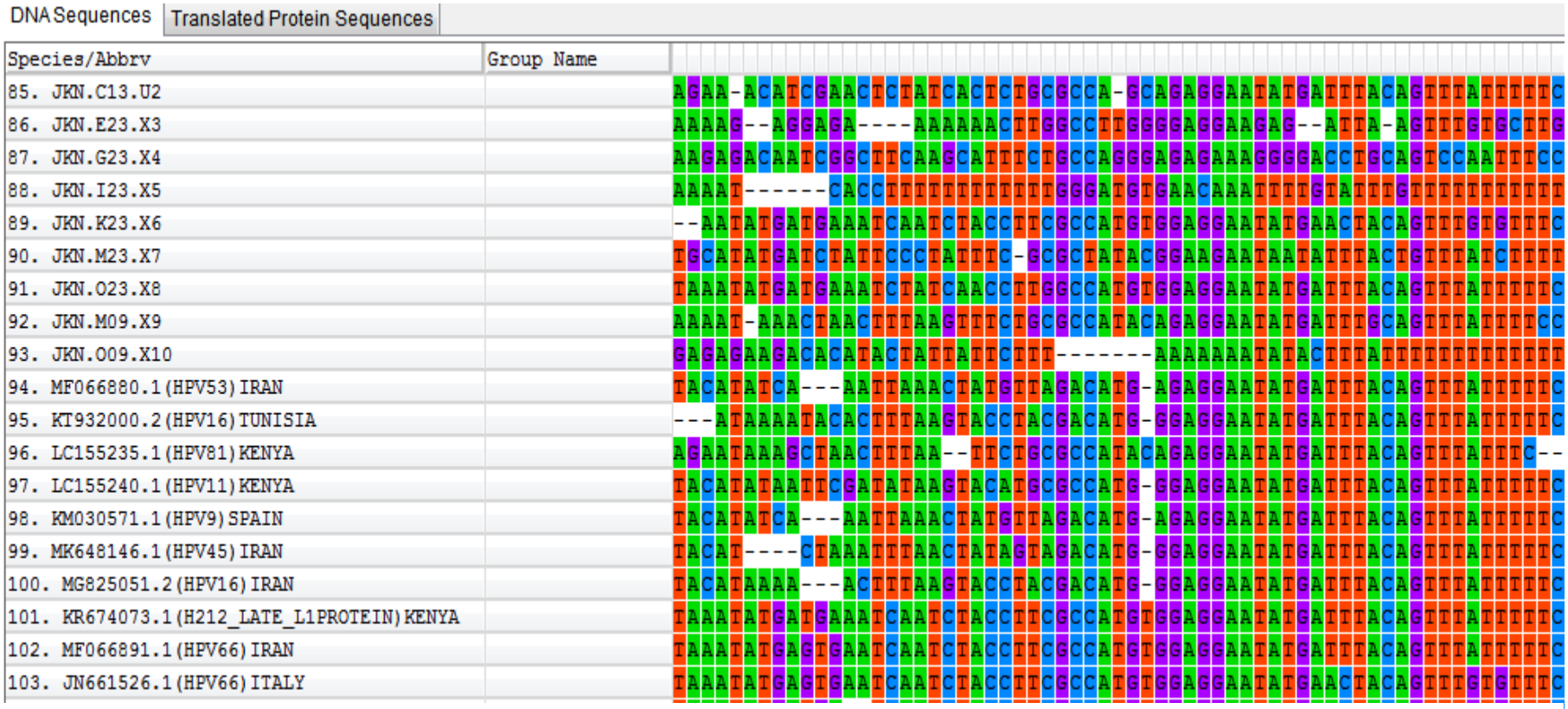

Figure 4- 3. A section of MEGA alignment of sample isolates and reference sequences.

4.3.2 Circular and Train phylogenetic analysis of Human Papillomaviruses sequences isolated in Eastern and Central Regions, Kenya.

The results of the Circular phylogenetic analysis of the HPV L1 genome showed particular geographic worldwide distribution (Figure 4-4). The total number of sequences was 157 comprising 81 (51.2%) study isolates and 76 (48.8%) reference sequences. A higher phylogenetic relationship with Asian, Middle East, and African HPV variants was evident where most study isolates clustered with reference sequences from Iran [22.3% (17/76)], Kenya [22.3% (17/76)], Bangkok [14.4% (11/76] and India [7.9% (6/76)]. Only one isolate JKN.C15.8P clustered with one HPV variant from North America (USA). There were only three reference sequences from South America (Brazil) that clustered with eight study isolates.

The circular tree shows two main clusters labelled A and B. Cluster A had a total of 73 sequences where [76.7% (56/73)] were study isolates and (23.2% (17/73)] were reference sequences. The predominant HPV genotype in this cluster was HPV81 and most reference sequences were mainly obtained from Bangkok [35.3% (6/17)] and Kenya [23.5% (4/17)]. The other main cluster (B) had mixed HPV genotypes whereas predominant genotypes were HPV66, HPV45, HPV11, and HPV16. Reference sequences were mainly obtained from Iran [23.8% (15/63)] and Kenya [19.4% (12/63]

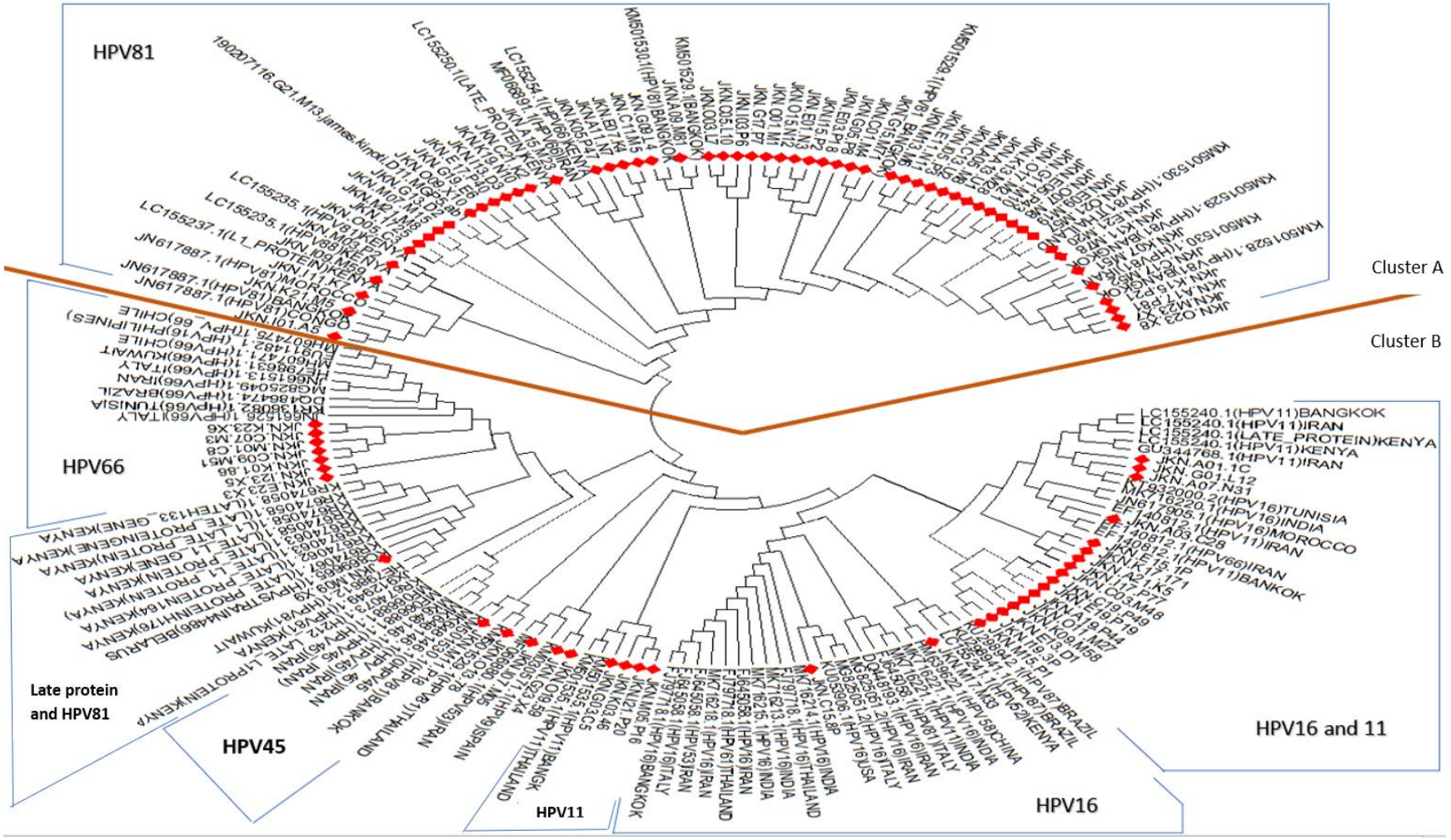

Figure 4-4. Phylogenetic tree diagram showing HPV distribution across the world.

The diagram shows a circle HPV phylogenic tree of study isolates and global reference sequences. A total number of 92 study isolate sequences were detected. The total number of reference sequences was 76. Study isolates are indicated by shaded triangles. Distribution of reference sequences across the globe: Brazil (3), Bangkok (11), Kenya (17), China (1) Philippines (1), Italy (5), Belarus (1), Iran (17), Thailand (4), USA (1), Chile (2), India (6), Congo (1), Tunisia (2), Morocco (1), Spain (1) and Kuwait (2)].

Fragmented Train HPV phylogenetic trees illustrate lines of evolutionary descent of sample isolates and reference sequences at different nodes from the main cycle. Bootstrap values and the origin of sample isolates from study sites are shown. Among the 92 study isolate sequences, 86 of them showed a close phylogenetic relationship of over 76% Bootstrap value with reference sequences of different HPV genotypes. Six sequences with bootstrap values less than 76% were not included in the subsequent analysis.

The HPV genotype sequences were obtained from samples collected from Meru County (JKN.M05.P16), Isiolo (JKN.I21.P20), and Kirinyaga (JKN.K03.46) and Embu (JKN.G03.C5) clustered with HPV11 detected in Bangkok (KM501535.1) (Figure 4-4 a).

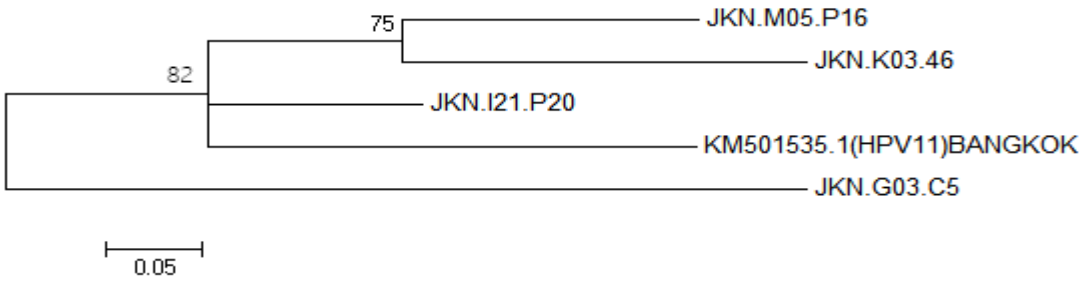

Figure 4-4 a. HPV 11 clade.

The Train phylogenic tree segment shown is constructed from phylogenic alignment illustrated in Figure 4.5 of the study isolates JKN.M05.P16, JKN.K03.46, JKNI21.P20, JKN.G03.C5 and reference sequence of HPV11 isolated from Bangkok. All study isolates and reference sequences showed a close relationship at over 75 bootstrap values.

Sample isolates from Isiolo County (JKN.I01.A5) had a close phylogenetic relationship with HPV81 reference sequences detected in Congo (JN617887.1(HPV81)CONGO)) and Bangkok (JN617887(HPV81)BANGKOK) while JKN.K21.M5) from Kirinyaga

County clustered with JN617887(HPV81)CONGO) reference sequence from Congo (Figure 4-4b).

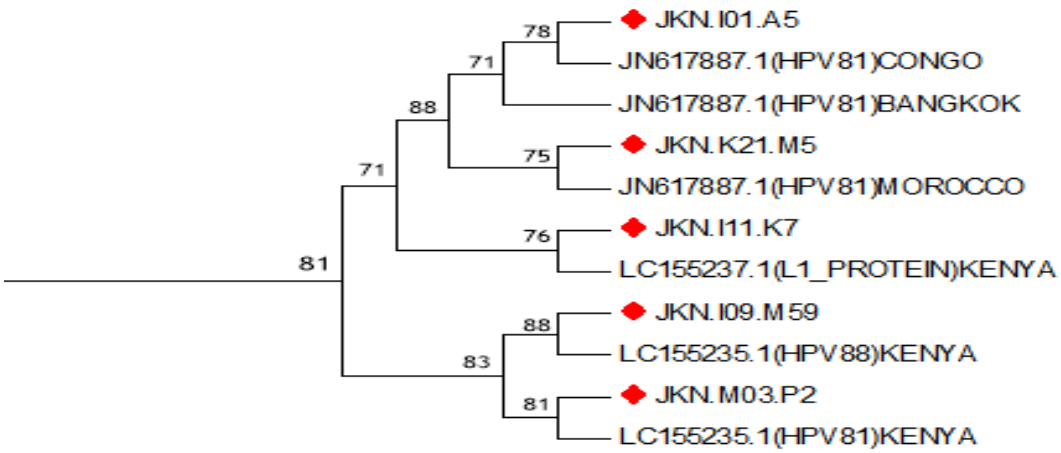

Figure 4-4b. HPV81 and HPV late (L1) protein clade.

The figure shows study isolate of HPV81: JKN.01.A5, JKN.K21.M5, HPV59: JKN.I09.M59 and JKN.MO3.P2JKN.I11.K7 and HPV late L1 protein: JKNI11.K7. Study isolates clustered with reference sequences from Kenya showed a higher phylogenetic relationship than reference sequences from Congo, Bangkok, and Morocco.

Human Papillomavirus sequences isolated from Meru (JKN.M11.P11) and Isiolo (JKN.O13.P81) Counties clustered with HPV81 detected from Thailand (KM501529.1) and Bangkok (KM501530.1) (Figure 4-4 c).

Figure 4-4 c. HPV 81 clade.

The figure shows a study isolates claustration with HPV81 sequences isolated from Thailand and Bangkok. High bootstrap values are indicative of close relation between study isolates (JKN.O13.78 and JKN.M11.P18) and reference sequences

HPV sequences isolated from Meru County (JKN.M09.X9) clustered with HPV81 sequences isolated from Kuwait [HE798612(HPV81)KUWAIT] and Kenya [JX912949(HPV81)KENYA] as shown in (Figure 4-4d).

Figure 4-4d. HPV81 clade.

The figure shows the close relationship between study isolate JKN.M09.X9 and reference sequences from Kenya and Kuwait at 79 bootstrap value and a foreclose relation with HPV strain 4486 from Belarus at 81bootstrap value.

HPV sequence isolates from Embu County (JKN.G01.L12), Isiolo (JKN.A01.1C) and Tharaka-Nithi (JKN.A07.N31) showed close phylogenetic relationship with HPV16 detected in Tunisia (KT932000.2(HPV16) TUNISIA) (Figure 4-4e).

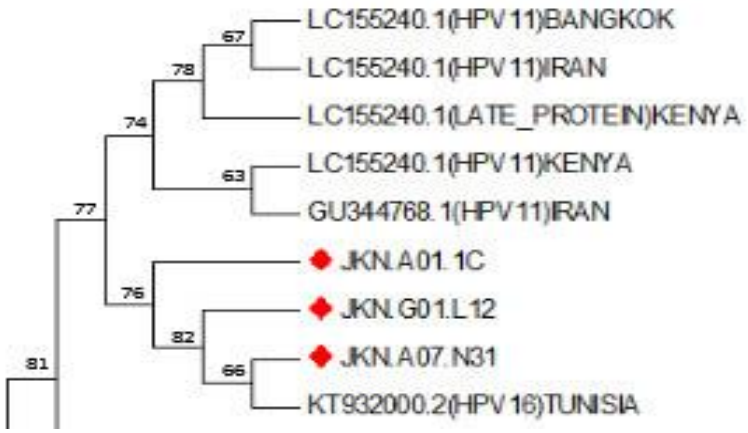

Figure 4-4e. HPV 16 and HPV11 clade.

The figure shows HPV16: JKN.AOI.1C, JKN.GO1.L12 and JKN.A07.N31 close phylogenetic relationship with an HPV16 reference sample from Tunisia at >76 bootstrap value.

Study isolates from Embu County (JKN.A03.C28) clustered with HPV sequences isolated from Iran [EF1408127(HPV66)IRAN] and Bangkok [1408121(HPV11)BANGKOK] as shown in (Figure 4-4f).

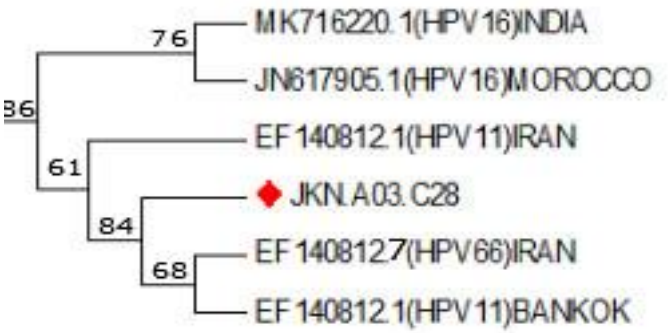

Figure 4-4f. HPV 66 and 11 clade.

The figure shows a close relationship between study isolate JKN.A03.C28 with HPV11 and HPV66 reference sequences from the same study.

Study isolate JKN.C15.8P from Isiolo County clustered with HPV16 reference sequence isolated Unites States of America [KU053906(HPV16)USA (

Figure 4-4g).

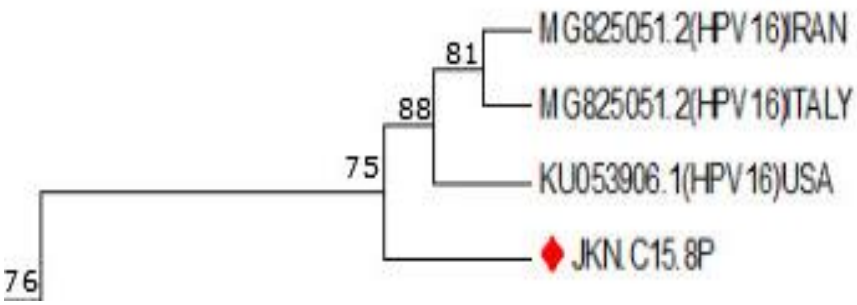

Figure 4-4g. HPV16 clade.

Study isolate JKN.C15.8P had a high sequence homology with a reference sequence from the USA, Iran and Italy at 75 bootstrap value.

Study isolate JKN.M17.M33 from Meru County clustered with HM6396221(HPV58) CHINA reference sequence from China as shown in (Figure 4-4h).

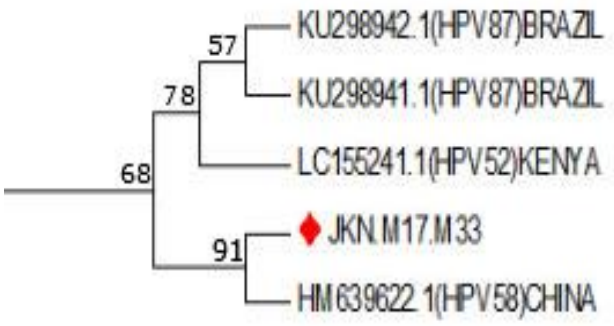

Figure 4-4h. HPV58 clade.

