ANALYSIS OF MANUAL LIQUID-BASED CYTOLOGY, HISTOPATHOLOGY AND HPV DNA TESTING AMONG HIV-POSITIVE WOMEN AT MACHAKOS LEVEL 5 HOSPITAL, MACHAKOS COUNTY, KENYA

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DECLARATION

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

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DEDICATION

I dedicate this work to my wife Diana and my parents Francis Mutuku and Susan Mutuku.

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OPERATIONAL DEFINITIONS OF TERMS

Carcinoma: A cancer type arising in skin cells or the epithelial tissue covering internal organs.

Cervical Cancer: A cancer type that affects cells of the cervix which is the lowest portion of the uterus connecting it with the vagina.

Colposcopy: A procedure for examining the cervix, vulva and vagina using a colposcope to detect abnormal changes in the cells, especially those suspicious for malignancy.

Conventional Pap smear: A technique for screening women for abnormal cervical lesions and malignancy of the cervix that involves collection of cervical smear using an Ayres spatula and cytobrush.

Dysplasia: The development of abnormal cells in a tissue which typically is followed by changes favoring development of malignancy.

Hysterectomy: A procedure done surgically to excise the entire uterus or part of it.

Liquid Based Cytology: A monolayer slide preparation technique for collecting cytological specimens in particular from the cervix by suspending patient specimen in a liquid fixative.

Squamocolumnar Junction: A junction between the ectocervix and the endocervix parts of the cervix where majority of cervical cancers and precancerous lesions occur.

LIST OF ABBREVIATIONS

ACCP: Alliance for Cervical Cancer Prevention

ACS: American Cancer Society

AGC: Atypical Glandular Cells

AIDS: Aquired Immuno Deficiency Syndrome

AIS: Adenocarcinoma In Situ

ALBC: Automated Liquid Based Cytology

ASCH : Atypical Squamous Cells cannot exclude High Grade lesion

ASCUS: Atypical Squamous Cells of Undetermined Significance

BV: Bacterial Vaginosis

CCC: Comprehensive Care Centre

CIN: Cervical Intraepithelial Neoplasia

CPS: Conventional Pap Smear

DNA: Deoxyribonucleic acid

GLOBOCAN: Global Cancer Incidence, Mortality and Prevalence

HIV: Human Immuno-deficiency Virus

HPV: Human Papiloma Virus

HR-HPV: High Risk – Human Papiloma Virus

HSIL: High-grade squamous intraepithelial lesion

IARC: International Agency for Research on Cancer

IUCD: Intra Uterine Contraceptive Device

KMPDC: Kenya Medical Practitioners and Dentists Council

KUERC: Kenyatta University Ethical Review Committee

LBC: Liquid Based Cytology

LSIL: Low Squamous Intraepithelial Lesions

MLBC: Manual Liquid Based Cytology

NACOSTI: National Comm ission for Science, Technology and Innovation

NCCPP: National Cervical Cancer Prevention Program

NILM: Negative for Intraepithelial Lesion or Malignancy

SCC: Squamous cell carcinoma

SCJ: Squamocolumnar Junction

SEKEB: South Eastern Kenya Economic Block

SIL: Squamous Intraepithelial Lesion

SOP: Standard Operating Procedures

SPSS: Statistical Package for the Social Sciences

UHC: Universal Health Coverage

WHO: World Health Organization

ABSTRACT

Liquid-based cytology is a technique of preparing a monolayer of cells by washing the cells in a phial of liquid fixative and spreading a thin layer of the sample on a glass slide. This enables a better morphological assessment of the cells, thus increasing the detection rate of cytological abnormalities. In the developed countries, the liquid based cytology technique is fully automated; however, in developing countries, the approach is undermined by its unavailability and related costs. Although conventional Pap smear examination is the primary approach for early detection of cervical cancer, it has shortcomings such as presence of obscuring materials like blood, mucus, and inflammation; hence, the sensitivity is reduced considerably. Contrary, manual liquid based cytology technique increases specimen adequacy rate and improves precursor lesions and cervical cancer detection by its ability to overcome the shortcomings experienced in Conventional Pap Smear. The main objective of this research was to compare the performance of manual liquid based technique versus that of histopathology and assess the utility of its remnant samples in detection of high risk Human Papilloma Virus. Through the use of a Manual liquid-based cytology, a prospective study of 400 cases was evaluated for pre-cancerous lesions and cervical cancer at Machakos County Hospital among women attending the Comprehensive Care Centre. Purposive sampling method was applied to obtain the study population. The principal investigator screened all the Pap smears and a pathologist reviewed all the abnormal smears. All participants with features of high-grade squamous intraepithelial lesions and above based on cytological results were referred for histopathology. In all remnant samples with abnormal lesions encompassing atypical squamous cells of unknown significance and above, detection of Human Papilloma Virus 16 and 18 was done using a real time Polymerase Chain Reaction. Chi-Square test was used to relate the groups' nominal categorical data appropriately. Cohen Kappa test was performed to establish the exact level of agreement among HPV DNA testing, histopathology, and Manual liquid based cytology. The prevalence of cervical lesions in this study was 7.8%. There were 41 (10.3%) cases of bacterial vaginosis recorded in this study. Out of the 25 samples tested for High-risk HPV DNA, 18 (72%) were positive while 7 (28%) were negative. Ten women were referred for biopsy and histopathological examination. Of those 10, 4 (40%) had CIN II, 3 (30%) had CIN III, 2 (20%) showed features of squamous cell carcinoma while 1 (10%) was found to have chronic cervicitis. There was moderate agreement between histopathology and high risk HPV DNA testing; k=0.574, (95% CI, .41 to .60), p =0.11. There was substantial agreement between histopathology and high risk HPV DNA testing; k=0.615, (95% CI, .61 to .80), p =0.035. Overall, manual liquid based cytology was found to have a moderate level of agreement with histopathology with ability to preserve remnant samples for adjunctive tests such as HPV DNA detection; therefore, it can be considered a substitute screening method in limited resource settings.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Cervical cancer remains a prevalent type of cancer in women with an increasingly high number of new cases, especially in the low and midle income countries where there are no well established screening programs (WHO, 2020). According to a recent data recorded by Global Cancer Incidence, Mortality and Prevalence (GLOBOCAN), cancer of the cervix was ranked the fourth topmost identified cancer and number four leading cause of cancer mortalities among women. There were 604,127 new cases recorded globally in 2020, which represents 6.5% of all cancers affecting females (Hull *et al*., 2020). In 2020, over ninety percent of all cervical cancer associated mortalities occurred in the low and middle-income countries (Sung *et al*., 2021). This number is anticipated to increase within the next ten to twenty years among women living in these countries unless control measures are implemented (Hull *et al*., 2020).

In the developing countries, the positivity rates of cervical cancer are still escalating. For instance, Africa alone recorded a total of 117,316 new cases and 76,745 deaths in 2020. Based on the 2020 International Agency for Research on Cancer‘s (IARC) report, cancer of the cervix was ranked the second frequent cancer type in Africa following cancer of the breast (Ferlay *et al.*, 2020). Of all the new cases in Africa, Eastern Africa had the highest incidence, recording a total of 54,560

new cases in 2020 (Sung *et al.,* 2021). In 2018, among the top twenty countries globally leading with cervical cancer new cases, nineteen of them were African (Bray *et al*., 2018).

Kenya was among the top twenty countries globally that recorded the highest positivity rates of cervical cancer in 2018 and was ranked position twenty (Bray *et al*., 2018). Consequently, cervical cancer is considered the second commonly detected cancer among Kenyan women and the first topmost frequent cancer amongst those females within the reproductive age group (Ferlay *et al.,* 2020). In 2020, Kenya reported 5,236 cases of cervical cancer and 3,211 cervical cancer fatalities (Sung *et al.,* 2021). According to Bruni *et al*. (2018), 9.1% of Kenyan women at any given time harbor the high-risk human papilloma virus (HPV) 16/18 infection and about 70 percent of cervical cancer cases results from these HPV subtypes.

Although its aetiology is suggested to be multifactorial, cervical cancer is said to be progressive and closely associated with prolonged infection with oncogenic strains of HPV (Zur Hausen, 2002; Ronco *et al*., 2010). Prolonged infection and persistence of high-risk strains of HPV is highly associated with the cervical cancer development, but it is insufficient to cause cancer on its own. It is estimated that 70% of cervical cancers cases are linked to infection with HPVs 16 or 18 strains (Woodman *et al*., 2007). Even though most sexually active women acquire HPV

infection during their life time at some point, in majority of the cases, the infection gets cleared after some few months. However, in some instances, persistent infection may occur, which increases the risk of developing precancerous lesions in the cervix that may progress to malignancy (Kjær *et a*l., 2010).

The progression of cervical cancer is slow, and this increases the chances of detecting pre-cancerous lesions and cervical cancer in early stages if regular screening is done (Wentzensen *et al*., 2009). Unlike most other cancer types, cervical cancer can be mitigated and intercepted, especially when pre-cancerous lesions are identified and managed at earlier stage. Thus, screening is paramount in reducing incidence rates and cervical cancer-related mortalities (Mishra *et al*., 2011).

When High Risk-Human Papilloma Virus (HR-HPV) enters the basal epithelial cells through epithelial abrasion, the virus may persist depending on the host immunity and other known risk factors; thus changing the integrity of the cells and leading to precancerous intraepithelial lesions. According to Usyk *et al*. (2020), when the lesions fail to regress, HR-HPV integrates into the host's cell genome, resulting in cell invasion and eventually invasive cancer.

For immunocompromised patients, particularly those affected by HIV/AIDS, the lesions preceding cervical cancer develop rapidly and are more aggravated

(Chambuso *et al*., 2018). Studies indicate that HIV and HPV persistence in immunosuppressed individuals pose a great risk of advancing invasive cervical and cervical dysplasia carcinoma. Women who are positive for HIV have higher chances of acquiring HPV, which is considered the primary causative agent of cervical cancer (Weldegebreal & Worku, 2019; Jolly *et al*., 2017). In immunocompetent women, the progression period of HPV infection is slower, usually 10–20 years to pre-cancer. However, among the HIV positive women, the rate is faster and more frequent, resulting in the rapid development of pre-cancer and invasive cancer (WHO, 2014).

Developing countries have lagged behind in combating the high mortality rate of cervical cancer because of limited resources and lack of a well-funded healthcare system (Beddoe, 2019). A study by Pankaj *et al*. (2018) documented the wide use of Conventional Pap Smear (CPS) test for cervical cancer detection. However, more insights have considered the technique less effective due to its inherent limitations. With CPS, many cells are uncaptured as only a portion of the specimen is smeared onto a microscope slide after collection. Furthermore, there is no representative transfer of the sample as the collection device is discarded, at times with more than 80% of the collected patient's sample still on the device (Kavatkar et al., 2008; Sherwani et al., 2007).

Another problem associated with CPS is clumping and overlapping, with more than one layer of cells formed, leading to a poor visualization of the cells. The CPS

specimen may appear clouded due to debris obtained from blood and mucus, thus obscuring visibility during microscopic examination (Duraisamy, Jaganathan and Bose, 2011). Drying artifacts may also be formed if the cells are not fixed immediately. Lastly, the collection device is discarded and thus a repeat sample is not available if needed. These limitations have been shown to reduce the sensitivity of CPS to less than 50% (Kavatkar et al., 2008).

A cervical cancer screening technique that applies the use of Automated Liquid-Based Cytology (ALBC) was invented to advance the sensitivity of CPS. However, the very high cost related to automated devices has hampered its implementation and use in the developing countries, like Kenya (Sharma *et al*., 2016). In Liquid Based Cytology (LBC), cervical cells are collected in a special container partially filled with a formulated fixative solution followed by further processing as opposed to CPS where a smear is made directly on a slide after collection. In LBC technique, the cellular structure is better preserved with reduced drying artifacts as fixing of the cells is done immediately without delay (Bentz, 2005).

Contrary to ALBC, a Manual Liquid Based Cytology (MLBC) technique is a cost-effective method as it utilizes reagents and equipment that are commonly available in most clinical laboratories (Mutuku *et al*., 2018). With MLBC, the rate of detecting precancerous lesions and cervical cancer is improved. Moreover,

specimen adequacy is improved as there are minimal artifacts, contaminating mucus, and blood; hence, cases of unsatisfactory smears are reduced (Nandini *et al*., 2012). The manual method allows proper mixing with evenly distribution of cells on slides, thus increasing the chances of detecting abnormal cytological lesions (Elnashar & Ghaffar, 2012).

Following the introduction of cytology-based screening programs over the past 50 years, mortality and morbidity from cervical cancer has significantly reduced (Safaeian, Solomon, & Castle, 2007). In most developing countries, including Kenya, the cytology technique is the primarily used method for screening invasive cervical cancer and its precursor lesions (Nygard, 2011). In cytological examination, a single screen is not satisfying to rule out cervical cancer, which necessitates multiple screens for higher sensitivity, making it a key limitation (Catarino *et al*., 2015).

Most abnormal cytological screening Pap smear results comprise Atypical Squamous Cells of Unknown Significance (ASCUS) and Low-grade squamous intraepithelial lesions (LSIL) (Schiffman, 2007). While these anomalies are generally cytomorphological actualization of “harmless”, ephemeral HPV infections, in 10–15% of women, the evident cytologic changes are followed by a fundamental histologic pre-cancer when histopathological examination is done (CIS/CIN3) (Sherman *et al*., 2003). Even though majority of ASCUS and LSIL are

likely to regress, there are cases in which they develop to higher lesions that may later advance to malignancy, especially in older women (Sundstrom *et al*, 2017). For detection of high-grade lesions, the ASCUS and LSIL cytology results should be further reviewed (Arbyn *et al*., 2013).

A study comparing the MLBC and CPS method affirmed the effectiveness of MLBC in giving better results than CPS in terms of specimen adequacy, specimen's preservation for future testing, clear background, and reduced cellular overlapping with increased detection of cervical cytology anomalies (Mutuku *et al*., 2018). In MLBC, specimen adequacy is improved due to the fact that at the time of sample collection; the entire brush with cells is transferred to the fixative solution, thus, minimizing wastage (Dhananjaya and Kumari, 2017).

1.2 Problem Statement

Developing countries are faced with the highest cervical cancer burden yet there are no alternative screening methods to overcome the challenges associated with CPS. The key challenges encountered include overlapping of cells and unclear background due to presence of mucus and debris. These shortcomings have been shown to reduce the sensitivity of CPS resulting to high number of false-negative rates. Another challenge facing the CPS is how to triage women with LSIL and ASCUS, which poses a risk of poor management of these patients. From the above challenges, therefore, it is necessary to adopt an alternative strategy for effective

screening and mitigation of cervical cancer amongst women living in countries with limited resources. This study seeks to compare MLBC with histopathology and asses the utility of remnant samples in detecting high-risk HPV in patients with ASCUS results and above.

1.3 Rationale of the Study

A screening technology that matches the limited resources we have in Kenya will benefit both the physician and the patient. There is a need to introduce adjunctive HPV testing in conjunction with cytology to triage patients with abnormal cytological smears, especially those with LSIL and ASCUS. This study assessed the performance of MLBC and compared it with histopathology. The residual samples for abnormal cytology were used to detect HR-HPV, thus increasing the utility of MLBC in screening for cervical cancer and its precursor lesions. This, in turn, is expected to improve the clinical management of the patients. This study made use of locally available reagents and equipment. The fixative and polymer solutions used were formulated from reagents available in the setup of resource limited facilities such as the ones in Kenya, and other developing countries. The findings from this thesis will be shared with the policy makers in the health sector and specifically Machakos county hospital department of emergency and health services. Overall, this is expected to aid in formulating policies that can enhance screening services for early diagnosis and prompt management of cervical cancer cases, especially in HIV positive women.

1.4 Research Questions

i. What is the prevalence and patterns of the cervical cytological lesions in HIV infected women?
ii. What is the prevalence of bacterial vaginosis among HIV infected women?
iii. What is the prevalence of high risk HPV in abnormal cytological finding of ASCUS, ASC-H, HSIL and LSIL?
iv. What is the agreement level between MLBC and histopathology?
v. How does histopathology test results and HPV findings compare?

1.5 Objectives of the Study

1.5.1 General Objective

To compare the performance of MLBC technique versus histopathology in screening for abnormal cervical cytology and assess the utility of its remnant samples in detection of high risk human papilloma virus

1.5.2 Specific Objectives

i. To determine the prevalence and patterns of cervical cytological lesions in HIV infected women.
ii. To determine the prevalence of bacterial vaginosis among HIV infected women.

iii. To determine the prevalence of high risk HPV in abnormal cytological finding of ASCUS, ASC-H, HSIL and LSIL.

iv. To determine the agreement level between MLBC and histopathology.

v. To correlate histopathology test results with HPV findings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cervical Cancer

Cervical cancer occurs in the uterine cervix's cells, the lowest part of the uterus that connects it to the vagina. The epithelium lining the cervix is both columnar and stratified with non-keratinizing squamous epithelia. The cervix has an area of transformation zone where cells in the columnar epithelium constantly change into squamous cells (ACS, 2020). It is in this transformation zone where most cervical cancers begin. However, there is no sudden transformation of cells to cancer. Normal cells slowly develop pre-cancerous changes which, in turn, progress to become malignant (Schiffman *et al.,* 2007).