The figure shows high homology between study isolate JKN.M17.M33 and a reference sequence (HPV58) from China at 91 bootstrap value.

The study isolates JKN.M05.P16 from Meru County, JKN.I21.P20 from Isiolo County, JKN.KO3.46 from Kirinyaga County and JKN.G03.C5 from Tharaka-Nithi County clustered with HPV11 reference sequences from Bangkok [KM501535.1(HPV11)BANGKOK and KM501535.2(HPV11)BANGKOK] (Figure 4-4i).

Figure 4-4i. HPV11 clade.

The figure shows the study isolates claustration with reference sequences from Bangkok, Thailand. Reference samples have the same accession numbers but low bootstrap values of homology (56) indicating typing error or wrong labelling of samples.

4.3.3 Accession numbers of Human Papillomavirus (HPV) sequences isolated.

Accession Numbers (Appendix 1) were obtained after submitting the Human Papillomavirus sequences isolated to:

DNA Data Bank of Japan
Bio information and DDBJ Center
National Institute of Genetics
Research Organization of Information and Systems
Mishima, Shizuoka 411-8540, Japan
https://www.ddbj.nig.ac.jp/ddbj/updt-form-e.html

Submission ID: 60489a403a01a5001809f684

Search Engine: http://getentry.ddbj.nig.ac.jp/.

4.3.4 Human Papillomavirus (HPV) genotypes associated with cervical abnormalities in Eastern and Central Regions, Kenya.

A total of thirteen HPV genotypes were detected from 86/317 (27.1%) participants. There were 105 HPV isolates occurring as single or multiple genotype infections. The most common low-risk HPV (lrHPV) genotype detected was HPV81 [58.6% (62/106)] and HPV11 [11.3% (12/106)] while the most common high-risk HPV (hrHPV) detected were HPV66 [9.4% (10/106)] and HPV16 [11.3% (12/106)]. The remaining HPV genotypes detected were lrHPV9, lrHPV44, lrHPV61, lrHPV87, lrHPV88, hrHPV45, hrHPV53, hrHPV52 and hrHPV58. Only hrHPV53 had a higher prevalence of 2.8% (Figure 4-5).

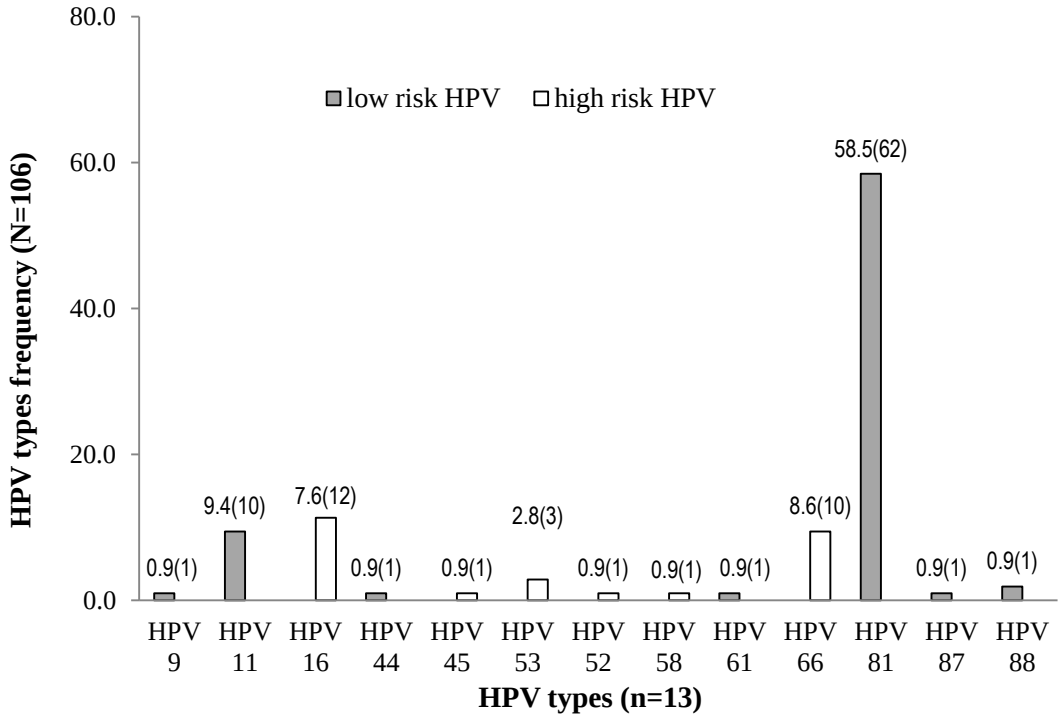

Figure 4-5. HPV type prevalence.

The graph shows Human Papillomavirus genotypes prevalence and diversity among women attending Reproductive Health Clinics in Eastern and Central Regions, Kenya. They were isolated as single or multiple HPV genotype/s infections from one or different participants (N=105). HPV genotypes with highest prevalence were high-risk (hrHPV) 16, hrHPV66, low-risk HPV (lrHPV) 81 and lrHPV11.

4.3.5 Identification of HPV genotypes as single or multiple infections among women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

A total of 67/317 (20.1%) participants were infected with a single genotype of low-risk HPV [HIV negative: HPV 81: 9/317 (2.8%) and HIV positive: HPV11: 7/317 (2.2%) and HPV81: 51/317 (16.1%)] while 9/317 (2.8%) were infected with multiple high–risk HPV genotypes. HIV-infected participants showed significantly high and more diverse HPV single and multiple HPV genotypes infection than HIV non-infected participants ($p<0.001$) (Table 4-12).

Table 4-12. Single and/or multiple HPV genotype infections in women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

HPV	HPV genotype	n (%)	HIV negative: [HPV type: n (%)]	HIV positive: [HPV type: n (%)]	p-value
Positive	**Single HPV genotype infection**				0.001***
	lrHPV	67(20.1)	HPV 81: 9(2.8)	HPV11: 7(2.2) HPV81: 51 (16.1)	
	hrHPV	4(1.2)		HPV16: 3(0.1) HPV66: 1(0.3)	
	Total	**71(21.3)**	**9(2.8)**	**62(19.5)**	
	Multiple HPV genotypes infection				
	lrHPV	2(0.6)		HPV81,44: 1(0.3) HPV81,88: 1(0.3)	
	hrHPV	9(2.8)	HPV16, 66: 3(0.9)	HPV16, 66: 4(1.2) HPV16,53: 1(0.3) HPV16,58: 1(0.3)	
	hrHPV and lrHPV	4(1.2)	HPV11,16,53, 61, 81: 1(0.3)	HPV 9,53: 1(0.3) HPV11,66: 1(0.3) HPV11,45,52,87: 1(0.3)	
Total		**15(4.6)**	**4(1.2)**	**11(3.4)**	
Positive		86(27.1)	13(4.1)	**73(23.0)**	
Total		**317(100)**	**156(49.2)**	**161(50.8)**	

***: the probability at the 0.001 level, n=317

4.3.6 Distribution of Human Papillomavirus genotypes by social demographic and risk factors associated with cervical dysplasia.

Human Papillomavirus genotype's distribution was diverse and high by HIV positive status than negative status across County of residence, age, choice of the family planning method, and the number of sex partners. The diversity in HPV genotype distribution was high and significantly associated with the number of sex partners and choice of family planning method ($p<0.005$) specifical women with more than one sex partner and those using non-hormonal contraceptives (Table 4-13).

Table 4-13. HPV genotypes prevalence by social-demographic and risk factors associated with HPV oncogenesis and HIV status among women attending Reproductive Health Clinics in Eastern and Central Regions, Kenya.

Category	HIV status	HPV genotypes infection													Total	p-value	CI
		High-risk HPV genotypes					Low-risk HPV genotypes										
		HPV16	HPV45	HPV53	HPV58	HPV66	HPV9	HPV11	HPV44	HPV52	HPV61	HPV81	HPV87	HPV88			
Residence																	
Embu	Neg	1(0.9)				1(0.9)						4(3.8)			**6(5.7)**	0.138	0.044 – 0.317
	Pos	3(2.8)	1(0.9)	2(1.9)	1(0.9)	2(1.9)	1(0.9)	4(3.8)		1(0.9)		13(12.8)	1(0.9)		29(27.6)		
Isiolo	Neg	2(1.9)				2(1.9)						1(0.9)			5(4.5)		
	Pos	1(0.9)				3(2.8)		3(2.8)	1(0.9)			11(10.5)		1(0.9)	20(19.0)		
Kirinyaga	Neg											1(0.9)			1(0.9)		
	Pos	2(1.9)				1(0.9)						8(7.6)			11(7.7)		
Meru	Neg	1(0.9)		1(0.9)				1(0.9)			1(0.9)	3(2.8)			7(6.6)		
	Pos	2(1.9)										15(14.3)			17(16.2)		
T. Nithi	Neg											1(0.9)			1(0.9)		
	Pos							2(1.9)				6(5.7)			8(7.2)		
Age in years																	
≤35	Neg	3(2.8)		1(0.9)		2(1.9)		1(0.9)				5(4.8)			12(11.4)	0.701	0.097 – 0.143
	Pos	3(2.8)	1(0.9)	2(1.9)		5(4.8)	1(0.9)	8(7.6)		1(0.9)		39(37.1)	1(0.9)	1(0.9)	62(55.8)		
>35	Neg	1(0.9)			1(0.9)	1(0.9)						4(3.8)			7(6.6)		
	Pos	5(4.8)				2(1.9)		1(0.9)	1(0.9)		1(0.9)	14(13.3)			24(22.8)		
Family planning																	
hormonal	Neg	1(0.9)				1(0.9)						4(3.8)			6(5.7)	0.036	0.08 – 0.221
	Pos	1(0.9)		2(1.9)		3(2.8)	1(0.9)	2(1.9)				10(9.5)			19(17.1)		
Other	Neg	3(2.8)		1(0.9)		2(1.9)		1(0.9)			1(0.9)	5(4.8)			13(11.7)		
	Pos	7(6.6)	1(0.9)		1(0.9)	4(3.8)		7(6.6)	1(0.9)	1(0.9)		43(40.9)	1(0.9)	1(0.9)	67(60.3)		
Parity																	
>3	Neg	1(0.9)				1(0.9)						2(1.9)			4(3.8)	0.490	0.087 – 0.181
	Pos	2(1.9)				1(0.9)		3(2.8)	1(0.9)			8(7.6)			15(14.3)		
>3	Neg	3(2.8)		1(0.9)		2(1.9)		1(0.9)				7(6.6)			14(13.3)		
	Pos	6(5.7)	1(0.9)	2(1.9)	1(0.9)	6(5.7)	1(0.9)	6(5.7)		1(0.9)	1(0.9)	45(42.8)	1(0.9)	1(0.9)	72(68.6)		
Number of sex partners																	
One	Neg	3(2.8)				3(2.8)						4(3.8)			10(9.5)	0.030	0.011 – 0.210
	Pos	3(2.8)	1(0.9)	2(1.9)	1(0.9)	1(0.9)		5(4.8)		1(0.9)		28(26.8)	1(0.9)	1(0.9)	44(40.0)		
>one	Neg	1(0.9)						1(0.9)			1(0.9)	5(4.8)			8(7.6)		
	Pos	5(4.8)		1(0.9)		6(5.7)	1(0.9)	4(3.8)	1(0.9)			25(23.8)			43(40.9)		
	Neg	**4(3.8)**				**3(2.8)**		**1(0.9)**			**1(0.9)**	**9(8.6)**					
Total		**12(7.6)**	**1(0.9)**	**3(2.8)**	**1(0.9)**	**10(9.5)**	**1(0.9)**	**10(8.6)**	**1(0.9)**	**1(0.9)**	**1(0.9)**	**62(58.5)**	**1(0.9)**	**1(0.9)**	**105(80.9)**		

N: negative; P: positive; **: the probability at the 0.001 level; *: the probability at the 0.005 level; T. Nithi: Tharaka-Nithi County β coff: beta coefficient; Neg: Negative; Pos: positive; CI: Confidence interval

4.3.7 Human Papillomavirus genotypes prevalence by visual cervical inspection.

A significantly high rate of HPV infection was established among HIV-infected [33.5% (27/81)] than HIV non-infected participants [25% (27/81)] who tested VIA/VILLI positive than those who tested VIA/VILLI negative (p<0.001). Low-risk HPV81 and high-risk HPV 16 were the most common genotypes isolated in both VIA/VILLI positive and negative outcomes (Table 4-14).

Table 4-14. HPV genotypes prevalence as single or multiple infections by cervical visual inspection and HIV status among women attending Reproductive Health Clinics in Eastern and Central Regions, Kenya.

Association between HPV genotypes prevalence and cervical visual inspection by VIA/VILLI results by HIV serostatus													
HPV genotypes infection													
VIA/VILLI negative test [n=236/317(74.4%)]													
HIV negative													
			7					2		0	1	127	137
			(3.0)					(0.8)		(0.0)	(0.4)	(53.8)	(58.1)
HIV positive													
5	1	1	36	1	1	1		2		1		50	99
(2.1)	(0.4)	(0.4)	(15.3)	(1.4)	(1.4)	(0.4)		(0.8)		(1.4)		(21.2)	(41.9)
Total													
5	**1**	**1**	**43**	**1**	**1**	**1**		**4**		**1**	**1**	**177**	**236**
(2.1)	**(0.4)**	**(0.4)**	**(18.2)**	**(1.4)**	**(1.4)**	**(0.4)**		**(1.7)**		**(1.4)**	**(0.4)**	**(75.0)**	**(100)**
Total HPV positive = 59/236(25%), p-value: 0.691													
VIA/VILLI positive test [n=81/317 (25.6%)]													
HIV negative													
			2					1				16	19
			(2.5)					(1.2)				(19.8)	(23.5)
HIV positive													
	2		15			1	1	2	1			38	62
(2.5)	(2.5)		(18.5)			(1.2)	(1.2)	(2.5)	(1.2)			(46.9)	(76.5)
Total													
2	**2**		**17**			**1**	**1**	**3**	**1**			**54**	**81**
(2.5)	**(2.5)**		**(21.0)**			**(1.2)**	**(1.2)**	**(3.7)**	**(1.2)**			**(66.7)**	**(100.0)**
Total HPV positive = 27/81(33.5), p-value: 0.001*													

***, the probability at the 0.001 level

Total HPV positive: 86/317 [VIA/VILLI positive: 59/317 and VIA/VILLI negative: 27/317.

Total VIA/VILLI negative cases are more than VIA/VILLI positive cases among participants with HPV infection. Most participants with multiple-HPV genotypes infection showed negative VIA/VILLI test results

4.3.8 Association of HPV genotypes with cervical histological results among HIV-infected and non-infected women.

Cervical histological outcome reported in association with single or Multiple HPV infection among HIV-infected and non-infected participants were: CIN1 [17 (5.4%)], CIN2 [16 (5.0%], CIN3 [6 (1.8%)] and invasive cancer [5 (1.5%)] ($p<0.001$). The majority of HIV-infected participants showed high single and multiple HPV genotype infection rates by all cervical histology outcomes than HIV-non-infected participants (Table 4-15).

Table 4-15. HPV genotypes are detected as single or multiple infections by the histological outcome in women attending Reproductive-health clinics.

HPV type of infection	Normal	§Histological analysis of abnormal cytology samples (n=78)						
		ASCUS	CIN 1	CIN2	CIN3	ICC	Total	p-value
HIV negative								
Single genotype HPV infection								
HPV 81	3(0.9)	1(0.3)	2(0.6)	2(0.6)	1(0.3)		9(2.7)	0.001*
Multiple geno**types of HPV infection**								
HPV 16,66	2(0.6)					1(0.3)	3(0.9)	
HPV11,16,53,81,61	1(0.3)						1(0.3)	
HPV Positive(total)	**6(1.9)**	**1(0.3)**	**2(0.6)**	**2(0.6)**	**1(0.3)**	**1(0.3)**	**13(4.1)**	
HPV Negative	**137(43.2)**	**6(1.8)**					**143(45.1)**	
Total (HPV positive/negative)	**143(45.1)**	**7(2.1)**	**2(0.6)**	**2(0.6)**	**1(0.3)**	**1(0.3)**	**156(49.2)**	
HIV positive								
Single genotype								
HPV 11	2(0.6)	1(0.3)	2(0.6)	1(0.3)	1(0.3)		7(2.2)	0.001*
HPV 16	1(0.3)			1(0.3)		1(0.3)	3(0.9)	
HPV 66				1(0.3)			1(0.3)	
HPV 81	21(6.6)	5(1.6)	12(3.8)	6(1.9)	4(1.2)	3(0.9)	51(16.1)	
Multiple genotypes								
HPV11, 66				1(0.3)			1(0.3)	
HPV 81,44				1(0.3)			1(0.3)	
HPV 81, 88				1(0.3)			1(0.3)	
HPV 9,53				1(0.3)			1(0.3)	
HPV 16,58				1(0.3)			1(0.3)	
HPV 16,66	3(0.9)	1(0.3)					4(1.2)	
HPV 66,53			1(0.3)				1(0.3)	
HPV 11,45,52,87	1(0.3)						1(0.3)	
HPV Positive (total)	**28(8.8)**	**7(2.1)**	**15(4.7)**	**14(4.4)**	**5(1.6)**	**4(1.2)**	**73(23.0)**	
HPV Negative	**68(21.4)**	**20(6.3)**					**88(27.8)**	
Total (HPV positive /negative)	**96(30.3)**	**27(8.5)**	**15(4.7)**	**14(4.4)**	**5(1.6)**	**4(1.2)**	**161(50.8)**	
Total (single/multiple inf.)	**239(75.4)**	**34(10.7)**	**17(5.4)**	**16(5.0)**	**6(1.8)**	**5(1.5)**	**317(100.)**	

ASCUS: Atypical Cells of Unknown Significance; CIN: Cervical Intraepithelial Neoplasia; ICC: Invasive cervical cancer; *: the probability at the 0.001 level, § abnormal cytology samples: Atypical Cells of Unknown Significance (ASCUS), Low-grade squamous intraepithelial lesion (LSIL), High-grade squamous intraepithelial lesion (HSIL), atypical squamous cells, cannot exclude HSIL (ASC-H) or Atypical glandular cells (AGC)].

4.3.9 Human Papillomavirus (HPV) genotypes prevalent by a history of other infections.

Majority of women who reported a history of genital warts [67/317 (21.1%)] and Urinary-tract infection (UTI) [68/317 (21.5%)] were mostly infected by low-risk HPV81 [48/317 (15.1%], HPV11 [genital warts: 6/317 (11.1%; UTI: 5/317 (2.1%)] and confected by high risk HPV16 and 66 [genital warts: 7/317 (2.2%); UTI: 5/317 (2.1%)]. Most HIV-infected women with a history of genital warts and recurrent UTI had more diverse HPV genotype infections than HIV-non-infected participants. There was a significant association between genital infections and HIV where most participants who reported a history of infection by warts and recurrent UTI were HPV positive ($p<0.001$) (Table 4-16).

Table 4-16. Association between the history of genital warts, rush and recurrent urinary tract infections among women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

History of infection (<10years)	HIV	HPV genotypes prevalence and history of warts and Urinary Tract infections (UTI) by HIV serostatus													Total with no history of infection	Total with/without a history of infection	*p-value*
		HPV 11	**HPV 16**	**HPV 66**	**HPV 81**	**HPV 11, 66**	**HPV 44, 81**	**PV 81, 88**	**HPV 9,53**	**HPV 16,58**	**HPV 16,66**	**HPV 66,53**	**HPV 11,45,52 ,87**	**HPV 11,16,53 ,81,61**	**Total with history of infection**			
Genital warts and/or rush																		
Yes	**Negative**				6 (1.8)						3 (0.9)			1 (0.3)	10 (3.1)	115 (36.3)	125 (39.4	***<0.001******
	Positive	6 (1.8)	2 (0.6)	1 (0.3)	42 (13.2)			1 (0.3)			4 (1.6)		1 (0.3)		57 17.9	68 (21.4)	125 (39.4)	
	Total	**6 (1.9)**	**2 (0.6)**	**1 (0.3)**	**48 (15.1)**			**1 (0.3)**			**7 (2.2)**		**1 (0.3)**	**1 (0.3)**	**67 (21.1)**	**183 (57.7)**	**250 (78.9)**	
None	**Negative**				3 (0.9)										3 (0.9)	7 (2.2)	10 (2.2)	
	Positive	1 (0.3)	1 (0.3)		9 (2.8)	1 (0.3)	1 (.03)		1 (0.3)	1 (0.3)		1 (0.3)			16 (5.0)	29 (9.1)	45 (14.2)	
	Total	**1 (0.3)**	**1 (0.3)**		**12 (3.8)**	**1 (0.3)**	**1 (0.3)**		**1 (0.3)**	**1 (0.3)**		**1 (0.3)**			**19 (6.0)**	**48 (15.1)**	**67 (21.1)**	
Recurrent UTI																		
Yes	**Negative**				7 (2.2)						2 (0.6)		1 (0.3)	1 (0.3)	11 (3.5)	110 (34.7)	120 (37.9)	***<0.001******
	Positive	5 (2.1)	2 (0.8)	1 (0.3)	41 (16.9)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	3 (1.2)				57 (18.0)	66 (27.2)	123 (38.8)	
	Total	**5 (2.1)**	**2 (0.8)**	1 (0.3)	**48 (15.1)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**5 (2.1)**		1 (0.3)	1 (0.3)	**68 (21.5)**	**176 (55.5)**	**243 (76.7)**	
None	**Negative**				2 (0.6)						1 (0.3)				3 (0.9)	33 (10.4)	36 (11.4)	
	Positive	2 (0.6)			10 (3.2)						1 (0.3)	1 (0.3)			14 (4.4)	22 (6.9)	36 (12.0)	
	Total	**2 (0.6)**			**12 (3.8)**						**2 (0.6)**	1 (0.3)			**17 (5.4)**	**57 (18.0)**	**74 (23.0)**	
Total HPV infection (HIV positive and negative)		**7 (2.2)**	**3 (0.9)**	**1 (0.3)**	**60 (18.9)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**7 (2.2)**	**1 (0.3)**	**1 (0.3)**	**1 (0.3)**	**86 (27.1)**	**231 (72.9)**	**317 (100.0)**	

***, the probability at the 0.001 level, UTI: Urinary Tract Infection

4.4 Knowledge, attitude, practice, and perception (KAPP) on factors associated with Human Papillomavirus (HPV) oncogenesis among women in Eastern and Central Regions, Kenya.

Knowledge of cervical cancer and its signs and symptoms, KAPP on cervical screening and vaccination, and risk practices associated with HPV oncogenesis varied by social-demographic factors of participants.

4.4.1 Knowledge of cervical cancer.

Almost all participants knew about cervical cancer [297(93.7%)] while only 20(6.3%) did not know about the disease (Figure 4-6).

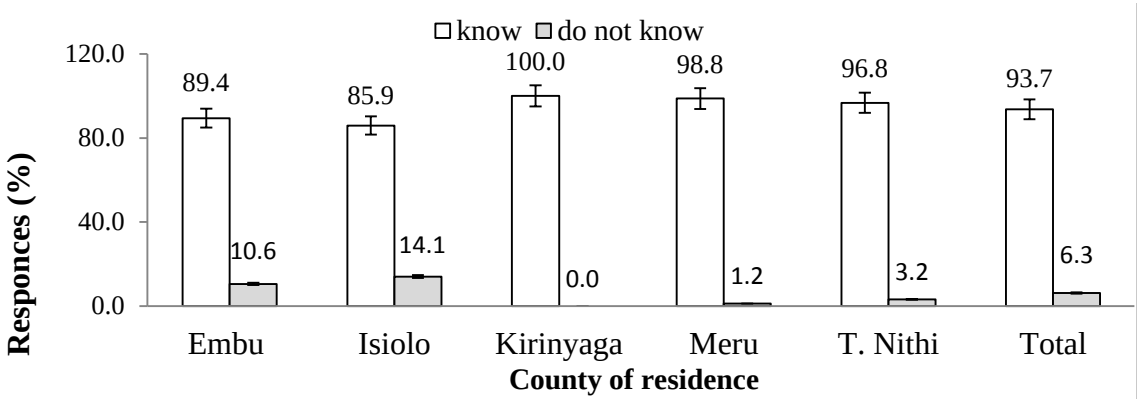

Figure 4-6. Knowledge of cervical cancer by County of residence among women attending Reproductive Health Clinics in Eastern and Central Regions, Kenya (*p=0.002*).

The graph shows the percentage frequency of 'Yes' and 'No' answers are given by participants from Eastern Kenya Counties upon filling out the questionnaire. The question asked to participants was: Do you know what cervical cancer is?

4.4.2 Awareness of symptoms of cervical cancer.

Most women were aware that having smelly vaginal discharge [281/317 (88.6%)] and persistent lower abdominal pain [283/317 (89.3%)] were symptoms associated with cervical cancer whereas most of them were not aware of dyspareunia [275 (86.8%)], unexplained post-menstruation [281/317 (88.6%)] (Figure 4-7).

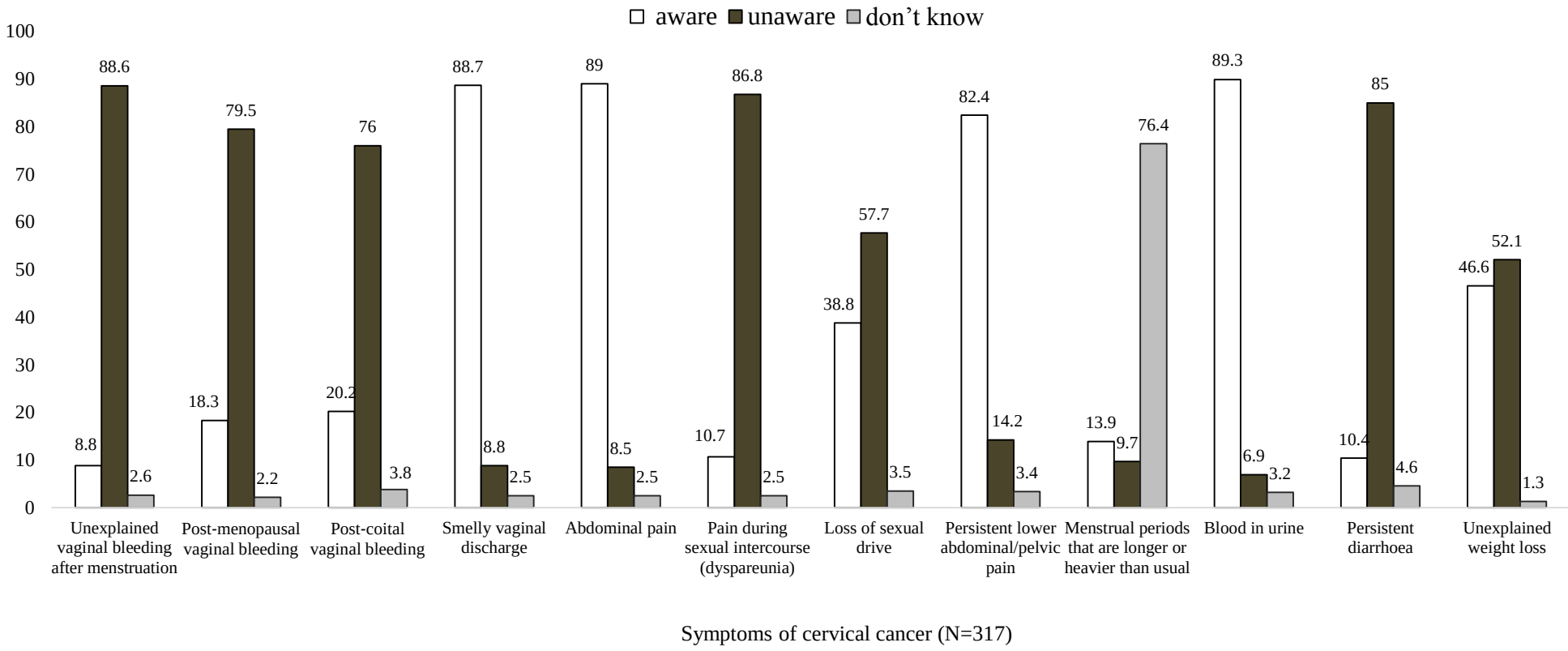

Figure 4-7. Knowledge of the symptom of cervical cancer among women attending Reproductive-health clinics in Eastern and Central Regions, Kenya (N=317).

The graph shows the number of 'Yes', 'No', and 'I don't know' responses given by participants upon filling out a questionnaire. Respondents were asked to tick symptoms associated with cervical cancer

4.4.3 Knowledge, attitude, practice and perception (KAPP) on cervical screening.

Overall, 134/317 (42.3%) of study participants had knowledge, 291/317 (91.8%) had a positive attitude, and 273/317 (86.1%) had a positive perception. and 34/317 (10.7%) reported a history of ever having undergone cervical screening. The majority of the participants with knowledge of cervical screening were from Tharaka-Nithi [17(54.8%)] and Meru Counties [41(50.6%)]. However, the County of residence did not influence knowing the procedure ($p>0.005$). Age was significantly associated ($p=0.042$) with knowing about cervical screening where most participants below 35 years of age [101(62.3%)] lacked knowledge of the procedure unlike those aged 35 years and above [73(47.1%)]. Education level was also significantly associated ($p=0.047$) with knowledge of cervical screening. The majority of participants with a university education level knew about cervical screening while those with primary school education lacked knowledge of the procedure. Most participants were in low-income status [65.7% (208/317)]. They reported a low cervical screening rate [9.6% (20/208)] and significantly ($p=0.012$) low knowledge of cervical screening procedures than high- and medium-income status (Table 4 – 17)

Table 4-17. Association between social-demographic characteristics and KAPP on HPV screening among women attending the Reproductive Health Clinics in Eastern and Central Regions, Kenya.

Characteristic	Category	Total	KAPP on cervical screening							
			Knowledge n(%)		Attitude n(%)		Practice n(%)		Perception n(%)	
			Positive	Negative	positive	Negative	Positive	negative	Positive	negative
Facility	Embu	**85(26.8)**	27(31.8)	58(68.2)	78(91.8)	7(8.2)	13(15.3)	72(84.7)	69(81.2)	16(18.8)
	Isiolo	**64(20.2)**	24(37.5)	40(62.5)	61(95.3)	3(4.7)	7(10.9)	57(89.1)	57(89.1)	7(10.9)
	Kirinyaga	**56(17.6)**	25(44.6)	31(55.4)	48(85.7)	8(14.3)	4(7.1)	52(92.9)	46(82.1)	10(17.9)
	Meru	**81(25.6)**	41(50.6)	40(49.4)	74(91.4)	7(8.6)	7(8.6)	74(91.4)	72(88.9)	9(11.1)
	Tharaka Nithi	**31(9.8)**	17(54.8)	14(45.2)	30(96.8)	1(3.2)	3(9.7)	28(90.3)	29(93.5)	2(6.5)
		p-value	*0.604*		*0.304*		*0.556*		*0.289*	
Age in years	<35	**162(51.1)**	61(37.7)	101(62.3)	149(92.0)	13(8.0)	15(9.3)	147(90.7)	140(86.4)	22(13.6)
	>35	**155(48.9)**	82(52.9)	73(47.1)	142(91.6)	13(8.4)	19(12.3)	136(87.7)	133(85.8)	22(14.2)
		p-value	*0.042***		*0.906*		*0.388*		*0.875*	
Education level	Primary	**96(30.0)**	33(34.4)	63(65.6)	87(90.6)	9(9.4)	16(16.7)	80(83.3)	82(85.4)	14(14.6)
	Secondary	**135(42.6)**	58(43.0)	77(57.0)	125(92.6)	10(7.4)	13(9.6)	122(90.4)	116(85.9)	19(14.1)
	College	**67(21.1)**	30(44.8)	37(55.2)	60(89.6)	7(10.4)	3(4.5)	64(95.5)	57(85.1)	10(14.9)
	University	**19(6.0)**	13(68.4)	6(31.6)	19(100.0)	0(0.0)	2(10.5)	17(89.5)	18(94.7)	1(5.3)
		p-value	*0.047***		*0.487*		*0.092*		*0.733*	
Marital status	Married	**226(71.3)**	85(37.6)	141(62.4)	208(92.0)	18(8.0)	23(10.2)	203(89.8)	191(84.5)	35(15.5)
	separated	**32(10.1)**	15(46.9)	17(53.1)	29(90.6)	3(9.4)	4(12.5)	28(87.5)	29(90.6)	3(9.4)
	Single	**41(2.9)**	20(48.8)	21(51.2)	38(92.7)	3(7.3)	5(12.2)	36(87.8)	38(92.7)	3(7.3)
	Divorced	**6(1.9)**	3(50.0)	3(50.0)	5(83.3)	1(16.7)	1(16.7)	5(83.3)	5(83.3)	1(16.7)
	Widowed	**12(3.8)**	11(91.7)	1(8.3)	11(91.7)	1(8.3)	1(8.3)	11(91.7)	10(83.3)	2(16.7)
		p-value	*0.009***		*0.945*		*0.982*		*0.753*	
Income status	Low	**208(65.7)**	76(36.5)	132(63.6)	191(91.8)	17(8.2)	20(9.6)	188(85.6)	178(56.2)	30(14.4)
	Medium	**95(30.0)**	52(54.7)	43(45.3)	87(91.6)	8(8.4)	14(14.7)	81(85.3)	83(87.4)	12(12.6)
	High	**14(4.3)**	6(42.9)	8(57.1)	13(92.9)	1(7.1)	-	14(100.0)	12(85.7)	2(14.3)
		p-value	*0.012***		*0.987*		*0.17*		*0.915*	
Total		**317(100.0)**	**134(42.3)**	**183(57.7)**	**291(91.8)**	**26(8.2)**	**34(10.7)**	**283(89.3)**	**273(86.1)**	**44(13.9)**

***, the probability at the 0.001 level; Income level: low (1.90), middle (1.9-5.5) and high (> 5.50) US$ PPP/day)

4.4.4 Awareness of the frequency of cervical screening.

Most women lacked awareness of the cervical screening cycle [43% (137/317)] whereas only 14% (43/317) were aware that the cervical screening cycle is 5 years (Figure 4-8).

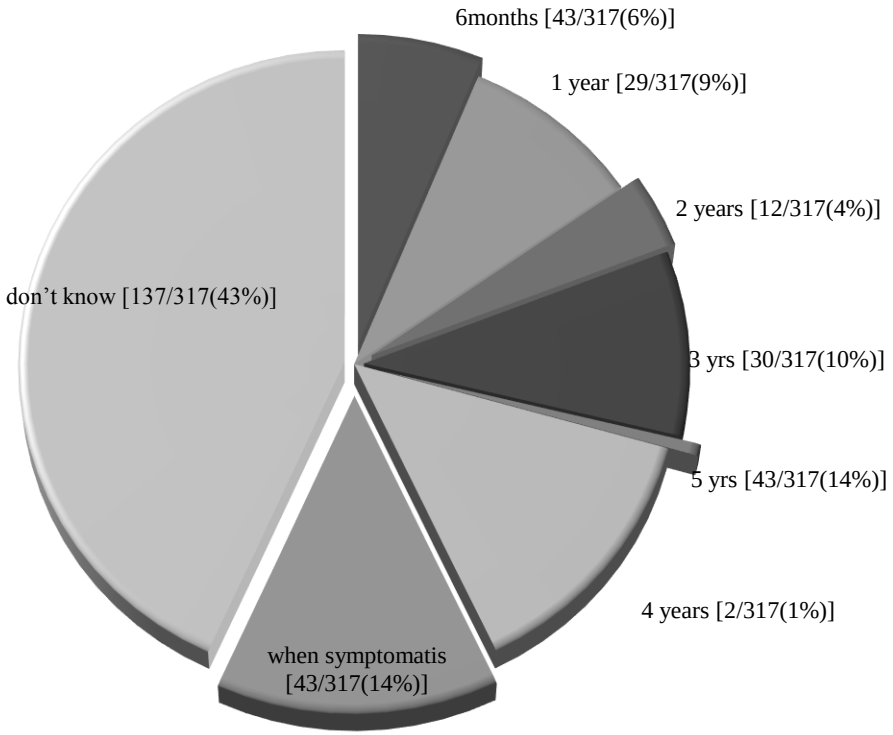

Figure 4-8. Awareness of cervical screening cycle (N=317).

The pie chart shows responses given by participants given by participants on the cervical screening cycle upon filling out a questionnaire. The question asked was: How frequently should one do a cervical screen?

4.4.5 Perspective on cervical cancer heredity.

The majority of participants who reported having a relative/s diagnosed with cervical cancer had undergone cervical screening [64.6% (113/175)] whereas most of those who reported no history of a close relative/s diagnosed with cervical cancer had not undergone cervical screening [84.5% (120/142)]. There was a significant association

between having a close relative diagnosed with cervical cancer and a participant's decision to undergo cervical screening (p<0.001). Family history of cervical cancer persuaded most participants to undergo cervical screening (Table 4-18).

Table 4-18 Association between relative/s history of cervical cancer and screening pattern.

		History of ever been screening [N(%)]		Total	p-value
		Ever screened	Never screened		
Participants with a relative/s diagnosed with cervical cancer	Yes	113(64.6)	62(35.4)	175(55.2)	0.001***
	No	22(15.5)	120(84.5)	142(44.2)	
	Total	**135(42.6)**	**182(57.6)**	**317(100.0)**	

***: the probability at the 0.001 level

4.4.6 Cervical screening rate by the source of information.

Television [70/317 {22.1%] with a screening uptake of 17/317(5.4%) was the most persuasive source of information for cervical screening compared to radio [72/317 {22.7%] with a screening uptake of 2/317(0.6%)}] and service providers [83/317 {26.2%] with screening uptake of 7/317(2.2%)}] (Figure 4-9).