Cervical cancer is categorized into two major types namely, adenocarcinoma, which occurs in the columnar epithelium, and Squamous Cell Carcinoma (SCC), which involves the squamous epithelium part. Notably, SCC accounts for 70% of cervical cancers (Lee *et al*., 2006). It occurs in the exocervix cells lining, and the cancer cells presents with squamous cells' features under microscopic examination. The development of SCC commences at the transformation zone, which is a junction located between the cervix's columnar cells and the squamous cells (Cohen *et al*., 2010).

The second type is adenocarcinoma, which accounts for approximately 18 percent of cervical carcinomas and arises from the mucus-secreting endocervical cells (Gien, Beauchemin, &Thomas, 2010). Other carcinomas of the cervix include adenosquamous and other malignancies or carcinomas constituting 4% and 5%, respectively (ACS, 2020).

2.2 Epidemiology of Cervical Cancer

2.2.1 Global

In terms of prevalence, cancer of the cervix is ranked as the fourth topmost identified cancer affecting females following breast, colorectal, and lung cancers. It is estimated that 604,127 new cases occurred in 2020, with 341,831 new deaths reported from cervical cancer. This represents 6.5% of all female cancer cases. Sadly, over ninety percent of mortalities related to cervical cancer were reported in middle and low-income nations. Of all the new cases reported in 2020 globally, Asia recorded the highest number of new cases (351,720) comprising over 50% of all new cases of cervical cancer globally (Sung *et al*., 2021).

The World Health Organization (WHO) reports that over 300,000 cervical cancer-related deaths are recorded globally every year (WHO, 2020). In the less developed countries, screening and treatment amenities are not well established, and thus, many women have no access to health services (Beddoe, 2019). As a result, many access medical services late, thus making the treatment more difficult and

expensive, and chances of cure are minimal. Screening allows for treatment in the asymptomatic precancerous stage. It is projected that the burden of cervical cancer will rise to 700,000 new cases and 400,000 deaths globally by 2030 unless effective control measures are adopted (WHO, 2020).

There is a high disparity in cervical cancer incidences between higher and lower income regions. Developed countries have experienced dramatic decrease in cervical cancer morbidity and mortality (Safaeian, Solomon, & Castle, 2007). In the western countries, incidence rates of cervical cancer dropped by more than 50% from 1975 through 2020 following an increase in screening as a way of detecting cervical cell changes before they turn cancerous (WHO, 2020).

The Third World countries, including those in the Sub-Saharan, Caribbean, and Latin American regions, experience the highest cervical cancer burden with over 85 percent of cases recorded (Sung *et al*., 2021). This is contrary to the low number of cases documented in the Western countries like North America and Western Europe. For instance, per every 100,000 population samples from Western Europe and North America, the range of Incident Rate (IR) was 6.6 to 7.3 (Sung *et al*., 2021). This was slightly higher when compared to the 4.4 and 5.5 IR recorded in Western Asia and New Zealand, respectively, per every 100,000 sample population. In 2020, Europe recorded 58,169 new cases of cervical cancer representing less than 10% of all new cases globally (Ferlay *et al.,* 2020).

The innovation of Pap smear in 1950 as a cervical cancer cytological screening technique has precipitated a comparative decrease of invasive cervical cancer incidences, especially in first world countries. This is due to the effective screening and treatment programs in these countries (Catarino *et al*., 2015). In the developing countries, implementation of Pap smear programs remains a big challenge due to limited resources; thus, cervical cancer continues to threaten the lives of women from these countries up to date (Ali & Wassie, 2012).

2.2.2 Africa

In Africa, cervical cancer is still a key health threat in women as it is ranked as the second commonly identified cancer affecting women. In the year 2020, a total of 117,316 new cases and 76,745 deaths were recorded in Africa alone (Sung *et al.,* 2021). Africa continent has approximately 270 million women aged 15 years and above who are predisposed to cervical cancer (WHO, 2020). Data from IARC ranks cancer of the cervix as the second leading commonly identified cancer in Africa after cancer of the breast (Ferlay *et al.,* 2020). Of all the new cases in Africa, Eastern Africa had the highest number of new cervical cancer cases, 54,560, in 2020 (Sung *et al.,* 2021).

In most of the African countries, limited access to reproductive health services, as well as lack of effective screening, has contributed to late diagnoses of most cervical cancers (Beddoe, 2019). Given the lack of well-established health care

facilities, most women in the less developed countries are diagnosed with cervical cancer at late stages, leading to poor prognosis following treatment. In 2018, among the top 20 countries in terms of new cervical cancer cases, 19 of them were African countries (Bray *et al*., 2018).

2.2.3 Kenya

In 2020, Kenya recorded a total of 5,236 new cases and 3,211 cervical cancer-related deaths. Cervical cancer leads in terms of cancer-related deaths in Kenya. As such, it is the second commonly identified cancer among Kenyan women after breast cancer (Sung *et al.,* 2021). Furthermore, Kenya was ranked position 20 globally among the leading twenty countries with highest cases of cervical cancer in 2018 (Bray *et al*., 2018).

The Kenyan population comprises 16.2 million females aged 15 years and above, implying that a great number of the Kenyan women are highly susceptible to developing cervical cancer if early screening and adequate health education are not implemented (Bruni *et a*l., 2019). . Approximately 9.1% of women in Kenya at any given time harbor the high risk HPV16/18 infection. Close to 70% of cervical cancers are as a result of HPVs 18 or 16 subtypes (Bruni *et a*l., 2019). According to WHO (2018) data, for every 100,000 Kenyan women, 33 are cervical cancer patients and about 22 succumb to the disease.

2.3 Etiology of Cervical Cancer and Risk Factors

From early studies, high-risk oncogenic HPV virus, which is sexually transmitted, has been strongly associated with cervical cancer (Bosch and Muñoz, 2002' Munoz *et al*., 1994). Sexual intercourse at an early age makes women highly vulnerable to the disease (Louie *et al*., 2009). In healthy women, HPV infection may be common, but their immune system is strong enough to eliminate the infection. Combined with other factors, the presence of high-risk HPV in the cervix can trigger the development and progression of pre-neoplastic lesions (Schiffman & Wentzensen, 2013).

The development of cervical cancer has been linked to persistent, high-risk HPV infection of the cervix (but not sufficient) (Moscicki *et al*., 2006). Evidently, development of cervical cancer is preceded by HPV infection in almost all the cases after which precancerous lesions develop (Ronco *et al*., 2010). Sexual activity is recognized as the primary pathway for HPV, but most infections may clear after one to two years (Moscicki *et al*., 2006). In contrast, if the high-risk HPV infection persists, specifically the HPV16 and HPV18, the effect is progression to either precursors or invasive cervical cancer (Schiffman *et al*., 2007). These precancerous lesions might take decades to develop and eventually can either progress to high grade cancer or to invasive type. The process can take between ten to thirty years (Schiffman *et al*., 2007).

High-risk HPV have been identified in most cervical cancer cases and the presence of HPV oncoproteins helps in the identification of the cancer phenotype. According to the IARC, 12 types of HPV are carcinogenic to humans. However, HPV 16 and 18 are classified as the most carcinogenic, accounting for approximately 70 percent of the global cervical cancer cases (IARC, 2012).

The prevalence of the major HPVs linked to cervical cancer varies slightly with geographical locations, although a fraction of cancers caused by other HPV types apart from 16 and 18 are higher in high-risk areas (IARC, 2012). The distribution of the high-risk HPV differs across geographical regions. This can be attributed to the discrepancy in the number of participants included in various studies as well as the varied cultural practices across the nations (Haghshenas *et al*., 2013). Women from different geographical areas have different sexual behaviors as well as moral and sexual practices, which affect their vulnerability of contracting HPV (Ali, Bedair and Abdi, 2019).

Cervical cancer is associated with the following risk factors: multiple sex partners, immunosuppression, chlamydia infection, intrauterine device use, long-term use of contraceptives, obesity, smoking, and family history of cervical cancer (ACS, 2020). Vinodhini *et al*., (2012) observed that women in the less developed regions were at a higher risk of acquiring HPV due to their increased exposure and living conditions as well as their lifestyle.

2.4 Natural History and Pathogenicity

Although it is likely that there is no single cause of cervical cancer, cervical neoplasia, sexual activity, and HPV are considered the causal agents based on early epidemiological findings (Muñoz *et al*., 1988). Therefore, HPV plays a significant role in cervical cancer development.

There are three categories of HPVs: high-risk type (16, 18, 31, and 45), which are found in approximately 90% of cervical cancers, intermediate type (33, 35, 39, 51, 52, 56, 58, 59, and 68), and the low-risk types (6, 11, and 42-44) (Burd, 2003). The low-risk HPV types are mostly linked with CIN 1 and cervical condylomas. The association between the high risk HPVs and cancer has odds ratios of greater than fifteen (> 15), which is stronger in methodologically sound case controlled studies (Snijders *et al*., 2011).

There exist more than 70 strains of HPV according to the classification, with some of these types such as HPV 6 and 11 being associated with warts. Other strains have oncogenic properties and the most common are types 16 and 18 (Schiffman *et al*., 2007). From the epidemiological findings, more than 90% of cervical cancers are attributed to a distinct HPV type. Apparently, a small percentage of cervical cancers is HPV-negative after laboratory testing, although this can be a false negative caused by failure in detection techniques to capture new HPV types (Eltoum *et a*l., 2007). Other causes of false negatives include failure to detect very low

concentration of known HPV genomes and sampling errors (Guyot *et al*., 2004; Jastania *et al*., 2006).

Transmission of HPV occurs through skin or mucosa contact during sex. If there are tears in the mucosa, the HPV infecting viral particles penetrate the epithelium, reach the germinal cells located the basal cell layer, and integrate into the host genome (Castellsagué, 2008). Integration induces an over-expression of viral E6 and E7 oncoproteins. HPV DNA breakage occurs in the E2 gene region that leads to up regulation of E6 and E7 (Ibeanu, 2011). Oncoprotein E6 interferes with host p53 gene function; E7 interferes with host tumor suppressor genes. Interference with p53 gene means there is no inhibition of apoptosis. Moreover, E7 causes cell cycle inhibition. Without cell cycle regulation, tumor cells keep replicating and proliferating (Longworth & Laimins, 2004).

The most important steps for cervical carcinogenesis include infection with HPV, persistence of HPV infection for a specific time, advancement to precancerous, and ultimately invasion. Additionally, there can be reversion that leads to HPV clearance in addition to regression of precancerous lesions, as shown in Figure 2.1 below (Schiffman & Kjaer, 2003).

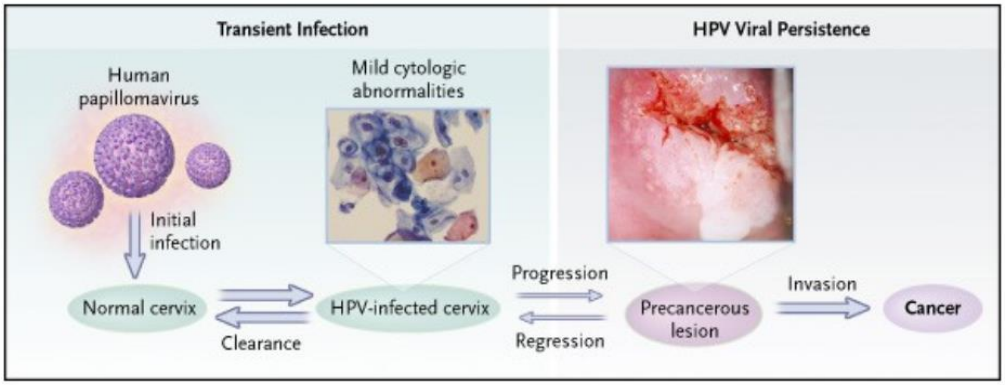

Figure 2.1: Stages of cancer progression referenced from *Wright & Schifmann New England Journal of Medicine 2003*

2.5 Diagnosis, Detection, and Screening of Cervical Cancer

2.5.1 Pap Smear (Cervical Cytology)

The Papanicolaou smear, abbreviated as Pap smear/cervical smear, is a cervical screening technique applied in detecting possibly cancerous and pre-cancerous cells in the cervix. Most healthcare practitioners considered it to be the most affordable cancer detection technique ever developed (Cibas & Ducatman, 2008).

Cervical cytology involves examination of cervical cell smears under the microscope. Cervical specimens are smeared on a glass slide using a cytobrush and then stained by special stains to detect lesions (Khan *et al*., 2005). The application of cervical cytology has contributed to early identification of cancerous cervical cells, reducing the advanced cervical cancer incidences that are linked to high mortality rates (Mukakalisa *et al*., 2014). Cervical cytology can be performed using the CPS cytology or Liquid-based cervical cytology.

2.5.1.1 Conventional Pap Smear Cytology

In the CPS technique, a smear is collected from the cervix and the endocervical canal using an Ayres spatula and cytobrush. Samples taken are then applied as a smear directly on a slide, which is followed by fixation using a cytology fixative. After staining, microscopic examination for cytological abnormalities is performed (Bibbo & Wilbur, 2014).

The use of CPS has resulted to reduction of cervical cancer incidences globally but mostly in the developed countries where screening services are well-established (WHO, 2020). However, in the middle and low-income nations, there has not been a widespread use of Pap smear due to lack of resources, and thus cervical cancer incidences are still high in these countries (Arbyn *et al*., 2020; Catarino *et al*., 2015).

Although the CPS has been widely used in diagnosing cervical cancer, newer techniques have been developed because the method has been linked to shortcomings such as inflammation and obscuring blood, which reduces its sensitivity (Kavatkar et al., 2008). Studies have shown that its sensitivity is less than 50% due to these inherent limitations (Sherwani et al., 2007; Kavatkar et al., 2008).

2.5.1.2 Liquid-Based Cytology

The technique of LBC was introduced to overcome limitations associated with the CPS method. Samples for LBC are collected similarly as those for the CPS, however, a cell smear is not made on a slide, but the collection brush is rinsed in a vial containing a liquid fixative, and then taken to the laboratory for further processing (Singh *et al.,* 2015).

The use of liquid-based methods, which has a preservative solution rather than the use of slide preserves the cellular structure of the specimen because the cells are immediately fixed. This process helps in overcoming challenges such as drying artefacts as well as removing yeast, red blood cells, bacteria, and most contaminating mucus (Cibas & Ducatman, 2008). This technique enables preparation of a monolayer because the cells are suspended in a liquid medium, therefore, enhancing effective morphological assessment. Due to cost and availability, developing countries are yet to fully implement the automated techniques compared to the developed countries where the technique is highly utilized (Pankaj *et al*., 2018).

In contrast, MLBC technique is considered less-costly and increases chances of precursor lesions' detection as well as improving specimen adequacy (Kavatkar *et al*., 2008; Sherwani *et al*., 2007). Compared with CPS, the diagnostic accuracy of cervical cytology has been shown to improve with LBC. A study by Singh *et al*.

(2015) found enhanced cyto-histological correlation rate and positive predicative value in liquid-based technique compared with CPS. There are three techniques used in LBC and they include MonoPrep Pap test, SurePath Pap test, and Thin Prep Pap test.

2.5.1.2.1 ThinPrep Pap Test

Using the ThinPrep Pap test, the sample can either be obtained with endocervical brush and plastic spatula combination or by use of a broom-type device. A methanol-based preservative is used to rinse or swirl the sampling device that is afterward transported and discarded in cytology laboratory. The transport medium aids in lysing red blood cells while the automated ThinPrep instrument holds each vial one at a time (Rosa *et al*., 2013).

Usually, the whole procedure takes approximately 70 seconds per slide, resulting in circular cells deposit with a diameter of 20mm. The slides used for diagnosis are mostly prepared using a fraction of a sample. The remaining sample may also be useful for further diagnostic testing, cell block preparation, or Thin Prep slide preparation (Cibas & Ducatman, 2008). In a study by Tao *et al.* (2019) involving a follow-up of 15,545 pap tests with histopathological analysis, as well as HPV DNA testing, of LSIL cytology between 2011 and 2016, ThinPrep detected 11.4% and 60.5% of CIN II and CIN I cases, respectively.

2.5.1.2.2 SurePath Pap Test

Contrary to the MonoPrep and ThinPrep techniques, the sure path Pap test involves cutting the tip of the sample collection device that is incorporated in the sample vial. In this method, slides are prepared using a Prep Stain sample processer and centrifuge. The sample mixing and dispensing onto the density reagent is mostly automated. The density centrifugation aids in eliminating some leukocytes and the red blood cells. A circular monolayer of cell deposits is produced, followed by staining of the individual slides (Cibas & Ducatman, 2008).

Many developed countries have embraced Sure Path LBC because it leads to increased CIN II detection rates. In a study by Rozemeijer *et al*. (2016), Sure Path was able to detect 12% of CIN 2 lesions in primary smears reported as borderline dyskaryotic diagnoses. When combined with CPS, the detection of CIN lesions increased to 20%. Tao *et al.* (2019) noted that SurePath could detect 12.4% and 56.7% of CIN II and CIN I lesions respectively in cytological samples diagnosed with LSIL using histopathology and HPV DNA.