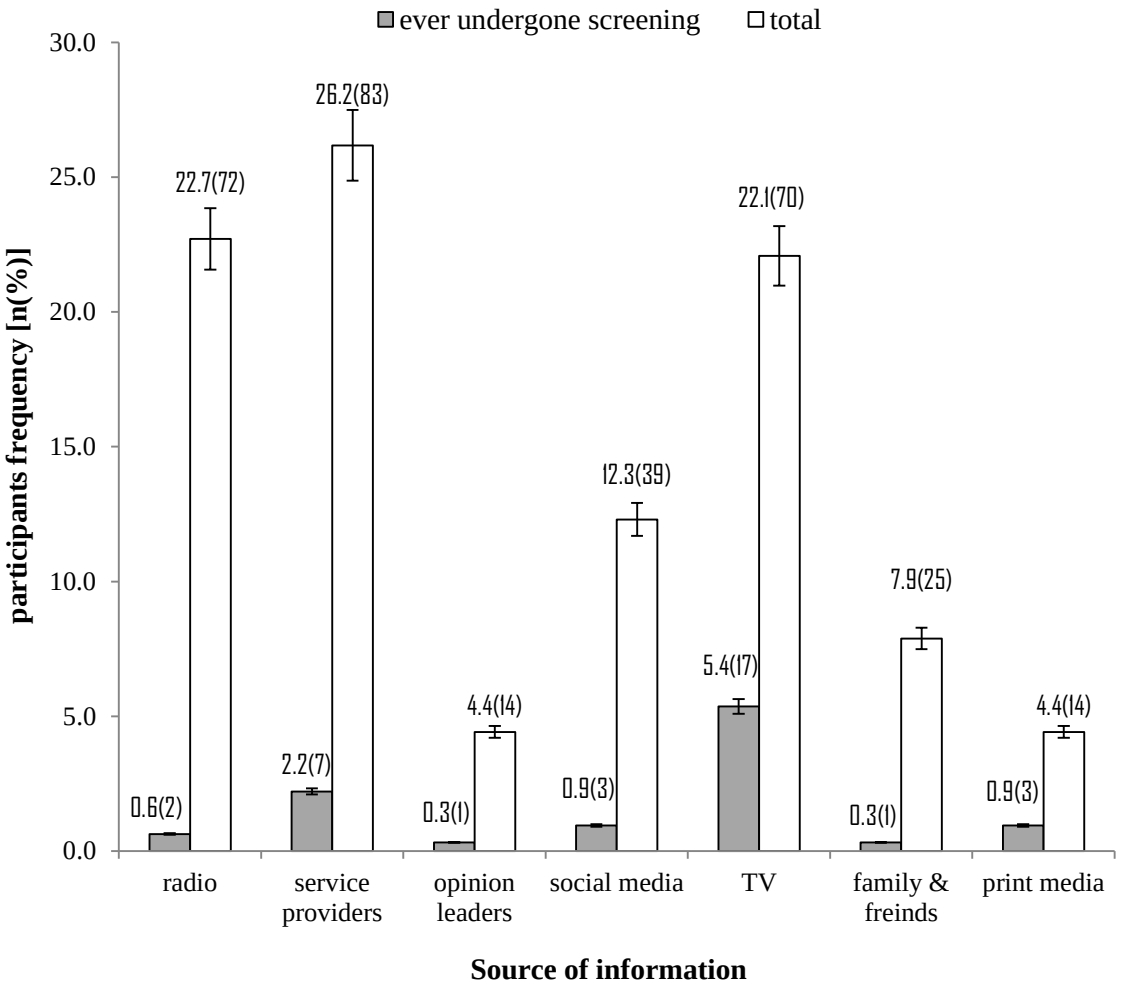

Figure 4-9. Relationship between the source of information on cervical cancer and history of cervical screening among women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

The graph shows responses given by participants on information sources on cancer of the cervix and reported history of cervical screening. The question asked was: Where do you mainly get information on cervical cancer? Responses were then compared with data on the history of cervical screening to determine if the source of information was persuasive in cervical screening uptake. Television was the main source of information that persuaded more women into cervical screening.

4.4.7 Association between social-demographic factors, cervical screening rate, and awareness of cervical cancer risk factors.

High odds [OR: 2.9(CI:1.02-8.6)] were reported by parity among women with awareness of the risk of smoking to the development of cervical cancer whereas those with three children and below had a higher cervical screening rate than those with more than three children. Participants across all education levels with the awareness that smoking habits and HIV infection are risk factors associated with cervical cancer were at significant risk of not undergoing cervical screening (Table 4-19).

Table 4-19. Relationship between cervical screening rate and awareness of risk factors associated with cervical cancer risk factors.

Characteristics	Category	Total [N/ (%)]	Screening rate by awareness of cervical cancer risk factors [Odd Ratio (CI)]									p-value
			CXC SR [n (%)]	Multiple sexes Partners	smoking habit	Prolonged hormonal contraceptive	high parity (>3)	Early sex debut¶	Recurrent warts infection	Recurrent UTI Infection	HIV infection	
Residence	Meru	81(25.6)	7(8.6)	1.4(0.6-3.0)	1.61(0.4-7.1)	0.83(0.6-1.1)	0.8(0.6-1.1)	0.8(0.6-1.1)	0.14(0.6-1.1)	0.84(0.6-1.1)	0.8(0.6-1.1)	0.556
	T. Nithi	31(9.8)	3(9.3)									
	Kirinyaga	56(17.6)	4(7.1)									
	Embu	85(26.8)	13(15.3)									
	Isiolo	64(20.2)	7(10.9)									
Age	<30	219(69.1)	24(11.0)	0.9(0.4-1.9)	0.9(0.4-2.0)	0.9(0.42-2.0)	0.9(0.4-2.0)	0.7(0.2-2.8)	1.3(0.61-2.9)	1.5(0.74-3.5)	1.07(0.5-2.2)	0.841
	>30	98(30.8)	10(10.2)									
Education	Primary	96(30.3)	16(16.7)	1.6(0.6-4.2)	0.3(0.0-0.8)	1.4(0.4-4.3)	1.17(0.4-9.)	0.8(0.2-2.9)	1.25(0.5-2.8)	0.61(0.3-1.3)	0.1(0.3-0.9)	0.042*
	Secondary	135(42.6)	13(9.6)									
	College/uni	86(27.1)	5(15.0)									
Marital status	Married	228(71.9)	23(10.2)	1.51(0.6-4.1)	1.62(0.4-7.1)	1.55(0.5-4.6)	1.1(0.1-8.6)	0.8(0.0-2.8)	1.36(0.6-3.1)	0.6(0.3-1.34)	1.57(0.8-3.3)	0.967
	Single/ separated	89(28.1)	11(33.0)									
Religion	Christian	255(80.4)	30(11.8)	1.5(0.5-3.9)	1.21(0.4-3.6)	1.66(0.6-4.9)	1.14(0.1-9.3)	0.77(0.2-2.1)	1.33(0.5-2.9)	0.61(0.3-1.3)	1.09(0.5-2.1)	0.225
	Muslim	62(19.6)	4(6.5)									
FP method	Hormonal	94(29.4)	11(3.5)	0.76(0.4-1.4)	0.77(0.3-1.8)	0.48(0.2-0.8)	0.85(0,5-1.5)	1.57(0.6-3.7)	0.69(0.4-1.2)	1.34(0.8-2.2)	0.86(0.5-1.4)	0.695
	Non-hormonal	223(70.3)	23(7.3)									
Parity	≤3	249(78.5)	25(7.9)	1.08(0.9-1.3)	2.9(1.02-8.6)	0.83(0.04-1.74)	0.39(0.1-1.4)	0.65(0.24-1.7)	1.03(0.5-2.05)	0.59(0.3-1.05)	0.6(0.36-1.0)	0.215
	>3	68(21.5)	9(2.8)									
Total		317(100)	34(10.7)									

¶Early sex debut defined from<12 years, CaCx SR: Cervical cancer screening rate, UTI: Urinary Tract Infection, HIV: Human Immunodeficiency Virus, FP method: family planning method, CI: confidence interval; **: the probability at the 0.005 level.

The table shows an association between baseline social demographic factors and cervical screening rate (p-value). It also shows the Binary Logistic regression association between social-demographic characteristics and cervical screening rate by awareness of risk factors associated with cervical cancer.

4.4.9 Knowledge, attitude, practice and perception (KAPP) on Human Papillomavirus vaccination.

The majority of participants had knowledge [77.1% (244/317)], a high willingness to undertake the HPV vaccine [85.1% (273/317)] and had positive perception [96.9% (307/317)] of the importance of HPV vaccination but no one had ever undergone HPV vaccination. County of residence, age, education level, marital, and income status were not predictor values for HPV vaccination (p>0.005) (Table 4-20).

Table 4-20. KAPP on HPV vaccination by social-demographic factors.

Characteristics	Total [N(%)]	Knowledge, attitude, practice and perception of HPV vaccination [N%)]							
		Knowledge		Attitude		Practice		Perception	
		Positive	Negative	Positive	negative	positive	negative	Positive	negative
facility									
Embu	**85(26.8)**	60(18.9)	60(18.9)	68(24.9)	17(38.6)	-	82(26.8)	81(26.4)	4(40.0)
Isiolo	**64(20.2)**	48(15.1)	16(5.0)	60(22.0)	4(9.1)	-	64(20.2)	63(20.5)	1(10.0)
Kirinyaga	**56(17.6)**	45(14.5)	11(3.5)	49(17.9)	7(15.9)	-	56(17.7)	55(17.9)	1(10.0)
Meru	**81(25.6)**	68(21.5)	13(4.1)	70(25.6)	11(25.0)	-	81(25.6)	78(25.4)	3(30.0)
Tharaka-Nithi	**31(9.8)**	23(7.3)	8(2.5)	26(9.5)	5(11.4)	-	31(9.8)	30(9.8)	1(10.0)
	p-value		*0.307*		*0.199*		-		*0.804*
Age in years									
<35years	**162(51.1)**	121(68.)	41(12.9)	140(44.2)	22(6.9)	-	162(51.1)	157(49.5)	5(1.6)
>35 years	**155(48.9)**	123(31.6)	32(10.1)	133(42.0)	22(6.9)	-	155(48.9)	1(1.6)	5(1.6))
	p-value		*0.324*		*0.943*		-		*0.943*
Education level									
Primary	**96(30.3)**	72(29.2)	24(32.9)	72(29.5)	15(34.1)	-	96(30.3)	92(30.0)	4(40.0)
Secondary	**135(42.6)**	105(43.)	30(41.1)	105(43.0)	21(47.7)	-	135(42.6)	131(42.7)	4(40.0)
College	**67(21.1)**	54(22.1)	13(17.8)	54(22.1)	7(15.9)	-	67(21.1)	66(21.5)	1(10.0)
University	**19(6.0)**	13(5.3)	6(8.2)	13(5.3)	1(2.3)	-	19(6.0)	18(5.9)	1(10.0)
	p-value		*0.673*		*0.493*		-		*0.748*
Marital status									
married	**226(71.3)**	172(70.5)	54(74.0)	190(69.6)	36(81.8)	-	226(71.3)	216(70.4)	10(100)
separated	**32(10.1)**	26(10.7)	6(8.2)	29(10.6)	3(6.8)	-	32(10.1)	32(10.4)	0(0.0)
single	**411(12.9)**	34(13.9)	7(2.7)	38(13.9)	3(6.8)	-	411(12.9)	41(13.4)	0(0.0)
divorced	**6(1.9)**	4(1.6)	2(2.7)	6(2.2)	0(0)	-	6(1.9)	6(2.0)	0(0.0)
widowed	**12(3.8)**	8(3.3)	4(5.5)	10(3.7)	2(4.2)	-	12(3.8)	12(3.9)	0(0.0)
	p-value		*0.306*		*0.286*		-		*0.439*
Income status									
low	**208(65.6)**	161(66.)	47(64.4)	174(63.7)	34(77.3)	-	208(65.6)	201(65.5)	7(70.0)
Middle	**95(30.0)**	72(29.5)	23(31.5)	86(31.5)	9(20.5)	-	95(30.0)	92(30.0)	3(30.0)
high	**14(4.4)**	11(4.5)	3(4.1)	13(4.8)	1(2.3)	-	14(4.4)	14(4.6)	0(0.0)
	p-value		*0.943*		*0.209*		-		*0.748*
Total	**317(100)**	**244(77.1)**	**73(23.0)**	**273(86.1)**	**44(13.9)**	**-**	**317(100)**	**307(96.9)**	**10(3.1)**

+ve; positive KAPP; -ve: negative KAPP

4.4.10. Association between social-demographic variable with knowledge, attitude, practice and perception on HPV vaccination.

Participants from Meru County [OD: 0.62, CI: 0.12-1.7] were less likely to know about HPV vaccination in comparison to other Counties while participants who used non-hormonal contraceptives were highly likely to lack information on the practice [OD: 0.26, CI: 0.74-0.97] (p-value<0.005). (Table 4-21).

Table 4-21. Association of social demographic factors with KAPP on HPV vaccination among women attending Reproductive-health clinics in Eastern and Central Regions, Kenya.

Category	n	Odds ratio (CI)			
		Knowledge	Attitude	Practice†	Perception
Residence					
Embu	85	0.46(0.16-1.33)	0.76(0.27-2.29)	-	0.67(0.73-6.28)
Isiolo	64	0.57(0.190-1.7)	2.88(0.7-11.61)	-	2.10(0.13-34.73)
Kirinyaga	56	0.48(0.157-1.4)	1.34(0.39-4.66)	-	1.83(0.11-30.37)
Meru	81	0.62(0.12-1.7) *	1.22(0.39-3.86) *	-	0.867(0.87-8.66)
Education level					
Primary	96	0.41(0.11-1.56)	0.47(0.55-4.13)	-	0.36(0.22-61.54)
Secondary	135	0.67(0.18-2.52)	0.30(0.04-2.42)	-	1.27(0.13-12.11)
College	86	0.51 (0.14-1.87)	0.030(0.4-2.38)	-	1.82(0.19-17.19)
Contraceptives use					
Hormonal	236	0.64(0.37-1.08)	0.98(0.50-2.02)	-	0.466(0.16-1.32)
Non-hormonal	81	0.93(0.93-2.69)	0.99(0.49-1.99)		0.26(0.74-0.97) *
Parity					
<2		1.41(1.28-1.5) *	1.72(1.5-1.9) *	-	3.27(2.6-4.17) **

M: Mean, SD: Standard deviation, - no data available; not vaccinated, ***: the probability at the 0.001 level; **: the probability at the 0.005 level. †: no practice i.e., vaccination.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Performance characteristics of cervical screening methods based on histopathology of abnormal cytology outcome among HIV-infected and non-infected women in Eastern and Central Regions, Kenya.

On average, HIV-infected women had significantly high abnormal cervical screening outcomes by all methods and HIV status (average: 27.4% (HIV-infected: 21.5%; HIV non-infected: 5.9%)). Pap smear tests reported high abnormal cervical screening outcomes (30.2%) than other methods. A high HIV infection rate was also established by Pap smear outcome of *Candida spp* and bacterial infection that is associated with cervical basal layer disruption allowing HPV and HIV entry (Li *et al.,* 2021; Xing *et al.*, 2021). HIV infection has been detailed to favour HPV acquisition and persistence. This could be the reason for the higher HPV infection rate and varying degrees of cervical inflammation from mild to severe among HIV-infected women in this study and others (Stelzle *et al.*, 2021; Pineros *et al.,* 2018). Abnormal histology results (63.1% (78/122)); ASCUS and higher lesions (CIN1, CIN2, CIN3 and SCC and ICC) showed significant association ($p<0.001$) with VIA test (25.6%), Pap smear (30.2%) and HPV DNA-PCR (26.5%) results. There is growing evidence regarding the impact of ART among HIV-infected women on HPV-associated lesions (WHO, 2021) which calls for further studies.

The diagnostic value of primary test with VIA (True-Positive (TP): 16.4%, True-Negative (TN): 61.2%, False-Negative (FN): 14.2% and False-Positive (FP): 8.2%) significantly reduced upon concurrently testing with Pap smear (TP: 14.5%, TN: 57.7%, FN: 6.3% FP: 14.5%) and HPV DNA-PCR. False-positive results that are incorrect outcomes of lesion-free women as CIN+ and FN results where CIN+ cases are not

detected are a common occurrence in cervical screening (Han *et al.,* 2017). Papillomavirus DNA-PCR primary testing produced negative results of ASCUS (7.3%) and in each category of CIN+ (0.3%) positive samples. However, CIN1, CIN2, CIN3 and ICC had 3.5%, 5.4%, 4.7% 1.6% and 1.3% HPV True positive tests respectively. These values decreased upon triage test of HPV DNA-PCR with VIA (2.2%, 4.4%, 3.8%, and 1.6% for ASCUS, CIN1, CIN2 and CIN3 respectively). Failure to detect HPV in CIN+ samples occurs in HPV False-negative cases as seen in this study or when the cervical abnormality is misclassified and when the cervical abnormality is HPV-independent (Xing *et al.,* 2021). When Pap smear abnormal samples were subjected to HPV DNA-PCR test, the number of TP (15.8%) and TN (65.6%) reduced to 14.8% and 58.4% respectively. Literature review triage testing produces minimal FP and FN than primary testing alone as established in this study. Therefore, primary results are applied in the "screen-and-treat approach", greater populace benefits but the approach may lead to unnecessary treatment of negative cases or ignoring positive ones hence allowing widow neoplastic changes to occur leading to cervical cancer as well as delay early detection (Marit *et al.,* 2021; Xing *et al.,* 2021; Han *et al.,* 2017)

In cervical screening, specificity, PPV and diagnostic accuracy of a screening method is reflex of FP outcome which can be a result of abnormal cervical cytology cases that spontaneously regress without progressing to cervical neoplasia upon biopsy (Xing *et al.,* 2021). In this study, the VIA test had the highest false-positive result 52/317(16.4%)] than Pap smear [32(10.1%)] and HPV DNA-PCR [31(0.9%)] resulting in low sensitivity, specificity, and PPV (probability that a positive test is a True positive) and NPV (negative test is a True negative) as reported in other studies (Xing *et al.,* 2021;

Han *et al.,* 2017). VIA sensitivity, specificity, NPV and diagnostic accuracy were notably high among HIV-infected than non-infected women.

Test sensitivity and Negative Predictive value increased when: 1) a triage testing approach was applied instead of primary testing, 2) when HPV DNA-PCR was part of any triage, and 3) when applied for the detection of CIN+ without ASCUS. Sensitivity in the detection of ASCUS with CIN+ by VIA (53.6%), HPV-DNA-PCR (61.9%) and Pap cytology (61.7%) increased to 92% (VIA – Pap), 84.3% (HPV DNA-PCR – VIA) and 90.4% (Pap – HPV DNA-PCR) hence reducing the number of women who would have been referred for colposcopy in a resource-constrained setting. Specificity and PPV were high in 1) primary and triage cervical screening approach when confirmed with CIN+ without ASCUS and, 2) among HIV-infected than non-infected women. The reason could be that there was a high number of true-positive cases obtained when CIN+ samples were applied in confirmation unlike when ASCUS and CIN+ were combined. Latent and initial stages of HPV infection often have a low incidence of cervical neoplasia and a higher chance of false-negativity as the viral load is too low to be detected using HPV-PCR (Pernille *et al.,* 2020; Pineros *et al.,* 2018).

HIV-infected women showed reduced FP and FN and increased TN results than HIV non-infected in both primary and triage tests hence increased specificity and sensitivity by all cervical screening methods. The reason could be HPV regression upon acquisition among HIV-infected women is low meaning that most infections will progress into cervical lesions and test positive by all cervical screening methods decreasing the number of False positives and increasing True positives.

This study, therefore, agrees with one of the seven prioritized WHO algorithms for HPV DNA detection in a screen, triage and treat approach Karagu *et al.,* 2018). The use of this algorithm in this study significantly reduced the high number of HIV non-infected than HIV infected women who would have undergone treatment if the primary screening approach alone was used.

5.2 Human Papillomavirus prevalence among HIV-infected and non-infected women in Eastern and Central Regions, Kenya in association with social-demographic factors.

This study established an overall HPV infection rate of 26.5% [cases: 67 (21.1%); control: 17 (5.4%)]. It agrees with the overall age-specific HPV infection rate of 27% in the neighbouring Nairobi region and disagrees with 40% (Bruni *et al.,* 2021) and 64% in a meta-analysis of pooled study of HIV-positive participants (Menon *et al.,* 2016) among women of the same age in Kenya.

A high HPV prevalence was reported in Embu and Meru Counties where HIV was most prevalent. There's a need to establish HPV prevalence in other regions of the country with a high HIV burden (Bruni *et al.,* 2021) that could be harbouring more HPV infection rates hence a raised overall national HPV prevalence (40%). This indicator should be prioritized by public health interventions to reduce cervical cancer morbidity and mortality.

This study, like others (McClymont *et al.,* 2020; Omire *et al.,* 2020; Yakub *et al.,* 2019; Mabeya *et al.,* 2018; Elmi *et al*., 2017) established a significant association between HIV infection and cervical abnormalities prevalence in all categories of residence age,

religion, marital status, and the number of sex partners where the majority of HIV-infected women reported higher prevalence than HIV non-infected women.

HPV infection and cervical cytology outcome were significantly associated with HIV serostatus across all Counties of residence ($p<0.05$). There was a minimal variation in HPV infection rate among HIV-infected and non-infected women across all Counties. This is an indication that women from all regions of Eastern Kenya are at a significantly equal risk of HPV acquisition and subsequent cervical dysplasia.

A higher HIV and HPV positivity rate and increased risk of contracting HPV (OR: 1.16, CI: 0.68-1.98) were detected among younger women (<35 years) than older women (above 35 years) as reported in other studies (Omire *et al.,* 2020). Younger women are more sexually active than their older counterparts hence a higher risk of contracting HIV, HPV and other sexually transmitted infections (Ermel *et al*., 2019; Shuling *et al.,* 2020). Upon contracting these infections, younger women are at more risk of cervical dysplasia but this risk is significantly reduced due to their high rate of HPV clearance and reduced persistence (Li *et al*., 2021; Huang *et al.,* 2018; Yakub *et al.,* 2019). Literature review the higher prevalence of CIN+ and cervical carcinoma among older than younger women (Chen *et al.,* 2018; Innes *et al.,* 2018) as seen in this study.

Other risky social-demographic factors for HPV acquisition established in this study (>1.0 Odds) and other studies (Shuling *et al.,* 2020; Elmi *et al*., 2017; Innes *et al.,* 2018) were having more than one sex partner that increases the chance of encountering an HPV-infected partner; hormonal contraceptives that have a tumorigenic effect on cervical transformation zone cells and, recurrent urinary tract and genital warts

infections that disrupts the protective cervical epithelium increasing the rate and load of viral entry (Shuling *et al.,* 2020; Akaaboune *et al.,* 2018).

The predominance of HPV among women with normal cytology 17.6% ($p<0.001$) was significantly lower than 20.7% (Bruni *et al.,* 2021) and 31.3% (Omire *et al.,* 2020) reported by other studies in Kenya and 34.3% in Morocco (Bruni *et al.,* 2021). Significantly, HIV-HPV co-infection with cervical cervicitis ($p>0.001$), *C trachomatis* ($p=0.012$) infection and abnormal cytology were established. The predominance of HPV in women with normal cytology in sub-Saharan Africa has been reported to be higher than in the rest of the world (Bruni *et al.,* 2021). HIV-positive participants who reported a history of bacterial vaginitis, cervicitis, cervical inflammation, and Candida *spp*. infection had a higher and more diverse HPV prevalence than HIV-negative participants. Studies have shown, that *C. trachomatis* and other sexually transmitted diseases increase the risk of HPV infection and persistence by increasing HPV's entry to the basal epithelium through micro-abrasions of the cervical epithelium and by interfering with efficient viral clearance (Huang *et al.,* 2018; Chen *et al.,* 2018; Innes *et al.,* 2018). Complex interactions between HPV and other pathogens to increase the oncogenicity of HPV in the cervical epithelium have been described in other studies (Chen *et al.,* 2018).

5.3 Phylogenetic relationships of Human Papillomavirus genotypes associated with cervical abnormalities among Human Immunodeficiency virus-infected women in Eastern and Central Regions, Kenya.

Human Papillomavirus genotype infection and more diverse genotypes of infection were common among HIV-infected compared to HIV-non-infected women in agreement with previous studies (Elmi *et al.,* 2017; McClymont *et al.,* 2020). The

foremost common HPV detected in Eastern and Central Regions, Kenya was lrHPV81 - *Alphapapillomavirus* 3 (α3) HPV species by both HIV serostatus and cytology outcomes. The predominance of HPV81 has moreover been detailed in women with anomalous cytology in Kenya (Omire *et al.*, 2020; Mabeya *et al.*, 2018) and Qatar (Elmi *et al*., 2017). It is associated with precancerous and cancerous lesions and is mostly detected among immunocompromised women (Li *et al*., 2021) as seen in this study.

Human Papillomavirus 16(α9) and HPV66(α6), were predominant high-risk genotypes associated with invasive cancer by HIV serostatus. Their predominance in abnormal cytology among HIV-infected women has been established (Li *et al*., 2021; Shuling *et al.*, 2020). HPV66 is classified as oncogenic due to its close genetic homology with HPV56(α9). This study and others, therefore, recommend routine testing for HPV16, HPV52, and HPV66 since these members of (α7) and (α9) dominate neoplastic tissues. Though they were also detected in normal cytology, a study has demonstrated the genetic variability and frequent mutating of L1, E6, and E7 genes mostly HPV52 and HPV53(α7) resulting in intratympanic variants with increased oncogenic potential (Li *et al.*, 2021; Molina *et al.*, 2020).

Single HPV genotype infection in CIN1+ showed diversity compared with multiple HPV genotype infections in CIN1+ among HIV-infected women. Diverse multiple HPV genotypes-infection was also seen among HIV-infected women. Other common HPV genotypes detected were HPV11, HPV44(α10), and HPV88 which do not feature in many studies as potential oncogenic genotypes. Here, their association with CIN+ could

have been increased by HIV infection as reported in other findings (Li *et al*., 2021; Molina *et al.,* 2020; Shuling *et al.,* 2020).

Invasive cancer and ASCUS were diagnosed among HIV-positive women infected with alpha 9 (α9) species high-risk HPV 16 and 66 co-infections and alpha 3 HPV 81. Common genotypes detected among three women with CIN2 were HPV 11, HPV16, HPV 66, and HPV81. Others were co-infected by HPV 11 and 66, HPV 81 and alpha 10 (α10) species HPV44, HPV 81 and 88, HPV 9 and alpha 6 species HPV 53 and HPV 16 and α9 HPV58. CIN1 was diagnosed among five HIV-positive women infected with HPV 81 and one woman with HPV 66 and alpha9 species HPV53. Non-oncogenic HPV58 and HPV81 association with cervical dysplasia in this study could also have been increased by HIV infection as reported in other studies (Li *et al*., 2021; Molina *et al.,* 2020; Shuling *et al.,* 2020). Members of *Alphapapillomavirus* 7 and *Alphapapillomavirus 9* dominate malignant tissues coding for a hydrophobic E5, hence considered oncogenic (Van-Doorslaer *et al.,* 2018).

A significantly low number of participants had infections with HPV genotypes of the same alpha group in this study. There were also a reduced number of hrHPV52 and 58 and an absence of most of the other hrHPV genotypes, notably HPV18. This could be attributed to antibody cross-reaction which is achieved within species unlike across species. Antibodies against HPV16 cross-react and protect against HPV52 and HPV58 of the same α9 species, HPV18 cross-react with HPV66, and HPV53 (α7) species and HPV81 will cross-react with HPV61 (Kovacevic *et al.,* 2021; Li *et al*., 2021).

Moreover, 19.5% of HIV-infected women harboured multiple HPV genotype infections as compared to 3.4% who harboured single HPV-genotype infections. Common high-risk genotypes detected in single and multiple HPV infections were HPV 16 and 66. HPV 66 (alpha 6 species) group is classified as oncogenic and has close genome homology with HPV56, an established carcinogenic genotype. A high prevalence of HPV 16 and alpha species HPV52 was reported in a meta-analysis study in Kenya among women with abnormal cytology (Van-Doorslaer *et al.,* 2018; Menon *et al.,* 2016).

This study and literature data in Kenya establish the predominance of mixed HPV genotypes among HIV-infected women other than those included in the current bivalent and quadrivalent vaccines in Kenya that protect against HPV6, 11, 16, and 18. Literature data have shown minimal effectiveness of the HPV vaccine against non-vaccine HPV genotypes (McClymont *et al.,* 2020; Innes *et al.,* 2018,). Hence, there is a need for increased effective HPV vaccination coverage among HIV-infected women with a diversified nonavalent (9vHPV) vaccine (Mabeya *et al.,* 2018) that protects against most genotypes detected in this study.

The phylogenetic analysis demonstrated multiple HPV genotypes identified within each participant and sample. Two plausible, unexclusive explanations for the occurrence are that the women are co-infected by different HPV genotypes as shown by 15 participants and different HPV genotype sequences originated within the same host. Cervical cell replication processes are utilized by HPV to multiply reducing the chances for human autosomal–like mutation.

5.4 Knowledge, attitude, practice, and perception of factors associated with HPV oncogenesis among women in Eastern and Central Regions, Kenya.

Almost all women in the study (93.7%) knew about cervical cancer across all counties of residence which in turn was a significant predictive factor for knowledge of cervical screening (p=0.002) as reported in another study (Yimer *et al*., 2021). Women in Tharaka Nithi County were highly likely to know (>1.0 Odds) and had willingness (>2.0 Odds) toward cervical screening and reported a lower HPV prevalence (16.0%). A higher proportion of women in other counties were less likely to have knowledge of cervical screening (<0.50 Odds) and in turn reported a higher HPV infection rate; Isiolo: 26.6% and Embu: 23.5%. Although cervical screening remains low in Africa, studies have shown the acceptability of the test to be high (Yimer *et al*., 2020; Murfin *et al.,* 2020; Wakwoya *et al*., 2020).

Majority of women aged ≤30 years were 57% highly probable to know about cervical screening, had a high willingness to undergo screening (63.1%), higher perception (58.7%) of the importance and effectiveness of the procedure and had high HPV-HIV co-infection prevalence rate (80.4%). They in turn reported a low screening rate (7.6%). Willingness on cervical screening uptake was high among HIV-infected than non-infected women. This study further established that HIV-infected women had a lower perception of the benefit of cervical screening and rarely did they undergo screening by either cervical screening technique during routine HIV viral load and CD4 check-ups.

Religion influenced KAPP on cervical screening as established by Yimer *et al* (2020). Muslim women had less knowledge, lower willingness, were less likely to undergo cervical screening and lower perception of the benefit of cervical screening than

Christians. They had a higher overall HPV prevalence (30.6% vs. 25.5%) and a higher HPV prevalence among HIV-positive women (22.6% vs. 20.8%) than Christian.

This study established that high proportions of women in Eastern and Central Regions, Kenya had secondary (42.6%) and post-secondary education (27.1%) but this was not a significant predicting factor for knowledge and uptake of cervical screening (18.3%). They were at significant risk of not undergoing cervical screening (OR:0.48, CI: 0.2-0.8). Despite high education attainment, they had a low perception (36.6%) of the importance of the procedure in detecting cervical cancer early. This could be the reason behind the highest HPV (52.6%) infection and HPV-HIV (90.0%) co-infection rates among women with university education. This study disagrees with Yimer *et al.*, (2020) that women with a high education status are highly likely to undergo cervical screening than those with low educational attainment. A minimal number of women with university (0.6%) and college (0.9%) level education reported ever undergoing cervical screening.

Knowledge of cervical screening was significantly associated ($p< 0.05$) with the choice of family planning method and number of sex partners ($p=0.039$). Women who reported to be using non-hormonal contraceptives were more willing to undergo cervical screening and highly likely to be aware of cervical screening than women who used hormonal contraceptives. Women with more than one sex partner were also highly likely to undergo cervical screening as reported by a study by Murfin *et al.*, (2020) but did not perceive this as a risk. They had a higher HPV prevalence (32.0%) than those with one sex partner (22.0%). Both groups were equally likely to undergo HPV screening and had a high perception of the importance of the procedure. Multiple sex

partners were significantly associated with HPV prevalence in this study as reported by Murfin *et al.,* (2020) and Mutambara *et al.,* (2017), but did not influence KAPP on cervical screening.

The cervical screening rate was slightly higher among persons who have a family member or friend with a history of the cervical disease (10.7%). Witnessing resource intensiveness and painful experiences involved in cervical disease management could have influenced this slight increase in screening uptake.

The majority of participants were not aware of the cervical screening cycle of 5 years (WHO *Cervical Screening Guidelines,* 2021). They however reported a minimal cervical screening rate. This means that they would wait for symptoms to appear before undergoing screening when the disease had advanced and was difficult to manage.

There were varying responses to symptoms associated with the disease. The foremost symptoms expressed in this study were persistent smelly vaginal discharge and abdominal pain. Most participants were not aware that dyspareunia, unexplained vaginal bleeding after menstruation, post- menopausal vaginal bleeding and post-coital vaginal bleeding are symptoms associated with cervical cancer (Murfin *et al.,* 2020). Literature review the low level of community awareness of cervical dysplasia signs and symptoms as the main challenge to increasing access and cervical cancer screening services in Sub-Saharan Africa (Yimer *et al*., 2020)

Many participants had knowledge, willingness, and perception of the importance of HPV vaccination but no one reported ever being vaccinated. Women from Isiolo and

Kirinyaga counties, those with post-primary school education were highly likely to have high acceptability of HPV vaccination. This study concurs with the WHO Vaccine Report (2018) that HPV vaccine acceptability is not a strong predictor of its uptake. The cost of the vaccine and its availability could be the reasons for low uptake.

Limited proportions of participants who had ever undergone cervical screening were aware and exposed to most risk factors associated with cervical cancer. The study established no link between awareness and exposure to these risks; the history of having undergone cervical cancer screening was not a predictor of awareness or exposure. Only 7.1% of the participants were aware of the dangers of smoking in the development of cervical cancer although few smokers were reported. African cultural norms discriminate against women smokers and this could be the reason for the very few reported cases of smoking among the participants. Awareness of risk factors associated with cervical cancer is limited in most African countries including Kenya (*National Cervical Cancer Prevention Program*, 2012), Nigeria (Okunowo *et al.*, 2018), and Cambodia (Touch and Oh, 2018).

Embarrassment, fear of results, and lack of time were significant reasons for failure to undergo cervical screening despite high KAPP on the procedures. Many participants had knowledge and perception of the importance of Pap smear test but low attitude and less screening practices. In a study conducted in western Kenya, 94% of women had never done HPV screening and cited barriers such as fear of results, lack of time, and knowledge. The main reason given for failure to do HPV screening in Cambodia (Kitchener *et al.*, 2018) was a lack of knowledge about the test. Other barriers

documented for failure to undergo cervical screening were embarrassment due to age difference between health personnel and patient, fear of results, and lack of time (Touch and Oh, 2018).

5.5 Limitation of the study.

This study was limited by the need for standard questionnaires to determine awareness and exposure to specific risk factors associated with cervical neoplasia in an African setting hence limiting the ability to compare results across studies.

Responses could have been altered by missing keywords like ‘cervical cancer’ in local dialect during questionnaire translation and back translation but caution was taken to give accurate translation and meaning during the study.

Accurate responses on self-reporting on the history of sexually transmitted diseases were not guaranteed during the study. However, participants were required to fill out the questionnaire in camera and assured of the confidentiality of their information to reduce this bias.

5.6 Conclusion.

1) Primary HPV DNA-PCR detection in the ‘screen-and-treat’ approach and ‘screen, triage and treat’ approach with cytology-triage were more accurate in parallel co-testing and sequential triage based on histopathology of abnormal cytology outcomes among HIV-infected and non-infected women. High sensitivity and specificity in the detection of CIN+ were established among HIV-infected than HIV non-infected women by all screening methods. High diagnostic values were obtained by all cervical screening methods when CIN+ histopathology outcome was used as a

reference than CIN+ with ASCUS.

2) Overall HPV prevalence was found to be 26.5% by Human Papillomavirus Polymerase Chain Reaction (HPV PCR) and 25.6% by visual inspection (VIA/VILLI) test which is higher than the national survey of 11.6%. Human Immunodeficiency virus-infected women had a higher overall HPV prevalence (21.1%) than HIV-uninfected women (5.4%) across all social demographic factors. HIV infection, higher parity (>3), hormonal contraceptives use, multiple sex partners, and recurrent vaginal infections were found to be risk factors associated with HPV oncogenicity among women of reproductive age.

3) Human Immunodeficiency infected women had a wider spectrum of HPV genotype infection than HIV non-infected. The most common HPV genotypes isolated among women with normal, abnormal cytology and histology outcome were low-risk HPV81 (CP8304) and high-risk non-vaccine HPV genotypes HPV66, HPV45, HPV52, HPV53, HPV58, and HPV16. A high predominance of HPV81 was evident. Most HPV sequences isolated in the study clustered mainly with HPV genotypes detected from Iran, Kenya, and Bangkok.

4) Knowledge, attitude, and perception of cervical screening and HPV vaccination were higher among HIV-infected than uninfected women. Those with higher parity (>3) and those with post-primary school education were highly likely to have high knowledge, positive attitude, and perception of the importance of cervical screening but this did not influence positive cervical screening behaviour. The study also revealed a lack of awareness of HPV genotyping and VIA/VILLI cervical screening

techniques and high awareness and willingness to undergo Pap smear tests. There was high awareness but low willingness to undergo screening by untrained traditional medicine men. Unexplained reasons were mainly linked to failure to undergo cervical screening.

5.7 Recommendations.

1) The Kenya Ministry of Health through County Health Boards should establish the HPV PCR test as a routine and complementary cervical screening technique to Pap smear and visual inspection in all teaching and referral health facilities in Eastern and Central Regions, Kenya. This will increase the sensitivity and specificity of cervical screening outcomes for cervical cancer management and follow-ups, especially among HIV-infected women.

2) The Kenya Ministry of Health Services should initiate continuous surveillance, development, and integration of effective population-based HPV screening programs alongside HIV among women of reproductive age to uncover predictor social-demographic risk factors associated with cervical neoplasia. This will also inform on cervical cancer disease burden in the country and potential HPV infection-oncogenic outcome based on social demographic factors and exposure to risks associated with cervical neoplasia.

3) All research health institutions together with the Kenyan Government should establish a pooled epidemiological data exchange centre of HPV genotypes from different regions of Kenya with programmatic considerations and constant updates

on HPV genotypes and their potential risk of cervical neoplasia. This will inform on biological evidence of oncogenicity by high and low-risk HPV genotypes notably HPV81 among HIV-infected women for effective vaccine design and other cervical cancer preventive strategies.

4) The Kenya Ministry of Health, Information and Communication, and the Media fraternity should recognize the need to develop educational strategies, and low-cost media campaigns with standardized content for broad Eastern Kenya community outreach focusing on risk factors for HPV oncogenesis, reproductive health-seeking behaviour to increase awareness, uptake, and coverage of cervical screening. There is also a need to advocate for the implementation of HPV-recommended vaccination schedules among HIV-positive women of reproductive age based on surveillance data.

5.8 Suggestions for further research.

1) There is a need for further research to determine HPV genotype's prevalence among women living with HIV in sub-Saharan Africa and establish possible cross-protection between prevalent HPV spectrum and genotypes included in the current vaccine for effective protection.
2) There is a need to establish preventive strategies against HPV81, HPV 44, HPV 58, and HPV 88 which were detected among most HIV-infected women with abnormal cervical screening outcomes.