2.5.1.2.3 MonoPrep Pap Test

In the MonoPrep Pap test, standard collection devices rinsed or swirled in a preservative, are used to obtain the MonoPrep sample, and the sampling device is later discarded. The transport media aid in lysing red blood cells. In the cytology

laboratory where the vials are delivered, a MonoPrep Processor, a completely automated instrument, is used in preparing the slides (Cibas & Ducatman, 2008).

In a split-sample clinical trial, MonoPrep prepared slides showed increased LSIL and more serious lesions detection than the conventional smear. Mono Prep also minimized the number of slides considered unsatisfactory with no difference in detecting benign conditions, transformation zone component, and/or the endocervical presentation of the cervix (Cibas et al., 2008).

2.5.2 HPV-DNA Testing

HPV is the primary causative agent of cervical cancer. HPV-DNA testing is a molecular-based test for screening cervical cancer which comprises screening for high-risk HPV strains. According to Louie et al. (2009), HPV testing has the ability to reduce mortality related to the advanced invasive cervical cancer as well as in HIV positive women.

Testing for HPV DNA is an effective method of triaging women with LSIL and can be used alone as the primary test or in combination with cytology for screening. In addition, it is applied in predicting treatment outcomes and in follow-ups (Cuzick et al., 2008). Specimens used in HPV-DNA testing can be obtained in two ways: the use of endocervical cytobrush and the liquid-based cytological cell suspension (Duraisamy et al., 2011).

It is not possible to propagate HPV in tissue culture, and thus detection is only by use of molecular techniques (Villa & Denny, 2006). HPV testing is more sensitive in identification of HSILs compared to cytology although the specificity is lower (Agorastos *et al*., 2015). However, this approach remains expensive since reading related samples requires sophisticated laboratory, and thus a challenge in many developing countries with unreliable laboratory equipment (Maine *et al.,* 2011).

2.5.3 Visual Inspection with Acetic Acid

Visual inspection of the cervix using acetic acid remains one of the simplest and cheapest cervical cancer screening methods as no sophisticated technology is required. This approach has helped reduce mortalities associated with cervical cancer, especially in low-income nations (Wright & Kuhn, 2012). In this method, a speculum is used to expose the cervix after a general examination and acquiring clinical history. The cervical mucosa is smeared with 5 percent vinegar or acetic acid, where the abnormal cells such as cancerous and dysplastic cells become acetowhite, while the normal cells remain unaffected (Duraisamy *et a*l., 2011).

The cells with sharp borders are regarded as the high grade while the ones with faint borders are regarded as low-grade (Catarino *et al*., 2018). This screening method allows immediate diagnosis and treatment of abnormal cells through the use of cryotherapy (Toliman *et al*., 2018). Research has affirmed the reliability,

cost-effectiveness, and sensitivity of the VIA, which is used in developing nations as an alternative to CPS (Duraisamy *et al*., 2011).

2.5.4 Visual Inspection with Lugol's Iodine

Visual Inspection of the cervix using Lugol's Iodine is another propitious technique for cervical cancer screening. At first, visual inspection with acetic acid is applied followed by Lugol's iodine solution which gives room for a second examination. The larger low-grade area makes it easier for the examiner to effectively observe the small high-grade lesion (Zhang *et al*., 2010; Garg, 2011).

There is a lot of glycogen stored in the normal squamous epithelial cells. In the presence of iodine solution, glycogen produces a mahogany-brown stain. The squamous epithelium's abnormal areas does not stain brown because it lacks glycogen (Sarian *et al.,* 2005). This method is considered more effective that Pap smear in detecting CIN yet simple and inexpensive as there is no need for expensive equipment (Duraisamy *et al*., 2011).

2.5.5 Colposcopy and Biopsy

Colposcopy is an approach used to examine both the vagina and cervix by a magnified and illuminated stereoscopic viewing (Camilleri & Blundell, 2009). By performing this procedure, it is possible to locate and examine the transformation zone, an area where the new squamo-columnar junction defines the internal border

or medial and metaplastic squamous epithelium develops (Waxman *et al*., 2017). The squamous metaplastic process may become abnormal, and when such abnormalities happen, they are usually graded based on iodine uptake, blood vessels, margins, and acetowhiteness (Urasa & Darj, 2011). Using the modern colposcope, the cervix's stereoscopic view can be done by varying the intensity of the light source. The selected magnification varies inversely with the depth of the focus and the field of view (Camilleri & Blundell, 2009). The lower genital track's reminder (perianal skin, vulva, and vagina) can also be assessed through colposcopy, especially among women without lesions but having abnormal cytological results. The HIV positive women are most affected by multifocal disease which involves perianal areas, vulva, and the vagina (Mustafa *et al*., 2016).

Colposcopy is considered the main stay of evaluating a normal smear in addition to positive results for high-risk HPV. If screening tests reveal abnormal cervix, the patient is referred for colposcopy to facilitate diagnosis and treatment. Colposcopy makes it possible to select the cervical area with severe modification for biopsy (Saslow *et al*., 2012), which, in turn, informs the need to remove the transformation zone. Additionally, colposcopy remains an essential tool for women who experience persistent abnormalities as well as those under follow-up following treatment (Jin *et al*., 2010). The indications for colposcopy include mainly abnormal cervical cytology, in addition to clinically-suspicious cervix. Besides,

colposcopy can be helpful in treatment evaluation for cervical dysplasia, as well as external genital warts and postcoital bleeding (Aydogmus *et al*., 2019).

On the basis of histopathological findings, pre-cancerous cervical changes can be categorized according to cervical intraepithelial neoplasia (CIN) terminology that grades histopathological changes as CIN1, CIN2, or CIN3 and/or cervical dysplasia (mild, moderate, severe, or carcinoma in situ) (Waxman *et al*., 2012). Preliminary CIN grading is possible through cytology or colposcopy, but a definitive grade requires histopathological examination (biopsy) (Akhter, Bari & Hayat, 2015).

Histopathological changes can also be described based on squamous intraepithelial lesion (SIL) terminology, in which specimens positive for SILs are categorized as LSIL or HISL (Waxman *et al*., 2012). The Squamous intraepithelial lesion terminology is subject to CIN subcategorization, for example, HSIL (CIN 2), which plays a critical role in clinical decision making and management. HSIL, which is included in The Bethesda System that is used to report cervical cytological results, comprises moderate dysplasia, severe dysplasia, and carcinoma *in situ* (Mukhopadhyay *et al*., 2013).

2.5.6 Onco*E6*™ Cervical Test

With advancement in technology, new rapid screening tests for cervical cancer have been invented, especially in the developed countries. The Onco***E6***™ cervical test

is a molecular based rapid screening test that detects the presence of HPV 16/18 E6 Oncoprotein. Previous research indicated that cervical cancer is likely to develop when HPV oncoproteins E6 and E7 are expressed for a prolonged duration (Jiang & Xue, 2015).

The levels of these oncoproteins are normally elevated in those HPV infections that culminate in invasive cervical cancer, but are in very low levels in HPV infections with no cellular transforming effect, as well as low grade dysplasia (Schmitt *et al*., 2011). Motivated by these findings, the Onco*E6*™ test was developed. It comprises a technology that aims to directly detect high levels of the E6 oncoproteins of HPV types 16 and 18 (Schweizer *et al*., 2010). The advantage of this test is its potential for application as a point of care test as it gives rapid results (Ndizeye *et al*., 2019).

2.6 Atypical Squamous Cells

Atypical squamous cells (ASC) refer to a category of cervical cytological changes suggestive of SIL though insufficient, both quantitatively and qualitatively, for such definitive interpretation. For effective ASC interpretation, the cells in question should possess three important features which include minimum nuclear changes (multinucleation, smudging, irregularity, chromatic clumping, and hyperchromasia), an escalated nuclear to cytoplasmic ratio, and the squamous differentiation (Solomon et al., 2002).

The 2014 Bethesda System divides the ASCs into two groups: atypical squamous cell cannot exclude a high-grade squamous intraepithelial lesion (ASC-H) and atypical squamous cells of undetermined significance (ASC-US). The cytologic criteria for identification of ASC-H and ASC-US cells are subjective, thus, the diagnoses incurs high intra-observer and inter-observer variation rates. The ASC-US category comprises about 90% of all ASC cases (Nayar & Wilbur, 2015).

2.6.1 Atypical Squamous Cells of Undetermined Significance

Atypical squamous cells of undetermined significance refers to cytological changes that suggest LSIL where the cellular features severity is incomparable to that of reactive squamous cells but lower than that of the SIL. Cells with known features are excluded during the ASCUS diagnosis. In addition to having nuclear changes that are non-diagnostic of SIL or LSIL, the size of the ASCUS cells are similar to that of superficial or intermediate squamous cells (Solomon et al., 2002).

The size of the nuclei is double the area of the squamous metaplastic cell nucleus, and close to thrice that of the normal intermediate squamous cell’s nucleus. There is escalated nuclear to cytoplasm ratio accompanied by irregular distribution of chromatin and partial nuclear hyperchromasia (Nayar & Wilbur, 2015).

2.6.1.1 Management of ASCUS

Patients with ASCUS are effectively managed by performing the oncogenic high-risk HPV DNA testing concurrently. According to Massad *et al*. (2013), colposcopy should be performed in HPV-positive, ASCUS women regardless of whether contesting or reflex HPV testing was done, while the HPV-negative, ASC-US women should embrace the 2012 ASCCP guidelines that encourage co-testing at three years interval.

In younger women, HPV infection is mostly triggered by a mixture of high-risk and low-risk viruses, therefore, to mitigate challenges related to interpretation of test results, women below 30 years should be exempted from the test. It is also acceptable to undergo an immediate colposcopy or Pap tests follow-up at 6-month intervals (Schiffman *et al*., 2015).

2.6.2 Atypical Squamous Cells, cannot Exclude HSIL

In all ASC cases, the atypical squamous cells, cannot exclude HSIL comprise 5–10%. The size of cells in ASC-H is comparable to that of metaplastic cells and may appear in clusters or singly showing features of HSIL. The cells are mostly sparse with several patterns such as post radiation changes, severe atrophy, markedly atypical repair, and atypical immature metaplastic cells (Nayar & Wilbur, 2015).

2.6.2.1 Management of ASCH

In comparison with ASC-US, the ASC-H positive results have a higher predictive value for CIN 2 or CIN3 (50% and 17%, respectively), and thus colposcopy should be recommended for patients with ASC-H cytological screening results. In absence of CIN2 or CIN3, either HPV DNA test or a 6-month interval Pap test should be done. A second colposcopy should be recommended in cases where the HPV DNA test gives positive results for high-risk virus or the repeated Pap test indicates a worsening ASC-US situation (Massad *et al*., 2013).

2.7 Squamous Intraepithelial Lesions

Squamous intraepithelial lesions include HPV-related noninvasive cervical epithelial abnormalities, ranging from transient HPV infectious to high-grade precursors and invasive cervical cancer (Kurman *et al*., 2014). HPV remains the principal causative agent for virtually all precursors and invasive cervical cancer with HPV 16 as the most prevalent (Solomon et al., 2002).

2.7.1 Low Grade Squamous Intraepithelial Lesion

The intraepithelial lesions encompassed in LSIL have features of preserved differentiation, organization, and maturation of squamous epithelium impound to parabasal and basal epithelial layers (Kurman *et al*., 2014). CNI and ‘mild dysplasia encompasses the squamous cell changes associated with HPV infections. Studies show that investigators have varying morphological techniques of differentiating

CN1 or mild dysplasia from koilocytosis with little clinical importance. The two lesions support a typical LSIL designation because they have similar clinical management, and biological behavior as well as sharing common HPV type (Castle *et al*., 2011).

Cytological abnormalities include superficial/ intermediate metaplastic cells with well preserved and well differentiated cytoplasm, slightly enlarged nuclei, finely granular, smooth nuclear membrane, mild hyperchromasia, slightly altered N:C ratio, and evenly distributed chromatin with presence of koilocytosis and prominent nucleoli (Solomon et al., 2002).

2.7.1.1 Management of LSIL

Under the 2012 ASCCP guidelines, HPV negative women aged 25 years and above should be cotested in three years interval; however, in presence of HPV positive cases colposcopic examination is highly indicated. Those young women (below 25years) with LSIL's cytologic interpretation should undergo cytology screening after one year. Similarly, those women with unknown status for HPV should also repeat cytology after one year (Massad *et al*., 2013).

2.7.2 High Grade Squamous Intraepithelial Lesion

This category is characterized by severe cellular dysplasia with mitoses, disorganized epithelial and absence of squamous differentiation (Solomon et al.,

2002). The cells can appear in syncytial-like aggregates, in sheets or singly. It is important to ensure those hyperchromatic crowded groups resulting from dysplastic cell changes are thoroughly screened when examining them for nuclear abnormalities. Compared to LSIL, the size of cells in HSIL is smaller. However, generally the overall size varies (Kurman, 2012).

The nuclear enlargement can be higher than that of LSIL cases. However, many HSIL have similar nuclear enlargement degree as the LSIL with decreased cytoplasmic area resulting to an increment of the nuclear cytoplasmic ratio (N: C). Despite of other cells having high N: C rations, LSIL's nuclei size is much higher and when compared to LSIL's N: C ratio, the HSIL's N: C ratio is comparatively higher (Bibbo & Wilbur, 2014).

2.7.2.1 Management of HSIL

Based on the 2012 ASCCP guidelines, cytological HSIL women aged 25 years and above should undergo colposcopy procedures immediately after a lesion is discovered and excision done concurrently. In cases where HSIL is not evident with colposcopy for women with HSIL cytologic interpretation, lesion may be revealed through immunohistochemistry, and by reviewing the histological and cytological materials (Massad *et al*., 2013).

2.7.3 Squamous Cell Carcinoma

Squamous cell carcinoma refers to the invasive epithelial tumor made of squamous cells with distinct differentiation degree (Kurman *et al*., 2014). Among all the cervical malignant tumors, invasive SCCs, which accounts for more than 60 percent remain the most frequent type. HSIL is an immediate precursor of cervical squamous cell carcinoma occurring mostly in women aged 50 years and above (Nayar & Wilbur, 2015).

Cellular cytological features include cells with bizarre configurations (spindle snakes and caudate tadpoles). Marked pleomorphism, orangeophilia associated with dense keratinization, eosinophilia may be more prominent. Occasional syncytial-like aggregates can be seen although cells occur singly. Nuclei are 1.5-3 times the intermediate cell nucleus's size. The chromatin is typically coarse, with pyknotic nuclei (Verma *et al*., 2014). Macronucleoli may be present and seen mostly in non-keratinizing squamous cell carcinoma. In contrast, the keratinizing type is mostly accompanied by evidence of hyperkeratosis, parakeratosis, atypical parakeratosis, and keratinizing dysplasia. A tumor diathesis consisting of old blood and necrotic debri is often observed (Solomon et al., 2002, Nayar & Wilbur, 2015).

2.7.3.1 Management of Squamous Cell Carcinoma

Colposcopy and biopsy for histopathological evaluation is recommended. Treatment options vary according to the disease stage as well as the patients' age

and clinical status. In addition, characteristics of the tumor, preferences of the patients, and availability of resources can influence the treatment strategy (Urasa & Darj 2011).

2.8 Glandular Abnormalities

2.8.1 Atypical Glandular Cells

Atypical Glandular Cells (AGC) category comprises cells of endocervical type with features of nuclear atypia exceeding obvious reparative or reactive changes without clear features of invasive adenocarcinoma or adenocarcinoma in situ. Based on the 2015 Bethesda classification, AGC is used to categorize glandular cells with cytological changes that are too significant to be called inflammatory or reactive but cannot be classified as malignant (Nayar & Wilbur, 2015).

Cells appear in strips and sheets with some crowding cells, pseudostratification and nuclear overlapping. A nuclear enlargement close to five times that of a normal endocervical cell is evident. Importantly, cells should be classified as of endometrial or endocervical origin when screening for AGC. The term “glandular” is used when cells’ origins are undetermined. Specific entities should be included if the features portrayed in atypical endocervical cells favour a particular identity such as neoplasia (Solomon et al., 2002).

2.8.2 Endocervical Adenocarcinoma of the Uterine Cervix

It comprises a third of all carcinomas of the cervix and occurs more regularly in the fifth decade. Most in situ and invasive endocervical adenocarcinomas occur among women over thirty years, a slightly older age group than patients with comparable squamous lesions. Adenocarcinoma in situ (AIS) is found in about 5% of cervical HSILs. Endocervical adenocarcinoma in situ is regarded as the glandular counterpart of cervical intraepithelial neoplasia (CIN3) and the precursor to invasive endocervical adenocarcinomas and adenocarcinoma in situ (Nayar & Wilbur, 2015). HPV 18 is predominantly associated with cervical adenocarcinoma. Endocervical mucinous carcinomas comprise the majority accounting for 70% to 90% of all cases, with endometrioid carcinoma being the second (Fujiwara & Devouassouxs, 2014).