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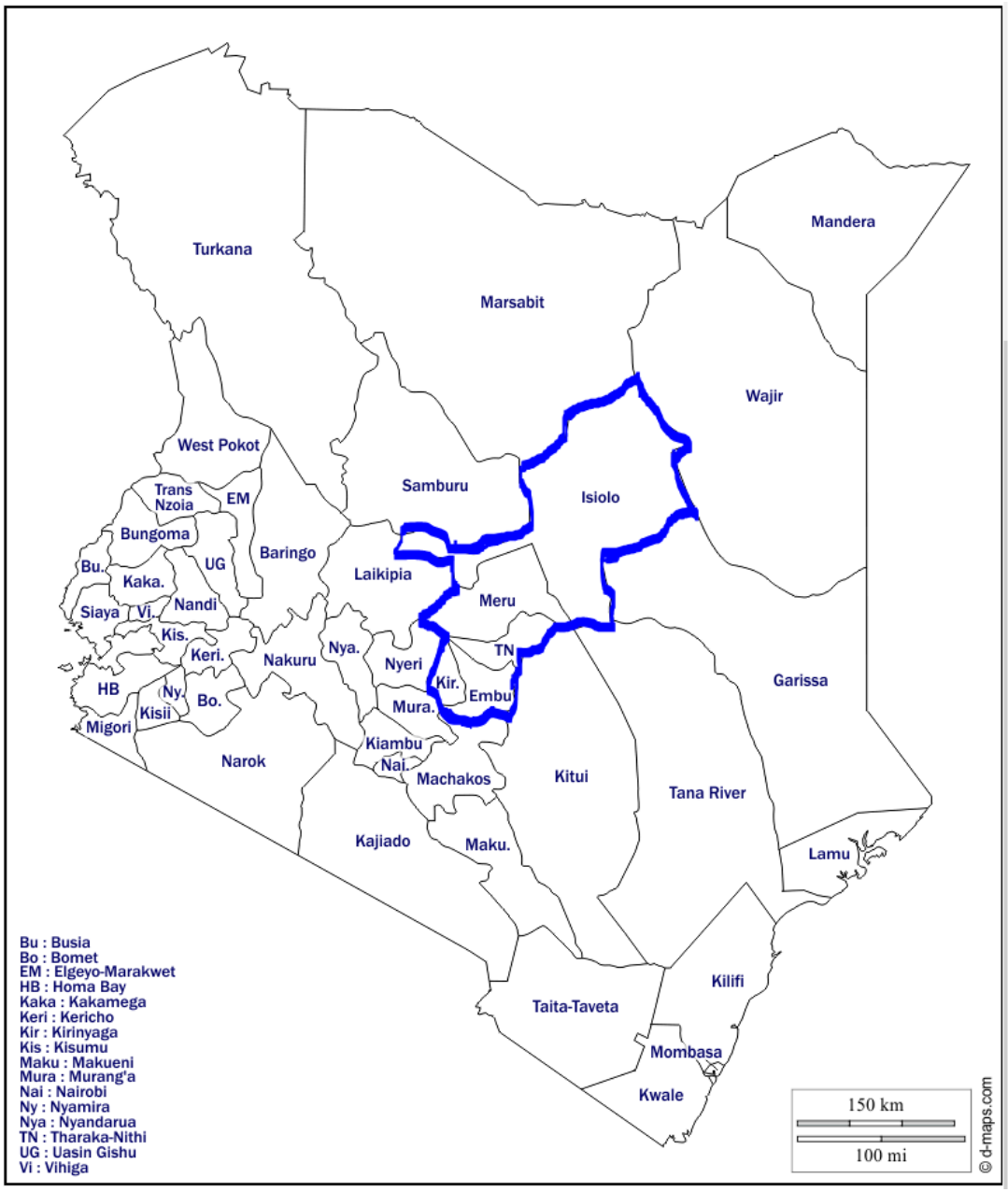
APPENDICES

Appendix 1: Accession numbers and Submission Identification of Human Papillomavirus Late Protein 1 Genome Sequences

Accession number	Submission Identification Sample number
LC618958	60489a403a01a5001809f684.o1378jknh7
LC618959	60489a403a01a5001809f684.e03p18jknc2
LC618960	60489a403a01a5001809f684.g09l4jknd5
LC618961	60489a403a01a5001809f684.a09m81jkna5
LC618962	60489a403a01a5001809f684.o03l7jknh2
LC618963	60489a403a01a5001809f684.c05l10jknb3
LC618964	60489a403a01a5001809f684.i03p6jkne2
LC618965	60489a403a01a5001809f684.g17p7jknd9
LC618966	60489a403a01a5001809f684.o01m1jknh1
LC618967	60489a403a01a5001809f684.O15n12jknh8
LC618968	60489a403a01a5001809f684.e01n3jknc1
LC618969	60489a403a01a5001809f684.i15p2jkne8
LC618970	60489a403a01a5001809f684.g05p8jknd3
LC618971	60489a403a01a5001809f684.c01m4jknb1
LC618972	60489a403a01a5001809f684.g15m71jknd8
LC618973	60489a403a01a5001809f684.m13m6jkng7
LC618974	60489a403a01a5001809f684.e11n11jknc6
LC618975	60489a403a01a5001809f684.i05p3jkne3
LC618976	60489a403a01a5001809f684.c13u2jknb7
LC618977	60489a403a01a5001809f684.a05m8jkna3
LC618978	60489a403a01a5001809f684.a13u1jkna7
LC618979	60489a403a01a5001809f684.k13m28jknf7
LC618980	60489a403a01a5001809f684.o17m21jknh9
LC618981	60489a403a01a5001809f684.m21m8jkng11
LC618982	60489a403a01a5001809f684.e05p43jknc3
LC618983	60489a403a01a5001809f684.o07m8jknh4
LC618984	60489a403a01a5001809f684.e09m19jknc5
LC618985	60489a403a01a5001809f684.o21m57jknh11
LC618986	60489a403a01a5001809f684.e21m20jknc11
LC618987	60489a403a01a5001809f684.k11m78jknf6
LC618988	60489a403a01a5001809f684.k07m51jknf4
LC618989	60489a403a01a5001809f684.c17m52jknb9
LC618990	60489a403a01a5001809f684.k15p21jknf8
LC618991	60489a403a01a5001809f684.a17p81jkna9
LC618992	60489a403a01a5001809f684.a15m23jkna8
LC618993	60489a403a01a5001809f684.o23x8jknh12
LC618994	60489a403a01a5001809f684.i01a5jkn_e1
LC618995	60489a403a01a5001809f684.k21m5jknf11

LC618996	60489a403a01a5001809f684.i11k7jkne6
LC618997	60489a403a01a5001809f684.i09m59jkne5
LC618998	60489a403a01a5001809f684.m03p2_kng2
LC618999	60489a403a01a5001809f684.m09x9jkng5
LC619000	60489a403a01a5001809f684.c158pjknb8
LC619001	60489a403a01a5001809f684.i07m05jkne4
LC619002	60489a403a01a5001809f684.m17m33jkng9
LC619003	60489a403a01a5001809f684.m23x7jkng12
LC619004	60489a403a01a5001809f684.k05p47jknf3
LC619005	60489a403a01a5001809f684.a21k5jkna11
LC619006	60489a403a01a5001809f684.e07k4jknc4
LC619007	60489a403a01a5001809f684.c11m5jknb6
LC619008	60489a403a01a5001809f684.e23x3jknc12
LC619009	60489a403a01a5001809f684.i23x5jkne1
LC619010	60489a403a01a5001809f684.k0186jknf1
LC619011	60489a403a01a5001809f684.m01c8jkng1
LC619012	60489a403a01a5001809f684.c07m3jknb4
LC619013	60489a403a01a5001809f684.k23x6jknf12
LC619014	60489a403a01a5001809f684.116m05p16
LC619015	60489a403a01a5001809f684.i21_p20jkne11
LC619016	60489a403a01a5001809f684.k0346jknf
LC619017	60489a403a01a5001809f684.g03c5jknd2
LC619018	60489a403a01a5001809f684.a011cjkna1
LC619019	60489a403a01a5001809f684.116g01l12jknd1
LC619020	60489a403a01a5001809f684.a07n31jkna4
LC619021	60489a403a01a5001809f684.a03c28jkna2

Appendix 2: Map of Kenya showing the study sites in the Eastern and Central Regions, Kenya



Appendix 3: Checklist of materials

The investigator should see these materials before starting any procedure (√)/(x)	
HPV DNA PCR genotyping	
HPV sequencing (Macrogen Netherlands™) - 84 +ve samples KIT	
MEM transport media (KEMRI)	
Maggio HighPrep™ Viral DNA/RNA (96 preps) kit	
Agarose gel powder (500 grams)	
Ethidium bromide (highqu™) 50gm	
Primers (Inqababiotech™) 4 sets	
Take5™ 100bd ladder Highqu - 5ml	
One Taq 2x Master mix (5xFIREPol mix®) - 15mls	
TBE - EDTA Buffer 1000gm	
Ethanol (absolute) 5 litres	
Isopropanol 5 litres	
Magnetic separation device (96-wells)	
Magnetic microplates (U-bottom)	
HIV determination	
Vironostika & reg; HIV-1 Plus O Microelisa HIV 383tests kit	
PIMA & reg; HIV CD4 counts kit, Alere Tech. - 25tests	
Alere Determine & reg; HIV-1/2 - 100 tests	
KHB Diagnostic kit for HIV (1+2) Ab - 50 tests	
Other consumables	
Disposable vaginal speculum - 24 pieces	
Pap smear kits - 30 pieces	
Non-sterile gloves, small and large size - 100 pairs/box	
Pipette tip, 1- 5ML, Universal, Blue, 1000/rack	
Pipette tip, 100 - 1000uL, Universal, Blue, 1000/rack	
Pipette tip, 10 - 100uL, Universal, Blue, 1000/rack	
Pipette tip, 1 - 10uL, Universal, Yellow, 1000/rack	
Vacutainer PLAIN 5ml - 100tubes	
Vacutainer EDTA 5ml - 100 tubes	
Epidof tubes (1000 pieces) pack	
Vacutainer needles - 100 neddles/box	
Blood lancets - 100 pieces/box	
Cotton wool roll, large	
Surgical spirit OMAERA - 5 litres container	
K-Y Jelly - 100mls tube	

PreservCyt transport solution - 50 ml pack	
Adhesive plaster Brown-hot melt Zinc Oxide large	
Biospot papers - 100 pieces /pack	
Hand washing soaps - 10 pieces and toilet paper	
Sub-total	
Documentation and publication costs	
Printing questionnaire, proposal, and binding	
Marker pens, pen pencils, white-out, and labels	
NACOSTI permit application fee - first year only	
Ethical clearance application fee - first year only	
Internet costs - for 3 years	

Appendix 4: Letters of Swahili translation

KENNEDY MELECKZADECK SHIUNDU
P.O. Box 29945 GPO 00100, Nairobi, Kenya.

15th July, 2016

To: Kenya Medical Research Institute Scientific Ethics Review Unit.

RE: KISWAHILI CONSENT DOCUMENT TRANSLATION

The translated document attached is an accurate Kiswahili interpretation of the English version listed below. Both of these documents are attached.

- Title of Translated Document: *"Determination of Human Papilloma Virus (HPV) variants and factors associated with cervical abnormalities among HIV-positive women in eastern Kenya".*
- Version Date of Translated Document: 10TH July, 2016.
- Principal Investigator: Njue James Kinoti - Kenyatta University.

I am native speaker with a Diploma in Social Work and Community Development (KNEC), Certificate in Social Development, vast experience in HIV community/social work and assure the Kenya Medical Research Institute Scientific Ethics Review Board that this translation is accurate to the best of my knowledge.

I also assure the KEMRI - SERU that I am acting independently from the principal investigator, and am not affiliated with any active study in any way in this PhD research.

If you have any questions, please contact me using the information below:

Mobile phone: +254 703 812 688.

Email: meleckzadecks@gmail.com.

Sincerely,

KENNEDY MELECKZADECK SHIUNDU
Community/Social worker.

Appendix 5: Swahili back translation

FROM: EUNICE GATWIRI
P.O. BOX549 – 60401, CHOGORIA
KENYA.

24th November, 2016

TO: KENYA MEDICAL RESEARCH INSTITUTE SCIENTIFIC ETHICS REVIEW UNIT.
NAIROBI, KENYA.

RE: **SWAHILI CONSENT DOCUMENT BACK TRANSLATION OF THE PROTOCAL TITLED: UAMUZI KUHUSU AINA ZA VIRUSI VYA PAPILOMA (HPV) NA MAMBO YANAYOCHANGIA KUWEPO KWA MAGONJWA YA MFUKO WA UZAZI MIONGONI MWA WANAWAKE WANAOISHI NA VIRUSI VYA UKIMWI MASHARIKI MWA KENYA.**

I certify that the translated document attached is an accurate and comprehensible English interpretation of the Swahili version stated above. The original consent and back-translated consent documents are hereby attached with the following details:

Version 1Date of Translated Document: 24th November, 2016.
Principal Investigator: Njue James Kinoti - Kenyatta University.
Other listed investigators: Dr. Margaret Muturi, Dr. Lucy Kamau and Dr Raphael Lwembe.

For any further clarification, reach me on: euniegatwi@gmail.comon +254717617892.

Sincerely.

EUNICE GATWIRI (BSc. Dip. Public Health).

Appendix 6: Consent - English

The Global Health Network Information Sheet and Consent Form

DETERMINATION OF HUMAN PAPILLOMA VIRUS (HPV) VARIANTS AND FACTORS ASSOCIATED WITH CERVICAL ABNORMALITIES AMONG HIV-POSITIVE WOMEN IN EASTERN KENYA.

Kenyatta University - Njue James Kinoti (PI), Margaret Muturi and Lucy Kamau.
Kenya Medical Research Institute (KEMRI) - Raphael Mwamtsi Lwembe.

Human Immunodeficiency Virus is a virus that causes AIDS (Acquired Immune Deficiency Syndrome) and is detected by the presence of its antibodies in blood. On the side, Human Papilloma Virus (HPV) is responsible for cervical abnormalities that include cancer. The study aim is to detect if you have cervical abnormalities and provide information on HPV genotypes which is valuable in prognosis, diagnosis and management of cervical abnormalities among HIV positive women.

This facility *e.g.* _____ *Hospital* works in collaboration with KEMRI and KU. KEMRI is organization that carries out medical research to find better ways of preventing and treating illness in the future for everybody's benefit. KU is an institution for higher education. One illness KEMRI and KU are currently trying to learn more about is cervical cancer. There is currently not enough information in Kenya about HPV genotypes in eastern Kenya among HIV positive women. To learn this we are asking 196 HIV-positive and 196 HIV-negative women aged 18-56 years to participate.

If you agree to participate in this research, we will use a small amount of the blood sample less than ½ tsp (2ml) from your arm and cervical cells using a swab while lying at athrotomical position, taken as part of your normal treatment. If there are any other research activities that the hospital staff would like you to participate in, staff shall explain and ask your permission first. Our priority for every patient is her care. Taking blood from the hand causes a small amount of pain, but the amount taken is too small to affect your health. Cervical cells harvest will course slight discomfort that will last for few minutes.

There is no direct benefit to you, but the results of this study will contribute in improving the care of people who have cervical abnormalities in the future. All participation in research is voluntary. You are free to decide if you want to take part. If you do agree you can change your mind at any time and withdraw from the research. This will not affect you now or in the future. Individual names are removed from all samples and replaced by codes, to ensure that samples can only be linked to the participants by people closely concerned with the research. Most of the research tests that will be done on the blood here in the hospital laboratories. However for some test that cannot be done in the laboratory and will be sent to KEMRI laboratories or exported overseas to Japan for further scientific analysis.

After the research, a small portion of the blood and cervical material will be stored. In the future, new research about HIV and cervical abnormalities may be done on these samples. Future research must first be approved by a national independent expert committee to ensure participants' safety and rights are respected. All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are closely concerned with the research will be able to view information from participants. An independent national committee and a committee in hospital administration have looked carefully at this work and agreed that the research is important, that it will be conducted properly and participants' safety and rights have been respected. You may ask any of our staff questions at any time.

You can also contact those who are responsible for your care and this research: Njue James Kinoti-Kenyatta University. If you want to ask someone independent anything about this research please contact: KEMRI - SERU Secretariat phone: 0717719477

Appendix 7: KEMRI Consent form for sample-only studies

DETERMINATION OF HUMAN PAPILLOMA VIRUS (HPV) VARIANTS AND FACTORSASSOCIATED WITH CERVICAL ABNORMALITIES AMONG HIV-POSITIVE WOMEN IN EASTERN KENYA.

I, _____, have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily.
Please insert the boxes below where relevant:

I agree to take part in this research
I agree to samples being stored
I agree to samples being exported

I understand that I can change my mind at any stage and it will not affect me in any way.
Participant's signature: _____ **Date** _____
Name _____ **Time** _____

I certify that I have followed the study SOP to obtain consent from the participant. She apparently understood the nature and the purpose of the study and consents to participate in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Investigator's signature: _____ **Date** _____
Investigator's name: _____ **Time** _____

Thumbprint of the subject as named above if they cannot write:

_____

Only necessary if the participant requires witness.

I attest that the information concerning this research was accurately explained to and apparently understood by the participant and that informed consent was freely given.

Witness' signature: _____ **Date** _____
Witness' name: _____ **Time** _____

***A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.**

Thumbprint of the witness as named above if they cannot write: _____

THE SUBJECT AND WITNESS WOULD NOW BE GIVEN A SIGNED COPY TO KEEP.

Appendix 8: Maelezo ya utafiti

UAMUZI KUHUSU AINA ZA VIRUSI VYA PAPILOMA (HPV) NA MAMBO YANAYOCHANGIA KUWEPO KWA MAGONJWA YA MFUKO WA UZAZI MIONGONI MWA WANAWAKE WANAOISHI NA VIRUSI VYA UKIMWI (VVU) MASHARIKI MWA KENYA.

Chuo kikuu cha Kenyatta - Njue J. Kinoti (Kiongozi), Margaret Muturi na L. Kamau.
Taasisi ya Utafiti wa Afya Kenya (KEMRI) - Raphael Mwamtsi Lwembe

Virusi Vya Ukimwi (VVU) huleta upungufu wa kinga mwilini na hupimwa kwa kuwepo kwa chembechembe zinazokinga mtu kutokana na virusi hivyo katika damu yake. Human Papilloma Virus (HPV) husababisha madhara kama saratani ya mfuko wa uzazi (SMU). Tunalo lengo la kufanya utafiti kujua uhusiano ya aina za virusi vya HPV na athari zake kwa jamii miongoni mwa wanamake wanaoishi na virusi vya ukimwi mashariki mwa Kenya. Utafiti huu utatuwezesha kutoa taarifa juu ya aina za HPV ambayo ni muhimu kwa ubashiri, utambuzi wa saratani ya mfuko wa uzazi miongoni mwawanawake wanaoishi na VVU.

Hospitali hii ya *Level 5 ya Meru* inashirikiana na KEMRI na KU kwa maswala mengi. Asasi ya KEMRI hufanya utafiti wa kimatibabu ili kutafuta njia bora ya kuzuia na kutibu kwa manufaa ya kila mtu kwa jamii. Kwa sasa hospitali ya Meru, KEMRI na KU zinashilikiana kujaribu kujifunza zaidi juu ya saratani ya mfuko wa uzazi. Kwa sasa hakuna taarifa za kutosha inchini kuhusuina na HPV mashariki ya Kenya miongoni mwawanawake wanaoishi na VVU. Ili kujifunza, tunaomba wanawake 196 wenye VVU na wanawake 196 wasiokuwana VVU wote wenye umri wa miaka 18-56 kushiriki.

Kama unakubali kushiriki kwenye utafiti huu, tutatumia kiasi kidogo cha sampuli ya damu chini ya ½ tsp (2ml) kutoka mono wako na seli kutoka mfuko wa uzazi wako wa uzazi kwa kutumia pamba ukiwa umelala kwa mgongo kwa njia iliyokubaliwa kiafya. Kama kuna utafiti mwingine wowote bali na utafiti huu, wafanyi kazi wa hospitali watakufahamisha mbele ya kukuhusisha. Kuchukua damu kutoka kwa mkono husababisha kiasi kidogo cha maumivu, lakini kiasi hichi ni kidogo mono na hakitathiri afya yako. Kuchukuwa seli kutoka kwa mfuko wako wa uzazi kutawasha lakini hautahathilika kiafya.

Hakuna faida ya moja kwa moja kwako, lakini matokeo ya utafiti huu itachangia katikia kuboresha huduma ya watu walio na marathi haya siku zijazo. Kushiriki katika utafiti ni kwa hiari. Uko huru kuchagua kama utashiriki. Kama haukubaliani, unaweza kubadili akili yako wakati wowote na kuondoka. Hii haitakuathiri wewe sasa ama katika siku zijazo. Majina ya mtu binafsi yataondolewa kutoka sampuli zote na kubadilishwa na nambari za siri ili kuhakikisha kwamba sampuli zinaweza tu kujulikana na wanaohusishwa na washirika wa utafiti huu. Vipimo vitafanyiwa hapa kwa mahabara ya hosipitali. Hata hivyo baadhi ya vipimo ambazo haziwezi kufanyika hapa katika maabala ya hospitali, zitapelekwa mahabala ya KEMRI ua ng'ambo Japan kwa utafiti wa juu wa kisayansi. Baada ya utafiti, sehemu ndogo ya damu na seli kutoka kwa mfuko wa uzazi zitahifadhiwa kwa utafiti wa siku zijazo. Utafiti wa siku zijazo lazima kwanza kupitishwa na kamati ya kitaifa ya wataalamu huru kwa kuhakikisha usalama wa waashiriki na haki zao zinaheshimiwa.

Kumbukumbu za utafiti huu zutifadhiwa katika makabati yatakayofungwa na namba ya siri ya kompyuta. Watu wachache tu ndio wanaweza kupata habali za washiliki wa utafiti huu, tume huru ya kitaifa na kamati katika utawala wa hospitali. Tume hulu imeangalia kazi hii na kukubaliana kwamba utafiti huu ni muhimu na kwamba ufanyike kwa kuzingatia maelezo yaliokubaliwa na haki za washiriki kuheshimiwa.

Unaweza kuliza wafanyikazi wetu swali yoyote wakati wowote. Unaweza kuwasiliana na mkubwa wa utafiti huu: Njue James Kinoti - Kenyatta University. Kama unataka kuuliza mtu hulu chochote kuhusu utafiti huu, tafadhali wasilianana: KEMRI Simu: 0717719477

Appendix 9: Fomu ya idhini ya KEMRI ya utafiti wa sampuli pekee.

UAMUZI KUHUSU AINA ZA VIRUSI VYA PAPILOMA (HPV) NA MAMBO YANAYOCHANGIA KUWEPO KWA MAGONJWA YA MFUKO WA UZAZI MIONGONI MWA WANAWAKE WANAOISHI NA VIRUSI VYA UKIMWI (VVU) MASHARIKI MWA KENYA.

Mimi, _____, nimesomewa na nimeelezwa yote ambayo yanayohusiana na utafiti huu na nikajibiwa maswali tata yote kikamilifu. *Tafadhali ingiza kisanduku hapa chini mahali ambapo panausika:*

Nakubali kushiriki katika utafiti huu
Nakubali sampuli zangu kuhifadhiwa
Nakubali sampuli zangu kupelekwa nje kwa utafiti

Naelewa kwamba naweza kubadili mawazo yangu katika hatua yoyote na haitaniathiri kwa njia yoyote.
Mhusika sahihi: _____ Tarehe _____
Jina la mhusika _____ Muda _____

Ninathibitisha kwamba nimefuata utafiti tonge kupata ihali kutoka kwa mshiriki. Yeye inaonekana kuelewa asili na lengo la somo na akakubali kushiriki katika utafiti. Naye amepewa nafasi ya kuuliza maswali ambayo yamekuwa na akajibu kwa kiwango cha kuridhisha.
Aliyechaguliwa / sahihi ya mtafiti: _____ Tarehe _____
Aliyechaguliwa / jina la mtafiti: _____ Muda _____

Alama ya kidole ya mshiliki kama hawawezi kuandika: _____

Muhimu tu kama mshiriki anahitaji mshahidi:

Mimi nimeshuhudia kwamba taarifa kuhusu utafiti huu ilikuwa sahihi, mhushika amelezewa kikamilifu na akaonekana kuelewa na kwamba ridhaa ya habari ilikuwa huru.
Sahihi ya shahidi _____ Tarehe _____
Jina la shahidi: _____ Muda _____

Shahidi ni mtu ambaye ni huru kutoka kesi au mwanachama wa wafanyakazi ambao hakuhusika katika kupata ridhaa.

Alama ya kidole ya mshahidi kama hawawezi kuandika: _____

MSHIRIKI NA SHAHIDI LAZIMA SASA WATAPEWA NAKALA HII YA KUWEKA.

Appendix 10: Questionnaire

QUESTIONNAIRE

Code number

HPV types and risk factors associated with cervical abnormalities among HIV-positive women in Eastern and Central Regions, Kenya.

Principal Investigator:
Njue J. Kinoti – Department of Medical Laboratory Science Kenyatta University
Co-Investigators/Supervisors
Dr Margaret Muturi – Department of Medical Laboratory Science, Kenyatta University
Dr Lucy Kamau – Department of Zoology Science, Kenyatta University.
Dr Raphael M.Lwembe – Centre for Virus Research –KEMRI

Hello. My name is Njue James Kinoti, a Ph.D. Medical Virology student at Kenyatta University. I am researching to determine Human Papilloma Virus variants and factors associated with cervical abnormalities among HIV-positive women in Eastern and Central Regions, Kenya". Kindly volunteer to take part in this study since your participation is very important in helping to determine the Human Papilloma Virus (HPV) disease burden and associated risk in the region. Questions will take less than twenty-five minutes. Some questions may border on your sexual behaviour and social-economic life. You may choose to participate in this study or decline. I kindly request you to answer each question to the best of your ability.

Part A: *Social-demographic factors*

A1	How old are you? [1] below 19 [2] 20-29 [3] 30-39 [4] 40-49 [5] 50-59 [6] 60 and above	
A2	Where is your residence?	
A3	What is your religion? [0] no religion [1] Muslim [2] Christian [3] Other	
A4	What is your education level? [0] none [1] primary school [3] secondary/A-level [4] college / University	
A5	What is your marital status? [0] single [1] married [2] separated [3] divorced [4] widowed	
A6	What is your profession? [0] unemployed [1] employed [2] student	
A7	What is your income index ($/day)? [0] low (US$1.90 per day) [1] middle (US$1.90 PPP - US$5.50 PPP per day) or [2] high (over US$5.50 PPP per day).	

Part B: *Factors associated with HPV oncogenesis*

B1	Do you use any family planning (FP) method? [0] no [1] yes	
B2	If yes, Which family planning method/s do you use? [0] Depo-Provera [1] Condoms [2] natural method [3] Norplant [4] Coil [5] other *(specify)*	
B3	How long have you used it/them?	
B4	Do you experience any problems with your FP method of choice? [0] n o [1] yes	
B5	Do you have children? [0] no [1] yes If yes, how many? [0] [1] [2] [3] [4] [5] [6] [7] [8]	
B6	When was your sexual debut? [0] never [1] below 15 [2] 15-20 [3] 21-25 [4] 26-30 [5] 30-35	
B7	How many sexual partners have you had in the last two years? [0] [1] [2] [3] [4] [5] [6] [7] or more	
B8	If more than two, do you always use protection [0] no [1] yes	
B9	Have you ever suffered/tested from these illnesses? If Yes, are you willing to share the results with me? (a) TB [0] no [1] yes ---------- (b) recurrent vaginal warts [0] no [1] yes ---------- (c) recurrent UTIs [0] no [1] yes ---------- (d) HIV [0] no [1] yes ---------- (e) Syphilis [0] no [1] yes ----------	
B10	Do you smoke? [0] no [1] yes If yes, how many cigarettes per day ----------	
B12	Do you take alcohol [0] no [1] yes If yes, what quantity per day/week/month ----------	

Part C: *Knowledge, Attitude, Practice and Perception towards HPV and HPV diagnostic techniques and vaccination*

C1	Have you ever heard of the Human Papilloma Virus (HPV)? If Yes, what is it ------- [0] knows [1] doesn't know	
C2	Can HPV be transmitted?	
C3	Can you protect yourself from HPV? If Yes, how ----------	
C4	Do you know Visual Inspection by acetic acid tests– *Explain* [0] no [1] yes (If yes, What is it?)	
C5	Have you ever undergone this test? [0] no [1] yes	
C6	Would you be willing to do this test [0] no [1] yes [2] I don't know	
C7	Do you think this test is helpful in the fight against HPV infection [0] no [1] yes	
C8	Do you know the Pap smear test– *Explain* [0] no [1] yes If yes, What is it?	
C9	Have you ever undergone this test? [0] no [1] yes [2] I don't know	
C10	Would you be willing to do this test [0] no [1] yes	
C11	Do you think this test is helpful in the fight against HPV infection [0] no [1] yes	
C12	Do you know HPV DNA PCR – *Explain* [0] no [1] yes (If yes, What is it?)	
C13	Have you ever undergone this test? [0] no [1] yes [2] I don't know	
C14	Would you be willing to do this test [0] no [1] yes	
C15	Do you think this test is helpful in the fight against HPV infection [0] no [1] yes	
	How often should a woman do the cervical screening?	

C16	[0] every 6 months [1] every 1 year [2] every 2 years [3] every 5 years [4] when symptoms appear [5] Don't know	

Part D: *HPV vaccination*

D1	Have you ever heard of the HPV vaccine or vaccination against cervical cancer? [0] no [1] yes If Yes, What is it? ----------	
D2	Have you ever been vaccinated against HPV [0] no [1] yes If no, why? ----------	
D3	Do you think HPV vaccination is helpful?	
D4	Can you advise your close relative or child to be vaccinated [0] no [1] yes	

Part E: *Cervical cancer, its symptoms, cervical screening frequency, source of information*

E1	Have you ever heard about cervical cancer? If Yes, What is it? - ----------	

What are the signs and symptoms of cervical cancer? *Tick*

a) unexplained vaginal bleeding after menstruation [0] no [1] yes
b) post-menopausal vaginal bleeding [0] no [1] yes
c) post-coital vaginal bleeding [0] no [1] yes
d) smelly vaginal discharge [0] no [1] yes
e) abdominal pain [0] no [1] yes
f) pain during sexual intercourse (dyspareunia) [0] no [1] yes
g) loss of sexual drive [0] no [1] yes

E2 How long should you stay before doing a cervical screen?
[0] 6 months [1] 1 year [3] 2 years [4] 3 years [5] 4 years [6] 5 years
[7] when symptoms appear [8] don't know

E3 Where do you get information on cervical cancer
[0] radio [1] service provides [2] opinion leaders [3] social media [4] TV
[5] Family members and friends [6] Print media

E4	Has any of your close relatives been diagnosed with cervical cancer [0] no [1] yes	
E5	In your perspective, is cervical cancer hereditary? [0] no [1] yes	

E6 Comments

Thank you for participating

Appendix 11: Questionnaire – Swahili version

DADISI | Namba ya Siri

HPV types and risk factors associated with cervical abnormalities among HIV-positive women in Eastern and Central Regions, Kenya.

Principal Investigator:
Njue J. Kinoti – Chuo kikuu cha Kenyatta
Investigators/Supervisors
Dr Margaret Muturi – Chuo kikuu cha Kenyatta
Dr. Lucy Kamau – Chuo kikuu cha Kenyatta
Dr Raphael M.Lwembe – Tasisi ya utafiti Ya KEMRI

Habari. Jina langu ni Njue James Kinoti, mwanafunzi wa PhD katikaChuo Kikuu cha Kenyatta. Nafanya utafiti wa aina za virusi vya HPVna maswala yanayousu saratani ya uzazi katika eneo hili la mashariki Kenya. Nakuomba ushiriki kwa sababu maoni yako yatakuwa ya muhimu sana kwa kutambua ukidhili kwa saratani ya uzazi eneo hili. Maswali yatachukua muda wa dakika ishilini na tano. Maswali mengine yatakuwa yanagusia tabia zako za ngono, uchumi na jamii. Kushiliki ni kwa ihali. Nakuomba kwa mwoyo mkujufu ujibu kila swali kikamilifu.

Sehemu ya A: *Jamii na mapato*

A1	Uko na miaka ngapi? [1] chini ya 19 [2] 20-29 [3] 30-39 [4] 40-49 [5] 50-59 [6] 60 na zaidi	
A2	Unaishi wapi?	
A3	Ndini lako ni lipi? [0] sina ndini [1] Islimu [2] Mkiristo [3] ndini nyingine	
A4	Umesoma mpaka wapi? [0] sijasoma [1] msingi [3] secondari [4] college [5] chuo kikuu	
A5	Hali yako ya ndoa? [0] sijaolewa [1] nimeolewa [2] tumetengana [3] nimetalikiwa [4] nimefiwa	
A6	Ujuzi wako ni upi? [0] sijaajiliwa [1] nimeajiliwa [2] mwanafunzi	
A7	Mapato yako ya siku ni pesa ngapi ($/siku)? [0] chini (US$1.90 kwa siku) [1] kati (US$1.90 PPP - US$5.50 PPP kwa siku) or [2] juu (juu ya US$5.50 PPP kwa siku).	

Sehemu ya B: *Maswala ya virusi vya HPV*

B1	Unatumia njia yeyote ya kupanga uzazi? [0] la [1] ndio	
B2	Kama ndio, Unatumia njia gani? [0] sindano - depo [1] Condomu [2] kitamanduni [3] Norplant [4] Coil [5] ingine *(eleza)*	
B3	Umetumia njia hii muda kiasi gani	
B4	Unapata shida yeyote kwa kutumia hii njia? [0] la [1] ndio	
B5	Uko na watoto? [0] la [1] ndio Kama ndio,wangapi [0] [1] [2] [3] [4] [5] [6] [7] [8]	
B6	Ulianza kufanya ngono lini? [0] sijawai [1] chini ya miaka 15 [2] 15-20 [3] 21-25 [4] 26-30 [5] 30-35	
B7	Umekuwa na wapenzi wangapi miaka mbili iliyopita? [0] [1] [2] [3] [4] [5] [6] [7] na zaidi	
B8	Kama zaidi ya mbili, unatumia kinga? [0] la [1] ndio	
B9	Umewai gonjeka/pimwa haya magonjwa? Kama ndio, unaweza kuniambia majibu? (a) Kifua kikuu [0] no [1] yes ---------- (b) warts kwa sehemu za siri [0] no [1] yes ---------- (c) maganjwa ya njia za mkojo an uzazi [0] no [1] yes ---------- (d) Ukimwi [0] no [1] yes ---------- (e) Kisonono [0] no [1] yes ----------	
B10	Unavuta sigara? [0] la [1] ndio Kama ndio, sigara ngapi kwa siku ---------	
B11	Unakunywa pombe [0] la [1] ndio Kama ndio, kiasi gani kwa siku/mwezi/mwaka----------	

Sehemu ya C: *Ufahamu, nia, utumizi na malengo kuhusu kupimwa na chonzo ya HPV*

C1	Umewai sikia virusi vya Human Papilloma Virus (HPV)? Kama ndio, ni nini ------- [0] unajua [1] hujui	
C2	Virusi vya HPV zinawezaenezwa? Kama ndio, vipi? ----------	
C3	Unaweza kujikinga na HPV? Kama ndio, vipi ----------	
C4	Unajua kipimo kiitwacho - Visual Inspection by acetic acid - eleza [0] la [1] ndio Kamandio, ni nini?	
C5	Umewaipimwa kwa hii njia? [0] la [1] ndio	
C6	Ungependa kupimwa nah ii njia [0] la [1] ndio [2] sijui	
C7	Unafikili kupimwa kwa hii njia kunasaidia kupingana na saratani [0] no [1] yes	
C8	Unajua kipimo cha Pap smear [0] la [1] ndio	
C9	Umewaipimwa kwa hii njia [0] la [1] ndio [2] sijui	
C10	Ungependa kupimwa kwa njia hii? [0] la [1] ndio [2] sijui	
C11	Unafikili kupimwa kwa hii njia kunasaidia kupingana na saratani [0] la [1] ndio [2] sijui	
C12	Unajua kipimo cha Pap smear HPV DNA PCR? – *eleza* [0] la [1] ndio Kama ndio, ni nini?	
C13	Umewaipimwa kwa hii njia [0] la [1] ndio [2] sijui	
C14	Ungependa kupimwa kwa njia hii? [0] la [1] ndio [2] sijui	
C15	Unafikili kupimwa kwa hii njia kunasaidia kupingana na saratani [0] la [1] ndio [2] sijui	
C16	Mwanamke anafaa kufanyiwa vipimo hivi baada ya muda gani? [0] miezi sita [1] mwaka [2] miaka miwili [3] miaka mitano [4] akiwa na dalili za ugonjwa [5] sijui	

Sehemu ya D: *Chanjo ya HPV*

D1	Umewaisikia chanjo bidhi ya virusi vya HPV? [0] la [1] ndio Kama ndio, ni nini?	
D2	Umewai pata chanjo bidhi ya HPV? [0] la [1] ndio Kama La, kwa nini?	
D3	Unafikili hii chanjo inasaidia kupigana na saratani ya HPV? [0] la [1] ndio	
D4	Unaweza kumshauli mtu wa jamii yako apate hii chanjo? [0] la [1] ndio	

Sehemu ya E: *Saratani ya ujazi, ndalili zake na njia za kupata habali*

E1	Umewai sikia saratani ya uzazi? Kama ndio, ni nini----------	

Ndalili za saratani ya uzazi ni nini *(tia alama)*

1) Kuvunja damu kiolela baada ya hedhi [0] la [1] ndio
2) Kuvunja damu baada ya miaka ya uzazi[0] la [1] ndio
3) Kuvunja damu baada ya ngono [0] la [1] ndio
4) Kutoa uchafu unaonuka kwa sehemu za siri[0] la [1] ndio
5) Uchungu wa tumbo [0] la [1] ndio
6) Uchungu wakati wa ngono [0] la [1] ndio
7) Kukosa hamu ya ngono [0] la [1] ndio

E2 Unafaa kukaa muda kiasi gani ndio upimwe saratani ya uzazi?
[0] Miezi sita [1] mwaka [2] miaka mbili [3] miaka tatu [4] miaka mine
[5] miaka mitano [6] ukipata ndalili [7] sijui