The criteria for Endocervical AIS include abnormal architecture: pseudostratified strips, rosettes, feathering, increased nuclear-cytoplasmic ratio, nuclear enlargement with pleomorphism of size and shape, coarsely granular evenly distributed chromatin, and nucleoli typically not prominent. Mitoses and apoptotic bodies may be present. Tumour diathesis typically is not present, but inflammatory background may be present (Solomon *et al*., 2002; Nayar & Wilbur, 2015).

2.9 Bacterial Vaginosis

Bacterial vaginosis (BV) is a common vaginal infection, particularly among women of childbearing age, and is linked to a myriad of health problems. The prevalence of BV varies across countries and regions. A recent systematic review reported a global burden of 23%-29% among the general population, with Europe and Central Asia and South Asia reporting the highest and lowest prevalence, respectively (Peebles *et al*., 2019). However, the burden of BV may also differ according to the population's characteristics such as race, hospitalization, number of sexual partners, pregnancy, and marital status (Kenyon *et al*., 2013 and Peebles *et al*., 2019). It arises when there is a decline in the count of helpful bacteria such as lactobacilli, as well as an overgrowth of harmful bacteria, including Enterobacteriaceae, *Streptococcus spp*, *Veillonella spp*, *Bacteroides fragilis*, *Ureaplasma urealyticum*, and *Gardnerella vaginalis* (Kamga et al., 2019). The harmful bacteria are opportunistic microbes that grow singly or in combination with other organisms.

Bacterial vaginosis is a non-inflammatory condition which in most cases lacks the typical symptoms such as vaginal discharge, odor, irritation, itching, erythema, and dysuria (Donders, 2010). Despite the absence of symptoms in most patients with BV, pregnant women are exposed to a high risk of endometritis and low birth weight (Bitew *et al*., 2017). These complications adversely affect the wellbeing and safety of both the mother and the neonate. Besides, BV has been linked with a high risk of pelvic inflammatory disease that is associated with chronic pelvic pain and

infertility (Bitew *et al*., 2017; Taylor *et al*., 2013). Patients with BV are also vulnerable to sexually transmitted diseases, and HIV (Bitew *et al*., 2017). Due to the absence and non-specificity of symptoms, many BV cases are undiagnosed, hindering the initiation of timely treatment and raising the risk of adverse health events.

Women who visit gynecologic clinics are likely to be tested for BV, especially when undergoing other examinations, such as Pap smear, to diagnose cervical cancer (Gillet *et al*., 2012). These patients may be undergoing routine screening or may have genital complaints. Notably, a recent systematic review and meta-analysis reported that BV and cervical cancer have a significant positive association (Gillet *et al*., 2012). A disturbance in the vaginal flora has been linked to cervical cytological abnormalities (Champer *et al*., 2018). Therefore, during cervical cancer screening, concurrent testing for BV is performed to inform the clinical decision-making of the healthcare provider. In Kenya, research on the diagnosis of BV during cervical cytological examination is limited. In this study, we sought to investigate the burden of BV among women participating in the screening for cervical cancer using a liquid-based cytological technique.

2.10 Treatment of Invasive Cervical Cancer

With suitable therapy, 80% of the cervical cancer cases detected at the earlier stages are curable. Inadequate medical infrastructures and resources have negatively

impacted early screening for cervical cancer and treatment in third world countries which is contrary to what is evident in developed countries (ACCP, 2004).

However, invasive cervical cancer treatment options are influenced by the disease stage at diagnosis. Additional factors like decision to have children, age, physical condition, the type of cancer, and the cervical region affect by cancer also affect the treatment decision. Radical hysterectomy or radiotherapy was previously used in treatment of early cervical cancer, and it resulted in 80–90% survival rates in every five years. For patients battling the advanced cervical cancer stages, both radiotherapy and chemoradiation are recommended. Results from a five randomized trials showed better survival with the combined therapy options (Tierney, 2009).

In absence of lymph node metastasis, small cervical tumors can be removed through surgical procedures such as hysterectomy which involves complete removal of the uterus. Hysterectomy procedures are categorized into two: simple and radical hysterectomy. With simple hysterectomy, both cervix and uterus are removed, and the recovery time is within few days of hospitalization because the surgery is performed laparoscopically (Skorupska *et al*., 2016).

In contrast, radical hysterectomy entails complete excision of the uterus, including cervix's soft tissues and two centimeters of upper vagina. Radical hysterectomy

may be performed laparoscopically, but the procedure can negatively impact functionality of the bladder and the bowel (Wright *et al*., 2012). At least six weeks of hospitalization with abdominal drainage and urine catheter removed within five days is recommended after a radical hysterectomy is done. Pain killers are also used for pain management (Long *et al*., 2007).

Distinct treatment modalities for different cervical cancer stages have been recommended by the National Cervical Cancer Prevention Plan (NCCPP). For instance, extended abdominal hysterectomy is recommended for micro invasive carcinoma to stage 1a, while adjuvant chemotherapy and radiotherapy or Wertheim's hysterectomy should be used for stage 1 to 2a. Lastly, adjuvant chemotherapy, palliative therapy, and radiotherapy are recommended for treatment of stage 2b to 4 (NCCPP, 2012)

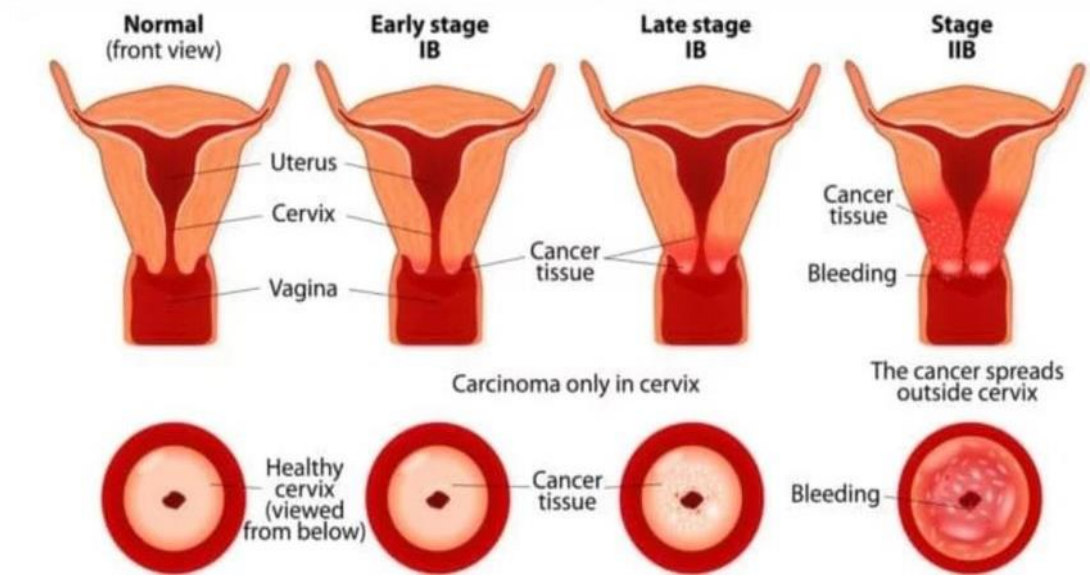

Figure 2.2: Stages of Cervical Cancer

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

This research was done at Machakos county hospital located at Machakos town in Machakos county; southern eastern part of Kenya. The study site was selected considering the availability of cervical cancer screening services at the Machakos cancer care and research centre. The study was done specifically at the Comprehensive Care Center (CCC) which serves patients infected with HIV AIDS, and at the cancer care and research center which is involved in diagnosis and treatment of cancer. This is the biggest referral hospital in the county serving patients from across the county and the neighboring counties. Comprehensive care centre is a care clinic for HIV/AIDS patients normally operated as an outpatient clinic serving over 4000 patients of which about 50% are women. On the other hand, the cancer care and research center offers cancer screening, diagnosis, counseling, chemotherapy treatment and management services free of charge to residents of Machakos County registered under the Universal Health Coverage (UHC) program. The Centre is also open to residents of other counties, with those from Makueni and Kitui, which are under the South Eastern Kenya Economic Block (SEKEB), being offered services at subsidized rates.

3.2 Study Design

This research utilized a comparative cross-sectional study design and was conducted between July 2020 and December 2020.

3.3 Target Population

The population of the current study was composed of women of 18 years and above, sexually active and attending comprehensive care centre clinic at the selected study site. The target population was chosen based on the fact that cervical squamous intraepithelial lesions are higher in women infected by HIV than the HIV–negative population. According to a research done by McKenzie *et al* (2011), among the HIV-positive Kenyan women, the cervical squamous intraepithelial lesions' prevalence rate is 46%.

3.3.1 Study Population

In this study, women attending comprehensive care centre at Machakos county hospital were the targeted study population.

3.3.2 Inclusion Criteria

Women aged eighteen years or above who were sexually active, attending comprehensive care center at the selected facility and consented to participate were included in this study.

3.3.3 Exclusion Criteria

Women who were pregnant or declined to give consent were excluded from the study. Women on treatment for precancerous lesion or cervical cancer and a previous hysterectomy were also excluded from this study.

3.3.4 Sample Size Determination

According to Mutuku *et al*. (2017), the abnormal cervical cytology's prevalence among women infected by HIV was 5.2%. The Fisher's *et al*, (1998) formula, was applied as follows to determine sample size;

$$n = \frac{Z^2PQ}{E^2}$$

Where: n = required sample size

Z=confidence interval at 95% (standard value of 1.96)

P=estimated prevalence of precancerous lesion (5.2%)

E= range of possible random error (5%)

Q= 1 – P or estimated proportion of failure

$$n = \frac{1.96^2 \times 0.052(1-0.052)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.052 \times 0.948}{0.05 \times 0.05}$$

= 75 participants

However in this study, 400 participants were included for a higher probability of detecting more cervical lesions.

3.4 Sampling Method

Purposive sampling method was applied in selecting the study participants. With this approach, patients available during the study period who met all the desired inclusion criteria and willing to take part were considered until the sample size was reached.

3.5 Recruitment of Research Participants and Data Collection

Recruitment of the research participants was carried out at the comprehensive care clinic. The Principal Investigator (PI) contacted the nursing officer in-charge of the clinic after seeking approval of the study by ethics review committee. The CCC in-charge then introduced the PI to a team of three clinicians comprising of one clinical officer and two nurses working at the clinic who assisted in collection of Pap smear samples from the research participants. Refresher training on Pap smear collection procedure was done for the team by the PI and an oncology nurse. The PI recruited four hundred (400) study participants who met all the desired inclusion criteria and signed written participation consent. Every day, recruited study participants were grouped and a short education session was given by the PI on the risk of cervical cancer, benefit of early detection, research study aims and procedure of the Pap smear in both English and Kiswahili. After the participants had given their written

consent (Appendix IA), the PI used a structured questionnaire (Appendix II) in the process of collecting demographic data after which samples were collected and send to the laboratory for processing.

3.6 Laboratory Methods

Cytological material was collected as follows:

Phase 1: Collection of Samples (n=400):

After obtaining a signed consent and collecting demographic data from the patient, a trained clinician then explained the procedure of Pap smear collection, assured and placed the patient in a comfortable and convenient position for sample collection.

A cervical cytology sample was collected using a cytobrush. The cytological material was then transferred with brushes into a formulated liquid fixative constituted from a mixture of isopropyl alcohol, 10% formalin solution, sodium citrate, and sodium chloride.

Brushes were broken off into the container of collection fluid. The collected samples were then transported to the Machakos cancer care and research center cytology laboratory for processing. The samples were vortex-mixed and then about 10ml transferred into a formulated alcoholic-agar (polymer solution made of alcohol, polyethylene glycol and agarose) in nipple-bottom test tubes. The

remaining samples were then kept at -80°C for detecting high risk HPV subtype 16 and 18 were appropriate. Test tubes were then centrifuged for 10 minutes at 2000rpm. A Pasteur pipette was used to make a smear on a glass slide after discarding the supernatant. For effective fixation, the prepared slides were placed in 50˚C hot air oven and left to dry for 15 minutes. Additional fixation of the prepared slides was done by dipping the slides in 95 percent alcohol for about 15 minutes (Mutuku *et al*., 2018).

Papanicolaou staining technique (Appendix III) was applied to stain all the smears. Screening of the Pap smears was done by the principal investigator. All abnormal smears were reviewed by a qualified pathologist. All the cytological abnormalities observed during examination were then reported using the 2014 Bethesda system (Appendix IV). Participants with HSIL and above from the cytological results were referred for histopathology.

Phase 2: Processing of Samples for High Risk HPV (subtype 16 and 18):

Processing of the HPV/DNA samples was done at CA Medlynks Kenya limited molecular laboratory by principal investigator. Quantitative detection of HPV 16 and 18, as well as genotyping were performed using the HPV 16/18 Real-TM Quant kit. On the assay day all the reagents were prepared using instructions on the user manual by the principal investigator. The equipment were set in preparation for the runs. Test samples were thawed and all working reagents brought to ideal working

temperatures. Isolation of DNA material followed by real time amplification was carried out as per the steps in HPV testing Standard operating procedure (Appendix V). The results were interpreted by the principal investigator with the supervising medical laboratory officer in-charge of the molecular section.

3.7 Quality Assurance

Experienced health professionals, who regularly perform Pap smears, carried out collection of samples. Refresher training on Pap smear collection was done to the clinicians prior to the study. Pap smears were stained in line with the recommended Standard Operating Procedures (SOPs) used for staining gynecological specimens at the Machakos cancer care and research center cytology laboratory. Appropriate SOPs were used to prepare the stains and reagents which were then filtered before use ensuring a high quality staining. Additionally, the faded overused stains were discarded and new stains prepared. Smears were examined by the researcher, and all abnormal smears were reviewed by a qualified pathologist; certified by the Kenya Medical Practitioners and Dentists Council (KMPDC).

After processing of the cytological samples, the remnant samples were preserved at the appropriate temperature (-80°C) for HPV DNA testing. Internal Quality Control samples were incorporated in the test kit and all the steps outlined in the HPV testing SOP (Appendix VI) critically followed up to the end of the procedure.

All the reagents were prepared using manuals as per the manufacturer's instructions and HPV testing done by the principal investigator.

Cervical biopsy samples for histopathology were collected by a qualified experienced gynecologist at the Machakos cancer care and research center. All the samples were processed and stained following the recommended standard operating procedures (SOPs) used for staining gynecological histopathology specimens at the Machakos cancer care and research center histopathology laboratory. The PI screened all the histopathology smears which were then reviewed and signed out by a pathologist.

3.8 Data Presentation and Analysis

Data analyses were conducted using the Statistical Package for the Social Science System (SPSS Version 18). In this case, variables are presented as percentages and absolute numbers. Comparison of nominal categorical data between the groups was done using the Chi-square test. Additionally, the results are presented as a percentage or mean±standard deviation. A P-value <0.05 was interpreted as statistically significant at confidence interval of 95%. Cohen Kappa test was used to determine the level of agreement among MLBC, histopathology, and HPV DNA testing.

3.9 Ethical Considerations

The research was approved by the Kenyatta University Ethical Review Committee (KU-ERC) and permit obtained from the National Commission for Science, Technology and Innovation (NACOSTI). Before collection of samples for screening was done, a voluntary and written consent was sought from the patients. All procedures were explained to the patients and clarifications made in a language they could understand. No participant was coerced into taking the cervical smear test without their permission. To minimize the anxiety or trauma that one may undergo during Pap smear collection procedure, assurance was offered to the participants to calm them. A qualified and experienced clinician collected Pap smears from the patients and all procedures done in a standard manner to avoid risks and maximize benefit to the patients. Patient privacy and confidentiality was strictly observed by use of numbers instead of names. All Pap smear results were communicated to the attending physician in a timely manner for further management. All information was kept confidential and the results send to the patients file records and effectively communicated by the nurse or doctor handling the patients in their following visit.

CHAPTER FOUR

RESULTS

4.1 Social-Demographic Features of the Study Participants

4.1.1 Age Distribution

Out of the 400 participants enrolled in this study, 32 (8.0%) were between 20 – 30 years, 111 (27.8%) between 30 – 39 years, 148 (37.0%) between 40– 49, 98 (24.5%) between 50 – 59 and 11 (2.8%) were aged 60 and above. The age range was 21 – 74 years. (Table 4.1).

Table 4.1: Distribution of Age

Age in years	Frequency	Percent
20 – 29	32	8.0
30 – 39	111	27.8
40 – 49	148	37.0
50 – 59	98	24.5
60 and above	11	2.8
Total	**400**	**100.0**

4.1.2 Marital Status

Out of the 400 women who participated in this study, 197 (49.3%) were married, 100 (25.0%) single, 64 (16.0%) widowed while 39 (9.8%) were divorced. (Table 4.2).

Table 4.2: Marital Status

Marital Status	Frequency	Percent
Married	197	49.3
Single	100	25.0
Widowed	64	16.0
Divorced	39	9.8
Total	400	100.0

4.1.3 History of Tobacco Smoking

In this study, only 7 (1.8%) women reported to have history of tobacco smoking (Table 4.3).

Table 4.3: History of Tobacco Smoking

Tobacco Smoking	Frequency	Percent
Yes	7	1.8
No	393	98.2
Total	400	100.0

4.1.4 Education Level

Majority of participants in this study had gone up to secondary level of education, 188 (47.0%) followed by primary education 158 (39.5%). Only 47 (11.8%) women had college/university education while 7 (1.8%) reported not to have gone to school. (Table 4.4).

Table 4.4: Education Level

Education Level	Frequency	Percent
Primary	158	39.5
Secondary	188	47.0
College/University	47	11.8
Not gone to school	7	1.8
Total	400	100.0

4.1.5 History of Pap Smear Screening

Out of the 400 women enrolled in this study, only 31 (7.8%) reported to have history of Pap smear screening test before this study. The rest, 369 (92.2%) had never had a Pap smear test done prior this study (Figure 4.1).

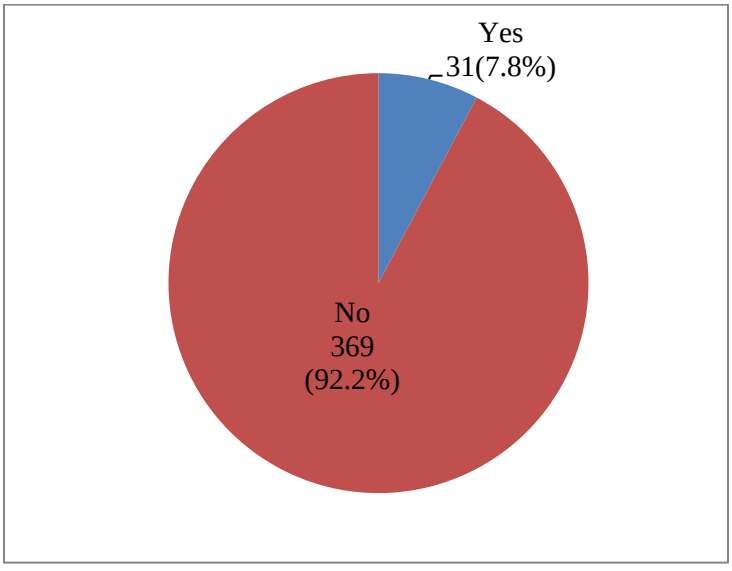

Figure 4.1: History of Pap Smear Screening

4.1.6 Family Planning Methods

In this study, condoms were the most commonly used method of family planning 179 (44.8%) followed by injection, 70 (17.5%). IUCD was the least popular method of family planning with only 14 (3.5%) women (Figure 4.2).

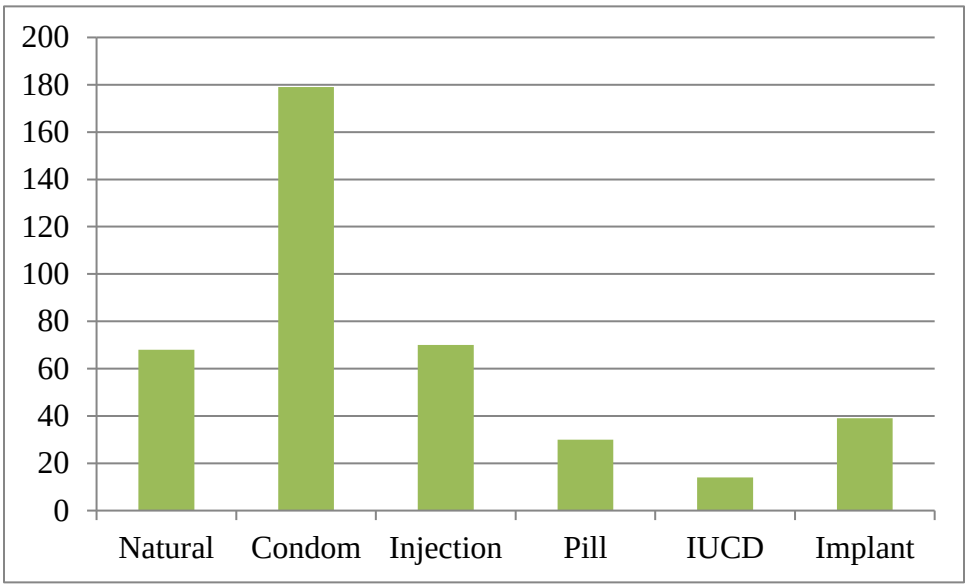

Figure 4.2: Family Planning Methods

4.1.7 Age at First Coitus

Many participants in this study engaged in their first sexual encounter at an age above 15 years, 257 (64.2%). Only 9 (2.3%) women had their first sexual encounter at an age below 10 years. (Table 4.5).

Table 4.5: Age at First Coitus

Age at first coitus	Frequency	Percent
10 and below	9	2.3
11 – 15	134	33.5
Above 15	257	64.2
Total	400	100.0

4.2 Prevalence and Patterns of Cervical Lesions on Manual Liquid Based Cytology

Out of the 400 women who took part in this study, 355 (88.8%) were Negative for Intraepithelial Lesion or Malignancy (NILM), 30 (7.5%) had abnormal Pap smear findings while 15 (3.8) had unsatisfactory smears for evaluation. The 15 participants whose smears were unsatisfactory for evaluation were excluded from the analysis in calculating the prevalence. In this study, the cervical cytology lesions' prevalence was 30 out of 385 (7.8%) (Table 4.6 and Figure 4.3).

Table 4.6: Pap Smear Findings on Manual Liquid Based Cytology

Pap smear Findings	Frequency	Percent
NILM	355	88.8
Abnormal smear	30	7.5
Unsatisfactory smear	15	3.8
Total	400	100.0

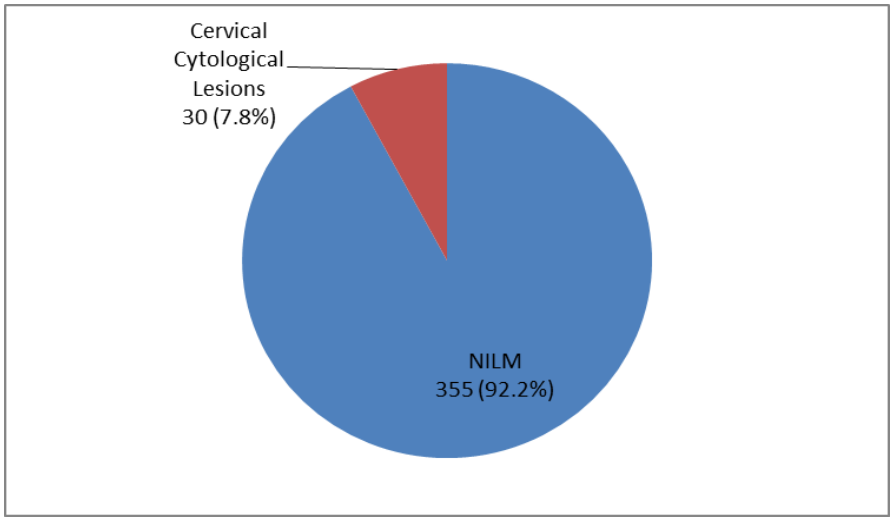

Figure 4.3: Prevalence of Cervical Cytological Lesions

4.3 Bethesda Classification of the Cervical Cytological Lesions

Of those with abnormal Pap smear findings, 9 (30%) women had ASCUS, 7 (23.3%) had High HSIL, 6 (20%) had LSIL, 5 (16.7%) had Atypical Glandular Cells (AGC), 2 (6.6%) had Atypical Squamous Cells cannot exclude HSIL (ASC-H), and 1 (3.3%) had Squamous cell carcinoma (SCC) (Figure 4.4).

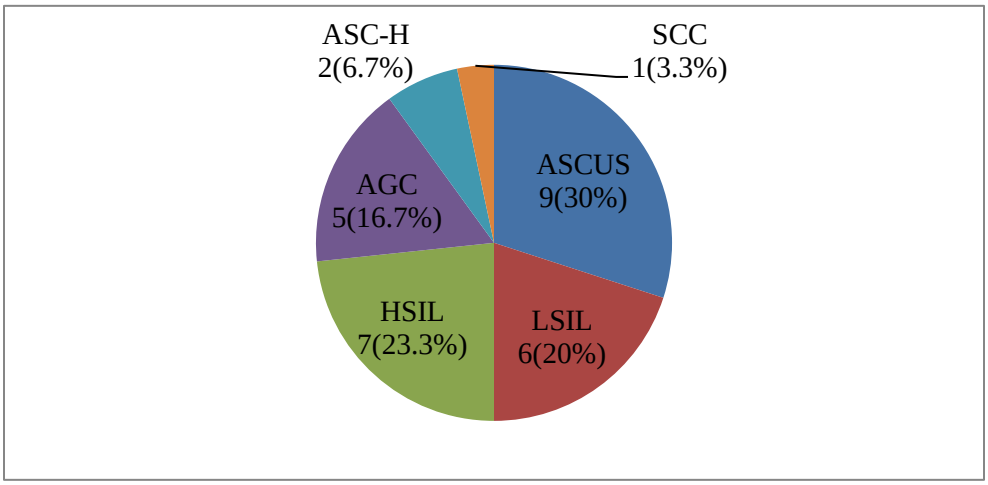

Figure 4.4: Bethesda Classification of the Cervical Cytological Lesions

4.4 Correlation of Pap Smear Findings and Social Demographic Characteristics

4.4.1 Age and Marital Status versus Pap Smear Findings

The age bracket 40-49 had the highest cases of abnormal cytological findings (n=15, 15%) while those aged 60 years and above had the smallest cases of lesions (n=1, 3.3%). Age and abnormal Pap smear findings had no statistically significant association (p value = 0.732). In regarding to marital status, women who were single had the highest number of abnormal Pap smear findings (n=9, 30%) while those divorced had the smallest number of abnormal Pap smear findings (n=1, 3.3%). However, cervical cytological lesions and marital status were not significantly associated (p value = 0.408) (Table 4.7).

Table 4.7: Age and Marital Status versus Pap Smear Findings

		Pap smear findings			
Age (years)	Total (%)	NILM (%)	Abnormal (%)	Unsatisfactory (%)	*P-value*
20-29	32(8)	29 (8.2)	2(6.7)	1(6.7)	0.732
30-39	111 (27.8)	102 (28.7)	7 (23.3)	2 (13.3)	
40-49	148 (37)	126 (35.5)	15 (50)	7 (46.7)	
50-59	98 (24.5)	88 (24.8)	5 (16.7)	5 (33.3)	
60 and above	11 (2.8)	10 (2.8)	1 (3.3)	0 (0)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	
Marital Status	Total (%)	NILM (%)	Abnormal (%)	Unsatisfactory (%)	*P-value*
Single	100 (25)	90 (25.4)	9 (30)	1 (6.7)	0.408
Married	197 (49.3)	170 (47.9)	16 (53.3)	11 (73.3)	
Divorced	39 (9.8)	37 (10.4)	1 (3.3)	1 (6.7)	
Widowed	64 (16)	58 (16.3)	4 (13.3)	2 (13.3)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	

4.4.2 Tobacco Smoking and Contraceptives use versus Pap Smear Findings

Out of the 400 participants included in this study, only 9 (2.3%) had history of tobacco smoking while 391 (97.7%) did not have any history of tobacco smoking in their life time. Notably, there was no statistical significant association between

tobacco smoking and cervical cytological lesions (P value = 0.106). In regards to the method of family planning, condom was the most common used method (n=179, 44.8%) while Intra uterine contraceptive device (IUCD) was the least used (n=14, 3.5%). Similarly Pap smear findings and contraceptives use were not significantly associated (P value = 0.921) (Table 4.8).

Table 4.8: Tobacco Smoking and Contraceptives versus Pap Smear Findings

		Pap Smear Findings			
Tobacco smoking	Total (%)	NILM (%)	Abnormal (%)	Unsatisfactory (%)	*P-value*
Yes	9 (2.3)	6 (1.7)	2 (6.7)	1 (6.7)	0.106
No	391 (97.7)	349 (98.3)	28 (93.3)	14 (93.3)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	
Contraceptive used	Total (%)	NILM (%)	Abnormal (%)	Unsatisfactory (%)	*P-value*
Natural	68 (17)	61 (17.2)	4 (13.3)	3 (20)	0.921
Condom	179 (44.8)	158 (44.5)	15 (50)	6 (40)	
Injection	70 (17.5)	61 (17.2)	6 (20)	3 (20)	
Pill	30 (7.5)	26 (7.3)	2 (6.7)	2 (13.3)	
IUCD	14 (3.5)	13 (3.7)	0 (0)	1 (6.7)	
Implant	39 (9.7)	36 (10.1)	3 (10)	0 (0)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	

4.5 Prevalence of Bacterial Vaginosis among the HIV Infected Participants

Out of the 400 women who participated in this study, 47 had infections. 41 (10.3%) had Bacterial Vaginosis (BV) while 6 (1.5%) had candida infections. A chi-square test was run to determine if there was any association between BV cases and the social demographic features. The highest cases of BV were observed in the age groups (30-39) years (34.1%) and (40-49) years (34.1%) while among those aged 60 years and above there was no any case of BV. Age was statistically associated with BV (P=0.002). Married women had the highest cases of BV (43.9%) while divorced women had the least cases (9.8%). There was no statistical significant association between marital status and BV (P=0.53). Those with secondary education as the maximum qualification had the highest cases of BV (61.0%) while those who had never gone to school had the least cases of BV (2.4%). Education level had no statistical significant association with BV cases (P=0.12). Based on the family planning method, women who used condoms had the highest cases of BV (43.9%) followed by injection (19.5%) with those using IUCD having zero cases of BV (Fig 4.5, Table 4.9 and Table 4.10).

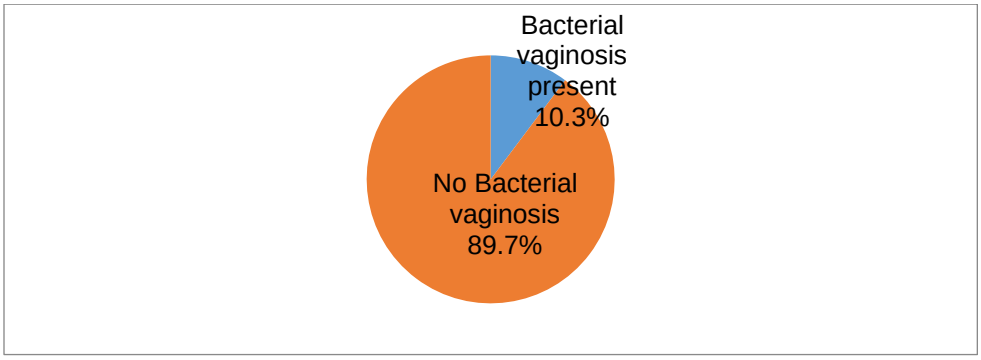

Figure 4.5: Prevalence of Bacterial Vaginosis

Table 4.9: Age and Marital Status versus Bacterial Vaginosis Cases

		Bacterial Vaginosis		
Age (years)	Total number (%)	Positive number (%)	Negative number (%)	*P-value*
20-29	32(8)	9 (22.0)	23 (6.4)	0.002
30-39	111 (27.8)	14 (34.1)	97 (27.0)	
40-49	148 (37)	14 (34.1)	134 (37.3)	
50-59	98 (24.5)	4 (9.8)	94 (26.2)	
60 and above	11 (2.8)	0 (0)	11 (3.1)	
Total number	400 (100)	41 (100)	359 (100)	
Marital Status	Total number (%)	Positive number (%)	Negative number (%)	*P-value*
Single	100 (25.0)	14 (34.1)	86 (24.0)	0.531
Married	197 (49.3)	18 (43.9)	179 (49.9)	
Divorced	39 (9.8)	4 (9.8)	35 (9.7)	
Widowed	64 (16.0)	5 (12.2)	59 (16.4)	
Total number	400 (100.0)	41 (100.0)	359 (100.0)	

Table 4.10: Contraceptives use and Education Level versus Bacterial Vaginosis Cases

		Bacterial vaginosis		
Family planning method	Total number (%)	Positive number (%)	Negative number (%)	*P-value*
Natural	68 (17.0)	6 (14.6)	62 (17.3)	0.784
Condom	179 (44.8)	18 (43.9)	161 (44.9)	
Injection	70 (17.5)	8 (19.5)	62 (17.3)	
Pill	30 (7.5)	4 (9.8)	26 (7.2)	
IUCD	14 (3.5)	0 (0)	14 (3.9)	
Implant	39 (9.7)	5 (12.2)	34 (9.5)	
Total number	400 (100.0)	41 (100.0)	359 (100.0)	
Education level	Total number (%)	Positive number (%)	Negative number (%)	*P-value*
Primary	158 (39.5)	9 (22.0)	149 (41.5)	0.116
Secondary	188 (47.0)	25 (61.0)	163 (45.4)	
College/University	47 (11.8)	6 (14.6)	41 (11.4)	
Never gone to school	7 (1.7)	1 (2.4)	6 (1.7)	
Total number	400 (100.0)	41 (100.0)	359 (100.0)	