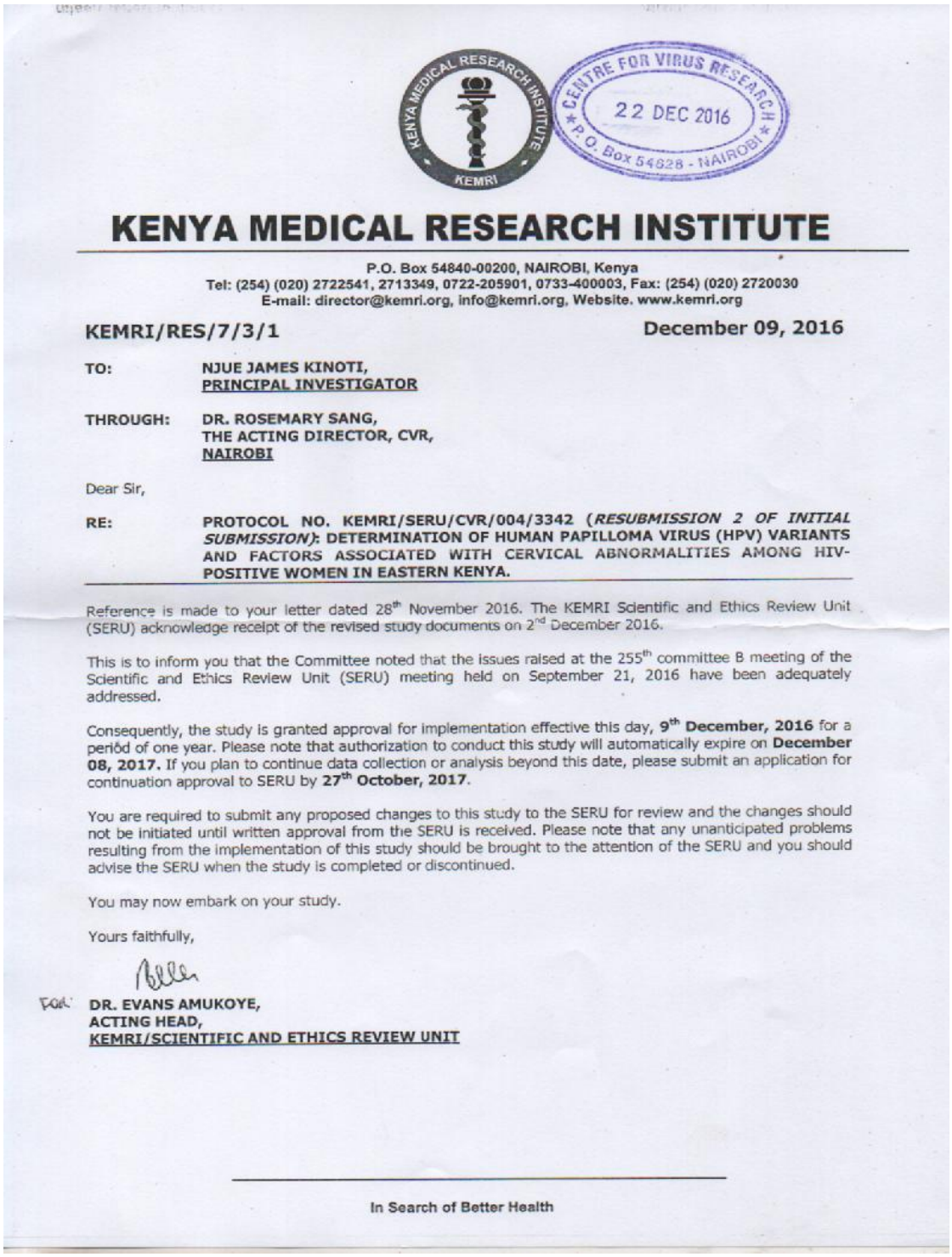
E3 Unapaswa habali za saratani ya uzazi kutoka wapi
[0] radio [1] madaktali [2] wakubwa wa afya [3] mtandao [5] TV [6] jamii
[7] magazeti

E4	Kuna mtu yeyote wa jamii yako amewaipata saratani ya uzazi [0] la [1] ndio	
E5	Unaweza kulithi saratani ya uzazi? [0] la [1] ndio	

E6 Maoni yako

Asante kwa kushiliki

Appendix 12: Ethical clearance letter



KENYA MEDICAL RESEARCH INSTITUTE

CENTRE FOR VIRUS RESEARCH
22 DEC 2016
P.O. Box 54628 - NAIROBI

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **December 09, 2016**

TO: **NJUE JAMES KINOTI,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. ROSEMARY SANG,**
THE ACTING DIRECTOR, CVR,
NAIROBI

Dear Sir,

RE: **PROTOCOL NO. KEMRI/SERU/CVR/004/3342 (*RESUBMISSION 2 OF INITIAL SUBMISSION*): DETERMINATION OF HUMAN PAPILLOMA VIRUS (HPV) VARIANTS AND FACTORS ASSOCIATED WITH CERVICAL ABNORMALITIES AMONG HIV-POSITIVE WOMEN IN EASTERN KENYA.**

Reference is made to your letter dated 28th November 2016. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledge receipt of the revised study documents on 2nd December 2016.

This is to inform you that the Committee noted that the issues raised at the 255th committee B meeting of the Scientific and Ethics Review Unit (SERU) meeting held on September 21, 2016 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **9th December, 2016** for a period of one year. Please note that authorization to conduct this study will automatically expire on **December 08, 2017.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **27th October, 2017**.

You are required to submit any proposed changes to this study to the SERU for review and the changes should not be initiated until written approval from the SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the SERU and you should advise the SERU when the study is completed or discontinued.

You may now embark on your study.

Yours faithfully,

FOR: **DR. EVANS AMUKOYE,**
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix 13: Research authorization letter

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
when replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. NACOSTI/P/17/85191/15335

Date: 2nd February, 2017

James Kinoti Njue
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Determination of human papilloma virus (hpv) variants and factors associated with cervical abnormalities among HIV-positive women in Eastern Kenya,"*** I am pleased to inform you that you have been authorized to undertake research in **selected Counties** for the period ending **1st February, 2018.**

You are advised to report to **the County Commissioners, the County Directors of Health and the County Directors of Education, selected Counties** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf**of the research report/thesis to our office.

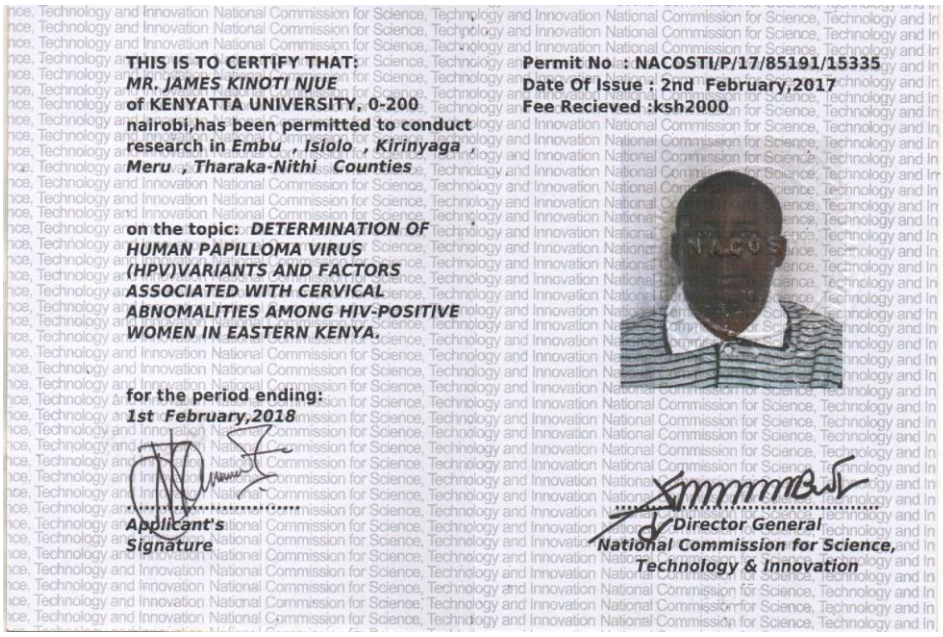
BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioners
Selected Counties.

The County Directors of Health
Selected Counties.

Appendix 14: Research permit



THIS IS TO CERTIFY THAT:
MR. JAMES KINOTI NJUE
of KENYATTA UNIVERSITY, 0-200 nairobi,has been permitted to conduct research in *Embu , Isiolo , Kirinyaga , Meru , Tharaka-Nithi Counties*

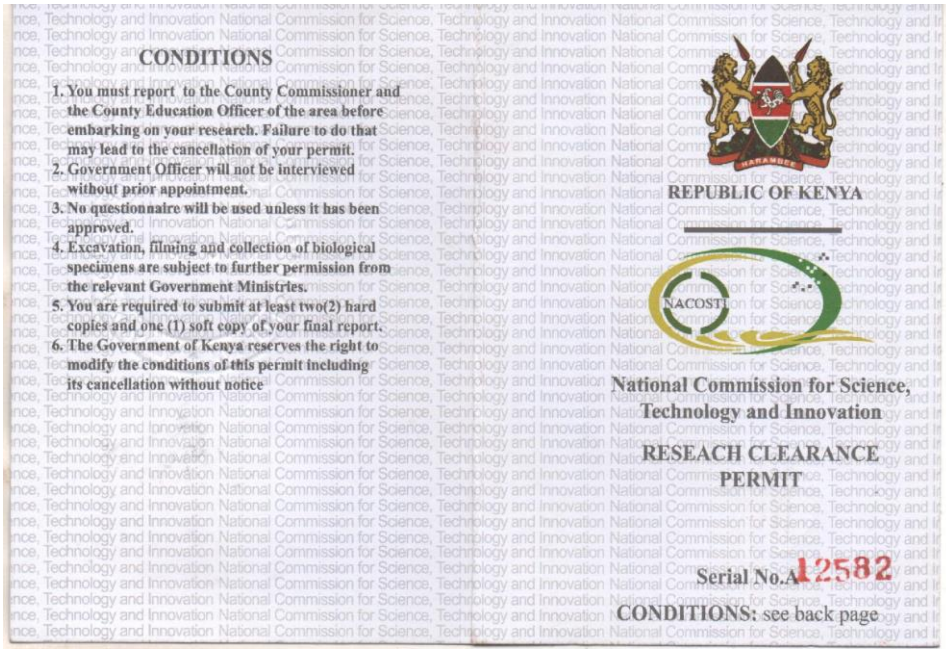
on the topic: *DETERMINATION OF HUMAN PAPILLOMA VIRUS (HPV)VARIANTS AND FACTORS ASSOCIATED WITH CERVICAL ABNOMALITIES AMONG HIV-POSITIVE WOMEN IN EASTERN KENYA.*

for the period ending:
1st February,2018

.....
Applicant's Signature

Permit No : NACOSTI/P/17/85191/15335
Date Of Issue : 2nd February,2017
Fee Recieved :ksh2000

.....
Director General
National Commission for Science, Technology & Innovation



CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officer will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

HARAMBEE

REPUBLIC OF KENYA

NACOSTI

National Commission for Science, Technology and Innovation

RESEACH CLEARANCE PERMIT

Serial No.A 12582

CONDITIONS: see back page

Appendix 15: Letter of authorization – Embu County

EMBU COUNTY GOVERNMENT
DEPARTMENT OF HEALTH

Telephone: 068-2231055/56
Cell Phone: 0704558949
Email: pghembu@gmail.com

When replying please quote our reference

Ref. SI4 VOL. II /101

OFFICE OF THE
MEDICAL SUPERINTENDENT
EMBU LEVEL 5 HOSPITAL
P.O. BOX 33
EMBU
Date 30th January 2016

To.
James Njue Kinoti

RE: APPROVAL TO CONDUCT RESEACRH IN THE LABORATORY AND PRODUCTIVE HEALTH UNITS

Approval is hereby granted to the above to undertake a research in the laboratory department and reproductive health unit having already paid Ksh. 4100 as hospital research fee.

Kindly accord him necessary assistance.

George Muthinji
For. Medical Superintendent
Embu Level 5 Hospital

MEDICAL SUPERINTENDENT
EMBU LEVEL 5 HOSPITAL
P. O. Box 33-60100, EMBU
Email: pghembu@gmail.com
Tel: 068-2230706 / Fax: 2230706

Appendix 16: Letters of authorization – Isiolo County

MINISTRY OF HEALTH

Telephone: Isiolo 064-52031
0722606180
0734841346
E-mail: isiolohealth@yahoo.com

OFFICE OF THE
MEDICAL SUPERINTENDENT
ISIOLO COUNTY REFERRAL
HOSPITAL
P. O. BOX 42
ISIOLO

REF.ISL/MED/H5 VOLII/392

2rd February, 2017

RE: APPROVAL TO CONDUCT RESEARCH IN THE LABORATORY AND PRODUCTIVE HEALTH UNITS FOR JAMES NJUE KINOTI.

This is to inform you that the hospital has no objection for Mr James Njue Kinoti to conduct his research.

The approval has been granted after his application to do research in PHD Degree program in department of Medical Laboratory Science at Kenyatta University was received.

Thanks.

ISIOLO DISTRICT HOSPITAL
MEDICAL SUPERINTENDENT
3 FEB 2017
P O Box 42-60300, ISIOLO

Aden R Golicha
Hospital Administrator
ISIOLO COUNTY REFERRAL HOSPITAL

Appendix 17: Letters of authorization – Kirinyaga County

KIRINYAGA COUNTY GOVERNMENT

COUNTY DEPARTMENT OF HEALTH

Telegrams: "MEDICAL", KERUGOYA
Telephone: (060) 21564, 21058
Fax (060) 21564
E- mail: dmohkirinyaga@yahoo.com
When replying please quote:

COUNTY DIRECTOR OF HEALTH
KIRINYAGA,
P. O. BOX 24,
KERUGOYA

REF; CDH/RES/VOL.I/248

30TH JANUARY 2017

THE MED SUPT
KERUGOYA COUNTY REFERRAL HOSPITAL

RE; APPROVAL TO CONDUCT RESEARCH IN KERUGOYA COUNTY REFERRAL HOSPITAL – NJUE JAMES KINOTI – REG.NO.P97/2811/2013.

We acknowledge the application for approval by the above named to conduct a research project titled "Determination of human papilloma virus (HPV) types and Risk Factors with Cervical Abnormalities among HIV- Positive Women in Eastern Kenya" in Kerugoya County Referral Hospital Kirinyaga County.

This is a course project in the Ph.D. degree programme in the Department of Medical Laboratory Science at Kenyatta University.

He is hereby granted approval to conduct this project in Kirinyaga County.

He is **Expected to Submit** the research findings to the County Department of Health with a final report being submitted before expiry of the approval period ending 27th October 2017.

He may embark on the study.

CHIEF OFFICER HEALTH
KIRINYAGA COUNTY
P. O. Box 260 - 10304
KUTUS

DR. ESBON GAKUO
COUNTY DIRECTOR OF HEALTH
KIRINYAGA COUNTY.

Appendix 18: Letters of authorization – Meru County

COUNTY GOVERNMENT OF MERU
DEPARTMENT OF HEALTH

Telegrams: "MEDICAL" Meru
Telephone: Meru 064-32370/1
Fax: 31242
Email: *hospitalmeru@yahoo.com*
When replying should be to:
Medical Superintendent

MERU TEACHING & REFERRAL HOSPITA
P. O. BOX 8 – 60200
MERU

Ref: MRU/MED/GEN/R.14

DATE: 2nd February 2017

Njue James Kinoti
Reg. No. (P97/28711/2013))
Kenyatta University
Department of Medical Laboratory Science

RE: PERMISSION TO COLLECT RESEARCH DATA

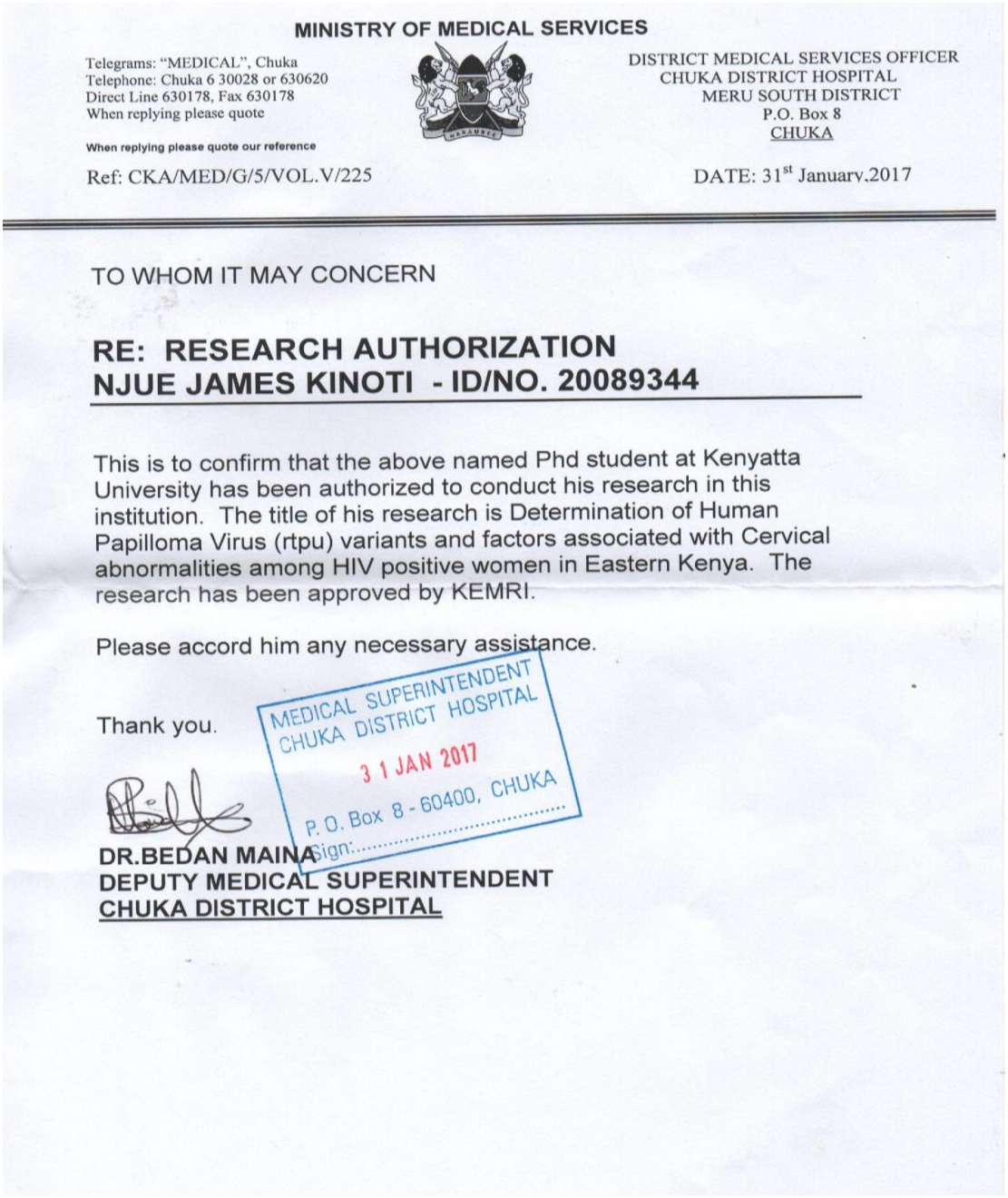
Following your request to collect data in Meru Teaching and Referral Hospital for your research on **"Determination of Human Papilloma Virus (HPV)types and factors associated with cervical abnormalities among HIV –positive women in Eastern Kenya**", you are hereby granted access to recruit study participants from clients accessing our services.

Note that on completion of the research, you are expected to submit one (1) Hard copy and soft copy to this facility.

Dr. Kanana Kimonye
Deputy Chief Executive Officer
Meru Teaching and Referral Hospital

THE COUNTY GOVERNMENT OF MERU
CHIEF EXECUTIVE OFFICER
MERU TEACHING AND REFERRAL HOSPITAL
P. O. Box 8- 60200 MERU

Appendix 20: Letters of authorization – Tharaka-Nithi County



MINISTRY OF MEDICAL SERVICES

Telegrams: "MEDICAL", Chuka
Telephone: Chuka 6 30028 or 630620
Direct Line 630178, Fax 630178
When replying please quote

When replying please quote our reference

Ref: CKA/MED/G/5/VOL.V/225

DISTRICT MEDICAL SERVICES OFFICER
CHUKA DISTRICT HOSPITAL
MERU SOUTH DISTRICT
P.O. Box 8
CHUKA

DATE: 31st January,2017

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORIZATION
NJUE JAMES KINOTI - ID/NO. 20089344

This is to confirm that the above named Phd student at Kenyatta University has been authorized to conduct his research in this institution. The title of his research is Determination of Human Papilloma Virus (rtpu) variants and factors associated with Cervical abnormalities among HIV positive women in Eastern Kenya. The research has been approved by KEMRI.

Please accord him any necessary assistance.

Thank you.

MEDICAL SUPERINTENDENT
CHUKA DISTRICT HOSPITAL
31 JAN 2017
P. O. Box 8 - 60400, CHUKA
Sign:.....

DR.BEDAN MAINA
DEPUTY MEDICAL SUPERINTENDENT
CHUKA DISTRICT HOSPITAL