4.6 Photomicrographs of Cervical Intraepithelial Lesions and Negative Smear

Figure 4.6 below shows photomicrographs of (**a**) High Grade Squamous Intraepithelial Lesion (HSIL), (**b**) Squamous cell carcinoma (SCC), (**c**) Low grade squamous intraepithelial lesion (LSIL), (**d**) Atypical glandular cells (AGC), (**e**) Atypical squamous cells of undetermined significance (ASCUS) and (**f**) Negative for intraepithelial lesion or malignancy (NILM) at X400 Field

a)

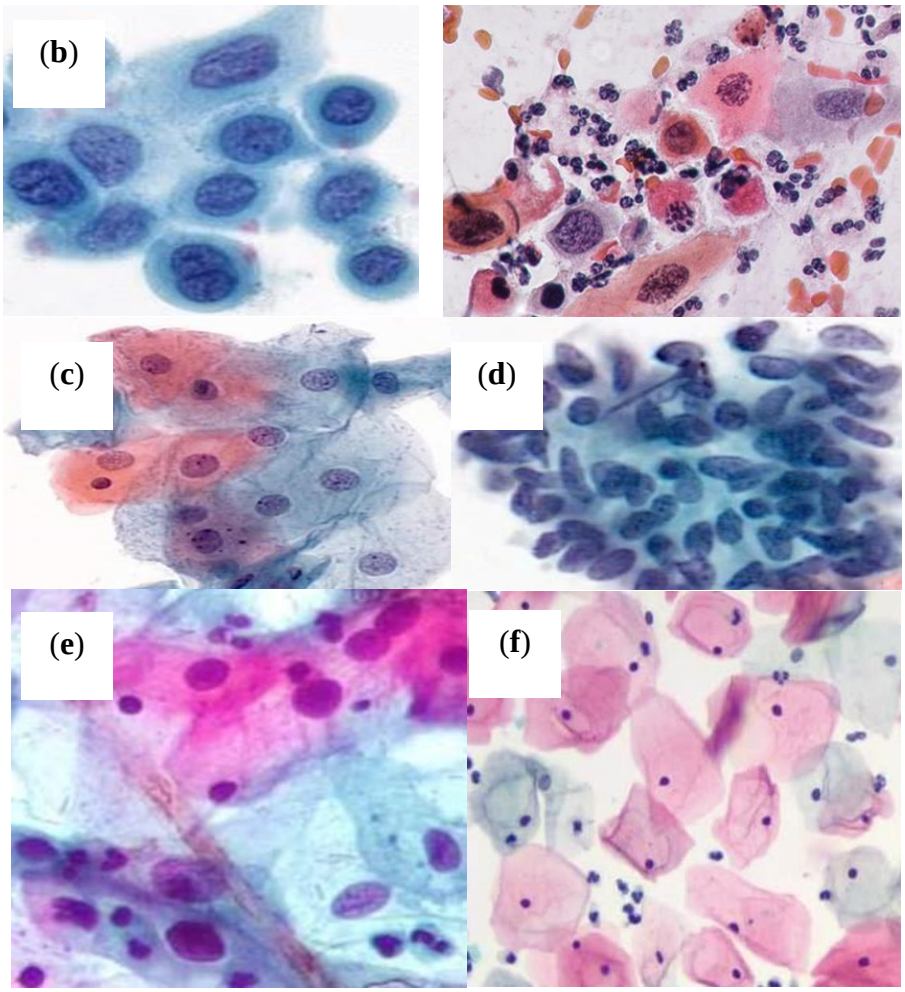

Figure 4.6: Photomicrographs of Cervical Intraepithelial Lesions and Negative Smear

4.7 Prevalence of High Risk HPV among Women with Abnormal Cytological Findings

Among the 400 participants enrolled in the current study, 25 had abnormal Pap smear findings of ASCUS and above. High risk HPV DNA testing for two genotypes (16 and 18) was done from their cytological remnant samples. Of these 25, 18 (72%) were positive for high risk HPV DNA while 7 (28%) were negative for high risk HPV. Genotype 16 was the most prevalent with 11 (61.1%) cases while genotype 18 was detected in 7 (38.9) cases (Tables 4.11, 4.12 and Figure 4.12).

Table 4.11: Results for Specific High Risk HPV Genotypes and MLBC Pap Smear Findings

	High risk HPV DNA results			
Pap smear findings	HR-HPV Negative (%)	HPV 16 Positive (%)	HPV 18 Positive (%)	Total (n)
ASCUS	3 (42.8)	4 (36.3)	2 (28.6)	9
LSIL	2 (28.6)	3 (27.3)	1 (14.3)	6
ASCH	1 (14.3)	0 (0.00)	1 (14.3)	2
HSIL	1 (14.3)	3 (27.3)	3 (42.8)	7
SCC	0 (0.00)	1 (9.1)	0 (0.0)	1
Total (n)	7	11	7	25

Table 4.12: Correlation of HPV DNA Test Results and Abnormal Pap Smear Findings

	High risk HPV DNA results			
Pap smear Results	Negative (%)	Positive (%)	Total	*P-value*
ASCUS	3 (42.8)	6 (33.3)	9	0.002
LSIL	2 (28.6)	4 (22.2)	6	
ASC-H	1 (14.3)	1 (5.6)	2	
HSIL	1 (14.3)	6 (33.3)	7	
SCC	0 (0.0)	1 (5.6)	1	
Total number	7 (100)	18 (100)	25	

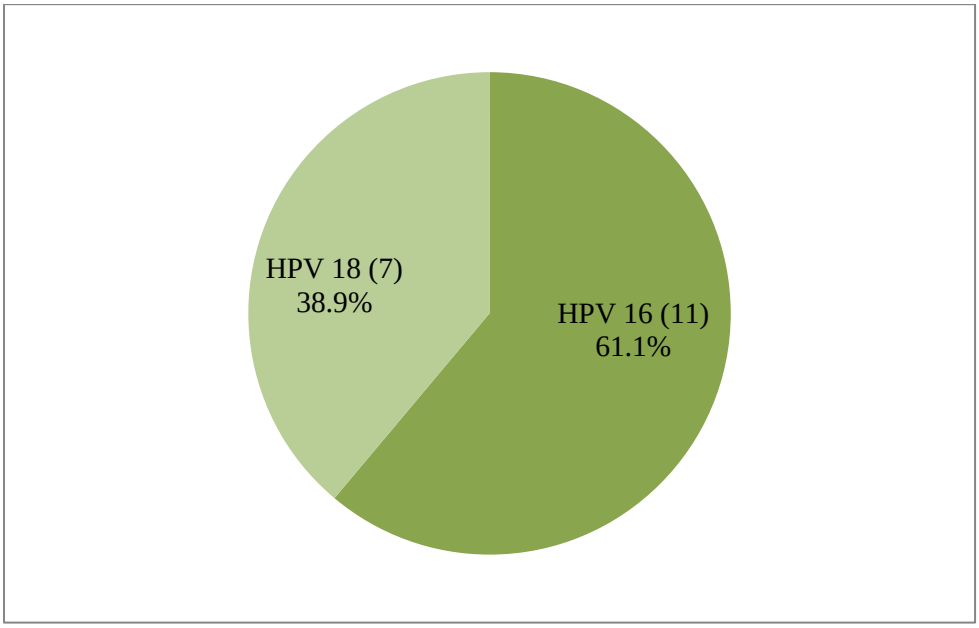

Figure 4.7: Frequency of HPV Genotypes

4.8 Histopathology Results

In the current study, 10 women had lesions of high grade and above from cytological examinations and were refereed for biopsy and histopathological examination. Out of this 10, 4 (40%) had CIN 2, 3 (30%) had CIN 3, 2 (20%) showed features of squamous cell carcinoma while 1 (10%) was found to have chronic cervicitis. (Figure 4.13, Table 4.13).

In order to establish the agreement level between Manual liquid based cytology and histopathology results, Cohen's kappa test was performed. The level of agreement between the two methods was moderate k=0.574, (95% CI, .41 to .60), *p* =0.11. The contingency coefficient for the two methods was 0.689 (Table 4.14)

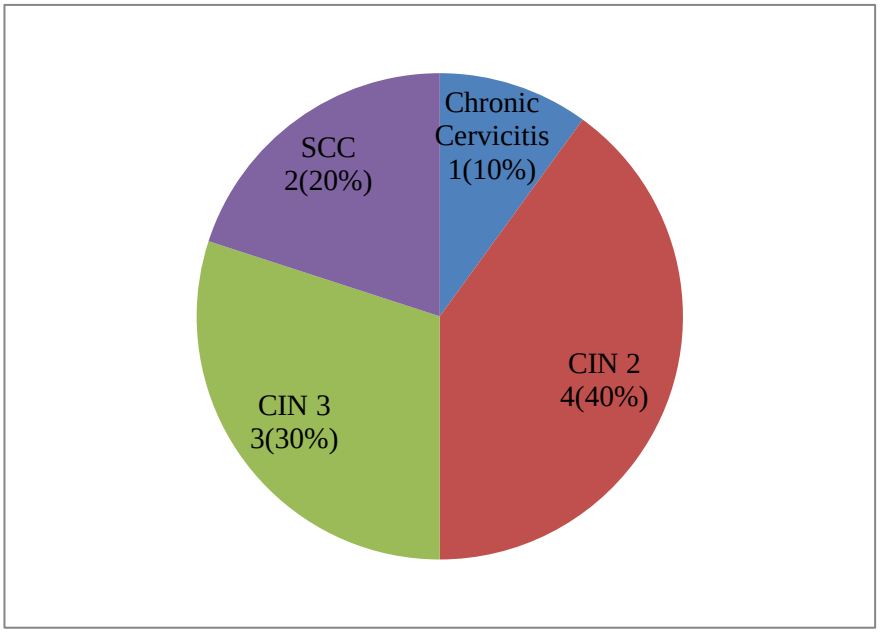

Figure 4.8: Histopathology Results

Table 4.13: Results for both Histopathology and MLBC abnormal Pap smear Findings

	Histopathology Results				
MLBC abnormal Results	Normal/chronic cervicitis (%)	CIN2 (%)	CIN3 (%)	SCC (%)	**Total**
ASC-H	1 (100.0)	1 (14.3)	0 (0.0)	0 (0.0)	2
HSIL	0 (0.0)	3 (85.7)	3 (100.0)	1 (50.0)	7
SCC	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	
Total	1 (100)	4 (100)	3 (100.0)	2 (100.0)	10

Table 4.14: Correlation of Manual Liquid Based Cytology and Histopathology Results

	Histopathology results						
MLBC abnormal results	Normal/chronic cervicitis (%)	CIN2/3 (%)	SCC (%)	Total	Kappa value	Contingency coefficient	P – value
ASC-H	1 (100.0)	1 (14.3)	0 (0.0)	2	0.574	0.689	0.11
HSIL	0 (0.0)	6 (85.7)	1 (50.0)	7			
SCC	0 (0.0)	0 (0.0)	1 (50.0)	1			
Total	1 (100)	7 (100)	2(100.0)	10			

4.9 Correlation of Histopathology Findings and HPV DNA Results

Out of the 400 women who took part in this study, 10 had lesions of high grade and above from cytological examinations. High risk HPV DNA testing for two genotypes (16 and 18) was done from the remnant samples. Out of these 10, 2 (20%) were negative for high risk HPV16&18, 4 (40%) were positive for HPV 16 and 4 (40%) were positive for HPV 18. (Table 4.15).

The 10 participants were also referred for biopsy and histopathological examination. To determine the agreement level between histopathology findings and HPV DNA results, Cohen's kappa test was performed. The two methods had a substantial level of agreement k=0.615, (95% CI, .61 to .80), p =0.035. The sensitivity and specificity of high risk HPV DNA testing with histopathology as the gold standard were 88.89% and 100% respectively (Table 4.16).

Table 4.15: Results for both Histopathology and Specific High Risk HPV Genotypes

	High risk HPV DNA results			
Histopathology Results	HR-HPV Negative (%)	HPV 16 Positive (%)	HPV 18 positive (%)	**Total**
Normal/cervicitis	1 (50.0)	0 (0.0)	0 (0.0)	1
CIN 2	0 (0.0)	2 (50.0)	2 (50.0)	4
CIN 3	1 (50.0)	1 (25.0)	1 (25.0)	3
SCC	0 (0.0)	1 (25.0)	1 (25.0)	2
Total	2	4	4	10

Table 4.16: Correlation of HPV DNA Results and Histopathology Findings

	Histopathology results						
HPV DNA results	Normal (%)	CIN2 & above (%)	Total	Kappa value	P-value	Sensitivity (%)	Specificity (%)
Negative	1 (100.0)	1 (11.1)	2	0.615	0.035	88.89	100
Positive	0 (0.0)	8 (88.9)	8				
Total	1 (100)	9 (100)	10				

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Social-Demographic Characteristics of the Study Participants

In this study, majority of women enrolled were aged between 40 to 49 years (37%). Only 11 (2.8%) were aged 60 years and above, while 32 (8%) were between 20 to 30 years. In a previous study done to establish the burden of cervical lesions among HIV-positive women in Machakos county hospital, majority of women who participated were between 40 to 49 years old (33.6%), which is close to the study's current results (Mutuku et al., 2017). In another similar study done in Rwanda, 43% of the women who participated were aged between 30 to 40 years, comprising the majority age bracket (Wanyoike, Kayumba & Khisa, 2014).

The comprehensive care centre at Machakos county hospital serves over 4000 patients, of which about 50% are women. According to Ziraba *et al*. (2018), women between 15-24 years are considered to have a high predisposition to HIV due to their high vulnerability reinforced by limited access to the vital education on reproductive health, unfavorable power relation, and women gender-based violence. The current study experienced a low turn-out among women aged 20 to 30 years old with only 32 (8%) participating. Most of them seemed not to be comfortable with the Pap smear collection procedure and declined to participate.

The present study showed that age lacked a statistically significant association with the prevalence of cervical cytological lesions. This agrees with other previous studies which found age not to have any statistically significant association with abnormal cervical cytology (Mutuku *et al*., 2017; Wanyoike et al., 2014; Gedefaw *et al*., 2013). However, the age bracket 40-49 had the highest cases of abnormal Pap smear findings in this study.

In the present study, a considerable number of female participants had a spouse (49.3%) while 25% reported to be single. Studies have had varying findings regarding the vulnerability to HIV among the singles and the married. A research undertaken in South Africa found that the risk of HIV infection among the individuals who were never married was twice that of the married (Tlou, 2019). Similarly, other studies have indicated that married people living with their spouses have low predisposition to HIV infection compared to the unmarried (Kposowa, 2013 & Shisana *et al*., 2016).

Notably, in the present study, marital status and cervical cytological lesions were not significantly associated. However, single women had the highest number of abnormal Pap smear findings (n=9, 30%) while those divorced had the smallest number of lesions. Studies have indicated that marital status is a key determinant in transmission of high-risk HPV which is closely associated with cervical cancer (Coskuner *et al*., 2014).

A study done in China to establish the prevalence and risk factors for HPV infection among women reported that women who had multiple sexual partners had a higher vulnerability to HPV infection. A low prevalence of high-risk HPV was reported among married women who used condoms frequently (Quan *et al*., 2019). Another study done in India reported increased risk of HPV infection among widows, and separated women due to their low socioeconomic status and vulnerability (Sauvaget *et al*., 2011).

In this study, only 7 women (1.8%) reported to have history of tobacco smoking. The current study has showed that tobacco smoking was not significantly associated with abnormal Pap smear findings. This contradicts previous studies, which have indicated that prolonged cigarette smoking contributed to cervical lesions' development (Mazarico *et al*., 2015; Eldridge *et al*., 2017). Matsumoto et al (2010) showed that prolonged tobacco smoking leads to slow regression of high grade lesions, thus making the cervical abnormalities to persist. In another study done in China, there was elevated risk of cervical cancer-related deaths among women who were smokers compared to those who were non-smokers (Jiang et al., 2015). However, in this study, only few women (n=9) were identified as smokers, and this could be the reason as to why we were not able to find any association between tobacco smoking and abnormal cervical cytology.

Abnormal cervical cytology was not associated with the family planning methods in the present study. This contradicts another study done in Australia which documented a high vulnerability to cervical cancer among women who used hormonal contraceptives for an extended period (Huilan *et al*., 2018). McKenzie *et al*. (2011) reported that reduced frequency of using condoms was associated with increased chances of squamous intraepithelial lesions. In a study by Roura *et al*. (2016), oral contraceptives use for over 15 years had a correlation with an increased predisposition to cervical intraepithelial lesions. Another study found that women who used intrauterine device had an elevated risk of developing cervical intraepithelial neoplasia compared to those who used oral contraceptives (Loopik et al., 2020).

5.1.2 Prevalence and Patterns of Cervical Lesion on MLBC

The current study has shown that the burden of cervical cytological lesions among HIV-positive women in Machakos county hospital in Kenya is 7.8% which is comparable to 6% documented in another study done in Nigeria (Ononogbu *et al*., 2013). This prevalence is also comparable to 6.3% reported in another study done in Northern Uganda in 2016 (Izudi *et al*., 2016). The prevalence documented in the current study is slightly higher than 4.3% that was reported in a previous study undertaken by Mutuku et al in 2017 (Mutuku et al., 2017).

However, this burden is lower compared to the one documented in prior studies conducted in Kenya and Africa. In a study done among HIV infected women to determine the burden and predisposing factors for precancerous cervical cancer lesions, the prevalence of abnormal cervical cytology was documented as 26.7% (Memiah et al., 2012). In another study by McKenzie et al involving HIV positive women receiving antiretroviral therapy in Kenya, the burden of cervical squamous intraepithelial lesions was found to be 46% (McKenzie *et al*., 2011).

In a research undertaken in Rwanda, the burden of cervical lesions among women infected by HIV was found to be 20% (Wanyoike *et al*., 2014). A study conducted in Southern Ethiopia documented a prevalence of 22.1% (Gedefaw *et al*., 2013). In other studies done in North-Central Nigeria, Swaziland, and South Africa, the burden of cervical cytological abnormalities in women infected by HIV was found to be 12.2%, 22.9%, and 19.6% respectively (Daniel et al., 2020; Jolly *et al*., 2017; Ntuli *et al*., 2020). Early initiation and adherence to antiretroviral therapy could have resulted to the low burden of abnormal cervical cytology documented in the current study as all women enrolled in this study were on antiretroviral therapy.

5.1.3 Prevalence of Bacterial Vaginosis among the HIV Infected Participants

In this study, the prevalence of BV among HIV-positive women in Machakos county hospital, Kenya was 10.3%. This prevalence is lower than that of India, which was 20.9% in a study done in 2020 (Joshi *et al*., 2020). Another study undertaken in the United States found a 17.3% burden of BV among HIV-positive women (French *et al*., 2011). This variation between our research and other studies could be due to methodological differences because we relied on cervical specimens only. The other two studies, however, used both cervical and vaginal specimens.

A statistical correlation between age and BV was found in the current study, with the highest cases observed among women aged 30-49 years. These research results are in tandem with those of Ranjit et al. who observed a higher burden of BV in women aged 30-40 years and lower prevalence in those aged 10-20 years and 50-60 years in Nepal. However, in the study by Ranjit et al., the association between BV and age was not statistically significant (Ranjit et al., 2018). Other studies done in Cameroon and Nigeria found a higher prevalence of BV in those aged 18-24 years. The age bracket 18-49 years corresponds to the reproductive age of women and is characterized by increased sexual activity (Ibrahim *et al*., 2014; Kamga *et al*., 2019).`

The increased sexual activity, one of the transmission methods of BV, could also explain the high prevalence of BV among married women and the lower rate in divorced and widowed women in this study. Assuredly, there was consistency between our findings and those of Gad et al., who reported a higher burden of BV among married women (Gad *et al*., 2014). Frequent sexual intercourse disrupts the normal flora of the vagina and prevents its restoration (Abdul *et al*., 2019). Another factor that is likely to influence the occurrence of BV is literacy level. Low literacy is associated with a higher likelihood of getting BV due to poor knowledge about preventive measures (Ranjit et al., 2018). In our study, the educational level had no significant association with BV, and those with secondary educational levels had the highest burden of BV. The effect of education could have been modified by other factors such as sexual activity, access to healthcare, and age.

Moreover, the family planning method has been linked to the risk of BV. While condom use is considered protective against BV due to the physical barrier provided, the burden of BV was elevated in women who used condoms than in those who used other contraceptive methods. However, the results for contraceptives use in our study were not statistically significant. Research has shown that consistent condom use is necessary for their effectiveness (Yotebieng et al., 2009). In our study, we did not evaluate the consistency of condom use, and this affects the accuracy of our findings. Women who used pills, implants, and injections had the lowest prevalence of BV. The low prevalence of BV among

women who use the aforementioned contraceptives has been associated with the protective effects of hormones against bacterial overgrowth in the vagina (Vodstrcil *et al.*, 2013).

5.1.4 Prevalence of High Risk HPV among Women with Abnormal Cytological Findings

In this study, the burden of high-risk HPV among HIV positive women with abnormal cytological findings of ASCUS and above was 72%. This prevalence is comparable to 62.8% reported by Weizhi et al in their study on high-risk HPV infection among individuals with abnormal cervical cytology (You et al., 2018). However, the prevalence in this study is higher than 54.87% that was reported by Wang et al in a research to investigate the distribution of genotypes and the burden of HPV among women with abnormal cervical cytology in Xinjiang, China (Wang *et al*., 2019). In another study done in Iraq on molecular genetics of high- and intermediate-risk genotypes of HPV among individuals with benign and malignant cervical lesions, the prevalence of HPV was 11.3%, which is lower than the one recorded in this study (Mushtak & Shaymaa, 2019).

In a study done by Karani *et al* at Kisii Teaching and Referral Hospital, the overall burden of high-risk HPV in VIA positive women was 20.49%. A significantly higher burden of high-risk HPV was reported among women with cervical cytological abnormalities at 34% compared to those with normal cytology at 1.46%

(Karani *et al*., 2020). Ngugi et al in his study to determine the burden of HPV infection stratified by cervical cytology and age in Thika indicated that women who had cervical lesions had an elevated burden of HPV compared to those who had normal cytology results on Pap smear (Ngugi *et al*., 2011). However, in the current study, women with normal cytology results from Pap smear were not tested for high-risk HPV, and thus, no comparison between the two groups.

Previous research has indicated that HIV-positive women have increased vulnerability to high-risk HPV infection. Due to their compromised immunity leading to persistent infection, HIV-positive women are susceptible to precancerous lesions and cervical cancer (Hawes *et al*., 2003; Rowhani *et al*., 2007; Clifford *et al*., 2016). In a research conducted in South Africa to investigate the burden, and predisposing factors to HPV among HIV negative and HIV positive women, the burden of high-risk HPV among HIV-positive women was 40.6% in comparison to that of HIV negative women at 21.4% (Taku et al., 2020).

In the present study, HPV 16 was more prevalent at 44% while HPV 18 had a prevalence of 28%. This is in agreement with previous studies undertaken in Africa and across the world which have documented HPV 16 as the most abundant high-risk HPV type. In a research done in Thika, the most prevalent strains of high-risk HPV were 16, 52, 56, 66, and 18 in that order (Ngugi *et al*., 2011). In another study by Karani *et al* (2020), the most prevalent genotype was HPV 16 at 30.8%, followed

by combination of HPV types 16, 18/45 at 22.6%, while the prevalence of HPV types 18/45 was 13.1%. Another study undertaken in Brazil to investigate the burden of high-risk HPV types 16 and 18, well as the prognosis of individuals diagnosed with stage I cervical cancer, reported a higher prevalence of HPV 16 at 65.3%, while that of HPV 18 was 33.3% (Zampronha *et al*., 2013). Other studies carried out in India, Colombia, and Nigeria have also shown that HPV 16 is the most common high-risk HPV type (Vinodhini et al 2012; Del Río *et al*., 2016; Fadahunsi *et al*., 2013).

In many developed countries, HPV DNA testing is replacing cytology in diagnosing cervical intraepithelial lesions and cervical cancer. HPV DNA testing has higher sensitivity in identifying precancerous cervical lesions than cytology (Dillner, 2019; Ogilvie *et al*., 2018). However, HPV DNA testing alone is reported to have a low specificity, and therefore, co-testing with cytology, which has higher specificity, is recommended for more accurate results (Felix et al., 2016). As a result of the high cost implications and lack of resources, HPV DNA testing has not been widely used in the developing countries, and thus, cytology remains the most widely used test in screening for cervical intraepithelial lesions and cervical cancer in these countries (Gupta *et al*., 2017).

5.1.5 Correlation of Manual Liquid Based Cytology and Histopathology Results

In the present study, a moderate agreement (57.4%) was observed between MLBC results and histopathological examination with a contingency coefficient value at 0.689. This level of agreement is comparable to 53% reported in a study by Zhu et al that sought to compare the performance of LBC, Pap smear, and histology (Zhu *et al*., 2007). Moreover, a research by Manoli et al in India documented a contingency coefficient value of 0.556 for MLBC and histopathology, which is inferior the value reported in the current study (Manoli et al., 2016).

In contrast, the rate of concordance reported in the present study is lower than 86% documented in a study by Nandini, which compared the performance of MLBC with CPS and histology (Nandini *et al*., 2012). In another study done by Macharia et al at Kenyatta National Hospital to compare the performance of CPS, LBC, and colposcopy clinical impression with histology as gold standard, the level of agreement between liquid based cytology and histology was 72.95%, which is higher than the one recorded in this study. Moreover, the present study has a higher concordance rate between MLBC and histology than the 43% documented in a study done in Thailand to evaluate the correlation between LBC and histopathology (Areeruk & Manchana, 2019).

Studies have shown that LBC is superior to CPS in screening for cervical intraepithelial lesions, and cervical cancer in terms of specimen adequacy, clear background and increased detection rate. The sensitivity and specificity are also higher in LBC than in CPS (Aboobacker & Shariff, 2020, Pankaj *et al*., 2018). A study done in India to compare the performance of MLBC and CPS with histology recorded a sensitivity of 81.8% for MLBC while that of CPS was 36.3% (Mittal, Kulkarni, & Aggarwal, 2018). In another study by Nandini et al, the sensitivity of MLBC was 75%, while that of CPS was 50% and thus MLBC was considered more sensitive than CPS (Nandini *et al*., 2012).

However, a study done in India reported that MLBC had a sensitivity of 22.22%, which was lower than that of CPS at 33.33% while specificity for both methods was 95.65% (Dhananjaya & Kumari, 2017). In another study done by Macharia et al at Kenyatta National Hospital comparing CPS, LBC, and colposcopy clinical impression with histology as gold standard, the sensitivity of CPS was 50%, which was higher than that of LBC at 13%. However, the LBC specificity in this study was 92% higher than that of CPS, which was at 57% (Macharia et al., 2014).

In this study, with MLBC, 385 (96.3%) samples were satisfactory for examination, which is marginally lower than 99% documented in a study undertaken in India to compare the performance of MLBC with histology as gold standard (Nandini et al., 2012). Other studies comparing the performance of MLBC with CPS found that

specimen adequacy in MLBC to be higher than in CPS. In their study, Dhananjaya and Kumari found MLBC to have a specimen adequacy of 88.7%, which was slightly higher than 86.6% for CPS (Dhananjaya & Kumari, 2017). Similarly, Mittal, Kulkarni, & Aggarwal, (2018), documented a specimen adequacy of 87.5% with MLBC which was higher than that of CPS at 85.5%. In another study by Mutuku et al comparing the performance of MLBC with CPS, specimen adequacy with MLBC was 95.9%, while that of CPS was 92.5% (Mutuku et al., 2018).

Other studies comparing the performance of automated LBC with CPS have also shown that specimen adequacy for LBC is higher than that of CPS. Pankaj et al found more unsatisfactory smears with CPS at 7.1%, while with LBC, the rate was 1.61% (Pankaj et al., 2018). In another study done in Pakistan to compare LBC and CPS, the rate of unsatisfactory smears with CPS was 1.3%, which was close to that of LBC at 1.2% (Hashmi *et al*., 2020). A study done in Egypt in 2019 documented a rate of unsatisfactory smears with CPS at 22%, which was higher than that of LBC at 2% (Ezzat & Abusinna, 2019). Similarly, in a study by Ranjana and Sadhna comparing the performance of CPS and LBC in screening for cervical intraepithelial lesions and cervical cancer, the rate of unsatisfactory smears with liquid-based cytology was at 1.67% while that of CPS was higher at 6.67% (Ranjana & Sadhna, 2016).

5.1.6 Correlation of Histopathology Findings and HPV DNA Results

In the present study, a substantial agreement (61.5%) was found between high-risk HPV DNA results and histology. Sensitivity and specificity of high-risk HPV DNA testing were calculated using histology as the gold standard. The current study reported sensitivity and specificity of HR HPV DNA testing as 88.89% and 100% respectively. One case, which had negative results for both high-risk HPV 16 and 18, had a histological diagnosis of CIN3. This could be attributed to either poor sampling or the possibility of other high risk HPV types which were not targeted in this study. However, there were no cases of false positives recorded with high-risk HPV DNA testing in this study.

The sensitivity recorded in the present study is compatible with the 90% documented by Andersen et al who compared high-risk HPV testing with LBC in screening for cervical cancer. However, in the same study, high-risk HPV testing had a specificity of 92.8%, which is lower than the one recorded in the current study (Andersen *et al*., 2020). A study undertaken in Greece by Agorastos et al, high-risk HPV 16 and 18 genotyping in diagnosing CIN2 and above had a sensitivity of 58.5%, which is lower than the one recorded in this study. The specificity in this study was 97.5%, which is close to 100% in the current study (Agorastos *et al*., 2015). In another study done in India to evaluate the sensitivity and specificity of Pap smear, as well as those of LBC and HPV in cervical cancer screening, the sensitivity of HPV DNA testing was documented at 89.9%, which is in tandem with

the reported in the present study. The specificity was 98%, which is also close to the value reported in this study (Pankaj et al., 2018).

A recent research in Korea by Kang et al to compare Pap smear and HPV testing reported that HPV testing had a sensitivity of 88.3% in screening for high-grade squamous intraepithelial lesion and squamous cell carcinoma that is slightly lower than 88.89% recorded in the present study. However, the specificity of HPV testing in screening for high grade squamous intraepithelial lesion and squamous cell carcinoma was documented at 54.92%, which is very low compared to the one recorded in the current study (Kang *et al*., 2020).

5.2 Conclusions

i. The prevalence of cervical cytological lesions using MLBC technique was 7.8% with ASCUS being the most prevalent lesion. None of the demographic features studied had a statistical significant association with cervical cytological lesions.
ii. The prevalence of bacterial vaginosis among the HIV-positive women was 10.3%. Age was significantly associated with incidence of bacterial vaginosis (P=0.002).
iii. The prevalence of high-risk HPV among women with abnormal cytological finding of ASCUS, and above was 72%, with genotype 16 being the most prevalent high-risk HPV subtype.

iv. There was moderate level of agreement between MLBC, pap smear findings, and histopathology results (k=0.574, 95% CI, p =0.11), and thus MLBC can be used as an alternative screening method in limited resource setups.

v. There was a substantial level of agreement between histopathology results and high-risk HPV DNA testing results (k=0.615, 95% CI).

5.3 Recommendations and Suggestions for Future Studies

i. Regular Pap smear screening should be introduced for all HIV positive women at Machakos county hospital critical care centre to ensure that there is early detection of precancerous lesions and cervical cancer for effective management of the patients.

ii. Future molecular based studies should be done to identify the dominant bacteria causing bacterial vaginosis among the HIV-positive women at Machakos county hospital.

iii. Future studies should be carried out to identify all the High-risk HPV genotypes circulating among women infected by HIV/AIDS at Machakos County hospital.

iv. Future studies with validation protocol for MLBC technique should be done.

v. A cost-benefit analysis should be done for MLBC to assess its cost effectiveness as an alternate cervical cancer screening method in resource limited settings.

vi. Future studies should be conducted to assess the utility of the remnant samples in detection of abnormal chromosomes and potential biomarkers for cervical cancer.

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APPENDICES

Appendix I: Client Consent Information Form

TITLE: ANALYSIS OF MANUAL LIQUID-BASED CYTOLOGY, HISTOPATHOLOGY AND HPV DNA TESTING AMONG HIV-POSITIVE WOMEN AT MACHAKOS LEVEL 5 HOSPITAL, MACHAKOS COUNTY, KENYA

My name is Onesmus Muia Mutuku, a postgraduate student at Kenyatta University (PhD in Medical Laboratory Science). I take this opportunity to kindly request your free will participation in the above study. The following consent form has detailed information on what my study entails and from such, you will get the insights on whether to be part of the study or not. Ultimately, I would request you to be at ease and raise any questions that may need clarification. Be free to ask any query related to the study purpose, your contribution and role to the depicted study, the shortcomings and benefits that you may be exposed to as a volunteer and your rights as a study participant. Additionally, nobody will be forced to engage in an activity that they feel not comfortable with. For the purposes of an evidence-based research, each participant will have a copy of the consent form for record keeping purposes.

BENEFITS

This study will be useful in facilitating early diagnosis of cervical pre-cancerous and cancerous lesions, as well as other abnormalities, that will enhance clinical decision-making for comprehensive management of your health problems. If cancer cases are detected, I will liaise with the patients to report to the CCC

whereby, together with the clinician, we will link them to a Referral Clinic for treatment. All women identified as having abnormal results of the Pap smear will be advised on routine follow up for management. Despite of the study having no financial advantages to the participants, the collected information will be vital to help in the making of informed decisions about the management and treatment of cervical cancer in the high-risk groups.

This study will also benefit the society at large in that, it is expected to develop clinically useful low cost manual liquid based cytology method that will improve the screening of pre-cancerous and cervical cancer. These may increase the survival of cervical cancer patients and thus improve their health condition and quality of life.

RISKS AND INCONVENIENCES

The interview process will involve a set of personal and quite sensitive questions to the study participants. Nevertheless, ethical considerations in the research will be adhered to and all collected information kept confidential. Furthermore, we will utilize the best approach to make sure that each participant is comfortable and any concern should be freely addressed to the researcher. Language may be sometimes a barrier in collecting data and we are much comfortable to switch to your language of choice to make the participants feel comfortable. Each participant will be free to withdraw themselves from the study if they feel unhappy about the process. It should be noted that the procedures will not involve any major complication but some discomfort may be felt during sample collection. For confidential purposes,

all signed and completed forms will be safely stored in locked cabinets that will be limited to access in the study site. No participants' details such as names will appear on the questionnaire data as they will be delinked. Indispensably, no financial costs will be asked from the participants as we solely appreciated your participation in making this study a success.

Pap smear collection procedure explanation for study participants

You will be requested to lie on an examination couch.

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

Sterile collection devices will be used to collect pap smear from suspicious areas.

The microscope slide containing the pap smear will be fixed using the fixative in the kit.

Then, the specimen will be taken by the principal investigator to the laboratory for staining and examination.

CONFIDENTIALITY

This study will be voluntary to every participant concerning the routine evaluations. As such, nothing will be altered about the services one may be targeting to realize. As mentioned earlier, you have the right to withdraw from this study and maintain the benefits depicted of being realized in this research. Study numbers will replace the names for confidential purposes. For purposes of data vulnerability and exposure, the questionnaires will only be kept for a maximum period of a year then be shredded. We promise to make sure that any presented information will be to

your own benefit and for the success of this study. After the evaluation process, the nurses and doctors assigned to you will communicate the results in the second visit and further send them to your file.

Who to Contact

Be free to contact the following persons any time you are comfortable with for any clarification you may be seeking regarding the study participation.

Onesmus Muia Mutuku (Principal investigator) on cellphone number 0712652085, my supervisors: Prof. Mutinda Kyama (cellphone number: 0711169526) and Dr. Scholastica Mathenge (cellphone: 0722936884) or Kenyatta University Ethics Review committee, P.O. Box 43844 – 00100, Telephone No; 0208710901/12

I…….. After having gone through the consent form and being given full explanation of the study purpose, do hereby provide consent for full participation in the diagnostic study and understand all the risks and benefits that I may be exposed to in the entire process.

Additionally, I am fully aware that I have the right to withdraw from the research without interfering with the benefits and quality of management to which I am part.

Participants Signature/Thumb print …… Date…….

Principal investigator…… Date……

Clinician (Research assistant)…… Date……...

Appendix II: Questionnaire

ANALYSIS OF MANUAL LIQUID-BASED CYTOLOGY, HISTOPATHOLOGY AND HPV DNA TESTING AMONG HIV-POSITIVE WOMEN AT MACHAKOS LEVEL 5 HOSPITAL, MACHAKOS COUNTY, KENYA

Before the specimen collection, all the participants will be obligated to fill this questionnaire by putting down a tick to each of the given choices.

Section A: Socio demographic information

Study number…….

Date ☐☐ ☐☐ ☐ ☐

DD/ MM/ YR

Residence……

1. Age…….

2. Marital Status

Single ☐

Married ☐

Divorced ☐

Widowed ☐

3. History of Tobacco/ Bhang smoking

NO ☐

YES ☐

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

How many cigarettes or packs per day…….....

4. Education

Primary ☐

Secondary ☐

College ☐

Not gone to school ☐

5. Last menstrual period…….

6. Ever had a Pap smear

Yes ☐

No ☐

If yes, when…….

7. Family Planning

Natural ☐

Condom ☐

Injection ☐

Pill ☐

IUCD ☐

8. No of sexual partners…….

Age/Year of first intercourse…….

Section B: Clinical history (CH)

Tick appropriately

Appearance of the cervix

1. Normal ☐
2. Eroded ☐
3. Inflamed ☐
4. Suspicious ☐

If other, specify……..…

Section C: For Investigator's Only

1. Specimen Adequacy

Satisfactory ☐

Unsatisfactory ☐

If Unsatisfactory, proceed to the end of the questionnaire.

2.Epithelial cell features

Negative ☐

ASCUS ☐

LSIL ☐

Inflammatory ☐

Reactive ☐

ASC-H ☐

☐

HSIL

SCC ☐

AGC ☐

AIS ☐

Adenocarcinoma ☐

COMMENTS

Refer ☐

Call Back ☐

Other, specify**...**……..

PRINCIPAL INVESTIGATOR'S NAME……

SIGN……..

PATHOLOGIST'S NAME…….

SIGN……..

DATE…….

Appendix III: Papanicolaou Staining Procedure, Progressive Method

Principle

A set of five dyes in three dilutions constitute the staining method in what can be termed as PAP classic form. The cell nuclei are stained by hematoxylin which is a nuclear stain. Keratin is stained by the Orange G. Consecutively, the bluing agent which preferably is the Scott's tap water substitute (STWS), applied after the hematoxylin has the role of making the nuclear dye be in place. The Eosin Azure (EA), which is the counterstain, is applied to provide a base for excellent staining of the cytoplasmic cells of the non-gynecological and gynecological samples. Notably, the EA constitutes three dies that are distinguished by numbers which describe the dyes' proportion. They include the EA-65, EA-50 and EA-36. The formulations are vital to provide a wide range of selection of the hues and color intensities. Equally important, Eosin Y is utilized to provide staining to the cilia, nucleoli, squamous cells and the red blood cells. The acidic nature of the Orange G is also essential to stain the keratin from its high affinity nature of the cytoplasm.

Procedure

1. Use a spray or the packaged fixative to fix the sample.
2. Submerge the sample and rinse in 95%, 80%, 70% and 50% alcohol respectively and then rinse with distilled water.
3. In the absence of acetic acid, using a stop watch for timing, stain in Hematoxylin Harris for a maximum range of ten to fifteen minutes.

4. Rinse in distilled water then transfer to a 0.5% hydrochloric acid aqueous solution and rinse three times.
5. Leave the sample for about a minute in Scott's tap water and use distilled water to rinse thoroughly.
6. In the following concentrations respectively, 50%, 70%, 80% and 95% alcohol dip the sample.
7. Use the PAP Orange G-6 solution to stain for a minute.
8. Use 95% alcohol present in two jars to rinse the sample.
9. Use PAP EA-36 to stain for a maximum of two minutes.
10. In different three jars containing 95% alcohol, rinse for about five to ten times.
11. Use absolute alcohol to rinse the sample before transferring to an equal part mixture of xylene and absolute alcohol, then allow the sample to dry.
12. Use a Mounting medium such as DPX to mount the sample.
13. Use a Microscope to observe and then record the results.

Interpretation of Results

Nuclei: Blue

Keratin: Orange-red

Eosinophil: Orange Red

Intermediate & Parabasal Cells: Blue Green

Superficial cells: Pink

Basophilic cells: Blue Green

Cytoplasm: Pink to pale pink

Candida: Red

Acidophilic cells: Red

Trichomonas: Grey green

Erythrocytes: Orange-red

Appendix IV: The Bethesda System for Reporting Cervical Cytology (2014)

SPECIMEN TYPE:
Indicate conventional smear (Pap smear) vs. liquid-based preparation vs. other

SPECIMEN ADEQUACY

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

GENERAL CATEGORIZATION (optional)

- Negative for Intraepithelial Lesion or Malignancy
- Other: See Interpretation/Result (*e.g., endometrial cells in a woman ≥45 years of age*)
- Epithelial Cell Abnormality: See Interpretation/Result (*specify* 'squamous' *or* 'glandular' *as appropriate*)

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY
(*When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report--whether or not there are organisms or other non-neoplastic findings*)

NON-NEOPLASTIC FINDINGS (*optional to report optional to report; list not inclusive*)

- Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes

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

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

OTHER

- Endometrial cells (*in a woman ≥45 years of age*)
  (*Specify if "negative for squamous intraepithelial lesion"*)

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

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

GLANDULAR CELL

- Atypical
  - endocervical cells (NOS *or specify in comments*)
  - endometrial cells (NOS *or specify in comments*)
  - glandular cells (NOS *or specify in comments*)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic

- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: *(specify)*

ADJUNCTIVE TESTING

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

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY

If case examined by an automated device, specify device and result.

EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (*optional*)

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

Appendix V: High Risk Human Papilloma Virus Real Time PCR Testing

Introduction

Infection of the genitals by HPV is considered to be a very common disease globally that is transmitted sexually. It accounts for over 40% of all viral STDS globally affecting mostly women who are under the reproductive age group. Cancer of the cervix is ranked the fourth topmost identified cancer and number four leading cause of cancer mortalities among women. The aetiology is closely linked with persistence infection with oncogenic strains of human papillomavirus (HPV). There are various genotypes of HPV which are placed into three categories depending on their risk to cause severe disease. These are; high risk which encompasses, ("16, 18, 31 and 45"), Intermediate which includes ("33, 35, 39, 51, 52, 56, 58, 59, and 68"), and Low-risk encompassing (6, 11, 42-44). Detection of HPV via PCR has offered a specific and more sensitive way of screening and quantification of the virus.

Purpose of the test

This is an in vitro test for detecting HPV 16 & 18 quantitatively in swabs collected from the urogenital areas. Packaged in the kit is an internal control "human beta-globin gene" which minimizes the possibilities of false negatives by way of controlling any possible cellular materials present in the collected sample.

Principle of the test

The test involves two processes; starting with DNA isolation followed by amplification which is done real time hence the name "real time". In cases where there is poor sample collection caused by excess mucous or not enough cells, detection of the internal control is not possible. Contained in the PCR mix-one tube are primers against HPV A7 and A9 regions for HPV 16 and 18. Included in the same tube is also a Beta-globin gene which plays the role of an internal control. It is in the Green/Fam channel where detection of results for amplification of genotype 16 DNA occurs while that of genotype 18 is detected in the Orange/Rox channel. Amplification for the internal control gene is detected in the HEX/Yellow/Joe channel. Amplification results of HPV 16 DNA, HPV 18 DNA, and β-globin gene used as Internal Control are detected on the Fam/Green, the Rox/Orange, and on the Joe/HEX/Yellow channels, respectively. The test contains standards with HPV known concentrations which allows for the determination of viral loads.

Materials and Reagents

i. Biological safety cabinet

ii. Micro centrifuge and Eppendorf tubes

iii. Non powdered gloves

iv. 65 ± 2°C heat block

v. Real time thermal cycler with 5 amplification channels

vi. Pipettes and sterile RNAse free pipette tips

vii. Lysis solution, washing solution and DNA eluent.

viii. Taq DNA polymerase, PCR mix1 and mix 2 buffer

ix. Negative control and QS HPV C1 and QS HPV C2(positive controls)

Safety/Risk Assessment (Environmental and Safety Controls)

i. Personal protective equipment (PPEs), including gloves, coats, and eye protector shields, should be used. Hands should be washed thoroughly.

ii. All specimens should be handled in a safety cabinet and always considered to be infectious. Any spill should be thoroughly washed using hypochlorite solution.

iii. Extracted materials including controls, samples and amplicons should be stored separated and should not come in touch with the other reagents

iv. The following activities/behaviors are barred from the working sections of the laboratory: consuming food or drinking, application of cosmetics, smoking, and handling of contact lenses.

v. It is important to ensure that there is one-direction for the laboratory process beginning from the area where extraction is done then to amplification and finally to the detection area. Samples, reagents and equipment should not be returned backward where previous step was done.

vi. All components should be thawed thoroughly before and assay is started and centrifuging done after thawing.

Sample Collection Storage and Transport

- The cervical cytology samples are collected using a cytobrush the same way for Pap smear test
- The brush is then broken and put in the collection container containing the MLBC formulated fixative solution and
- After the samples are processed and extraction of cells for cytological screening, the remnant cells are stored at -80^{o} C waiting HPV DNA testing.
- The samples are transported to the molecular laboratory while maintaining the cold chain.

Procedures

DNA Isolation

i. Bring the lysis and wash solution to room temperature until disappearance of crystals, if previously kept at 2-8°C.

ii. Prepare the required amount of 1.5ml polypropene corresponding to the amount of samples and add a Negative Control.

iii. Add 300 microliters of the lyse solution followed by 20 microliters of the DNA sorbent and 100 µl of samples and controls.

iv. Vortexing and Incubation at a temperature of 65°C is done for 5 minutes.

v. Add 300 µl of absolute alcohol and vortex.

vi. Transfer the solution to a column using a micropipette tip with a barrier tip.

vii. Centrifuge at 8000g for 1 minute and discard the carrier tube of the column replacing it with another.

viii. Add 500 µl of wash solution and centrifuge at 13000g for 3 minutes. Repeat this process while discarding used carrier tubes and replacing with new ones to minimize inhibition of PCR or cross contamination.

ix. Re-suspend the column with 100 µl of DNA eluent and incubate at 65 °C for 5 minutes with caps open while vortexing periodically.

x. Centrifuge at 12000g for 1 minute and the carrier tube now contains the DNA material required for PCR. In cases where amplification is delayed to another day, storage of the samples should be done at temperature of 2 to 8^{o} C for a period not exceeding five days. If amplification will be done later after five days, the samples should be frozen at -20 or -80^{o}C.

PCR Preparation

i. Thaw PCR MIX 1 and 2 together with the Taq polymerase. This is followed by brief vortexing and centrifuging.

ii. For N amount of samples prepare the mastermix as follows;

(N x 10 µl)+1, (N x 5 µl) +1, (N x 0.5 µl) vortex and centrifuge briefly.

iii. Transfer 15 µl of mastermix on PCR tubes and add 10 µl of samples and controls.

iv. Load the samples to the amplifier and PCR reader (Qiagen Rotorgene) or and read the results.

A temperature profile is created as follows:

Phase/step	**Temperature in degree Celsius**	**Time in minutes/seconds**	**Detection of fluorescence**	**Number of repeats**
Hold	95	15 min	–	1
Cycling	95	15 sec	–	45
	60	30 sec	FAM/Green, JOE/Yellow, ROX/Orange	

Results Interpretation and Data Analysis

Interpretation of the results is done by software inbuilt in the instrument. This is based on crossing of the fluorescence curve with threshold line. Detection of the

internal control, genotype 16 and genotype 18 occurs on the Yellow/Hex/Joe, Green/FAM and Orange/ROX channels respectively. Notably, threshold line is not supposed to cross the baseline but rather the sigmoid curves of signal accumulation of positive samples only. The threshold should be set at level where fluorescence curves are linear and do not cross curves of the negative samples.

Diagnostic Characteristics of HPV 16/18 Real-TM Quant Kit

Samples type	**Diagnostic sensitivity[2], %**	**Diagnostic specificity[3], %**
Scraping of membrane mucosa of cervix uteri, endocervical Scraping	100	98
Swab of vaginal mucosa	100	94

Appendix VI: Publication One

International Research Journal of Oncology

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Prevalence and Patterns of Cervical Cytological Lesions among HIV-Positive Women in Machakos County Hospital Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MOM and KCM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors WGN and SGM managed the analyses of the study. Author TKK managed the literature searches and reporting of the slides. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Cervical cancer remains to be a major threat to health among women globally with highest incidences in the developing countries. Studies have showed that HIV-positive women are at higher risk of HPV infection which is the causative agent of cervical cancer. The aim of this study was to determine the prevalence of cervical cytological lesions among HIV infected women in Machakos county hospital Kenya.

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Study Design: Cross-sectional study.
Place and Duration of Study: Machakos County Hospital Comprehensive Care Centre and Machakos Cancer Care and Research Centre, between August 2020 and December 2020.
Methodology: A total of 400 women who were HIV-positive and attending the comprehensive care center at the facility were enrolled in this study. Cytological samples obtained using a cytobrush were processed using manual liquid based cytology technique. All smears were stained using the Papanicolaou staining method and examined microscopically for cervical cytological lesions. Chi-square test was performed to evaluate the association between cervical cytological lesions and the demographic variables.
Results: A total of 400 participants were enrolled in this study. 15 had unsatisfactory smears and thus excluded from analysis. The prevalence of cervical cytological lesions in this study was 30 out of 385 (7.8%). Of the cervical cytological lesions observed, Atypical Squamous Cells of Undetermined Significance (ASCUS) had the highest number of cases 9 (30%) while Atypical Squamous Cells cannot exclude High grade (ASC-H) had the least number of cases 2 (6.6%). Of all the demographic variables studied, none was found to have any statistical significant association with cervical cytological lesions.
Conclusion: The prevalence of cervical cytological lesions among HIV-positive women in this study was 7.8%. There was no statistical significance association between any of the demographic variables studied and cervical cytological lesions.

Keywords: Cervical cytological lesions; HIV-positive women; pap smear; cervical cancer.

1. INTRODUCTION

Cervical cancer remains to be a major threat to health among women globally with highest incidences been recorded in low and middle income countries. According to data by World Health Organization, cervical cancer was ranked the fourth most frequent cancer in women with an over 500,000 new cases in 2018 representing 6.6% of all female cancers. It was estimated that 90% of deaths from cervical cancer occurred in low and middle-income countries [1].

Precancerous cervical lesions and cervical cancer are considered to be more aggravated and progress rapidly in immunocompromised patients particularly those with HIV infection. Studies have indicated that HIV and HPV Persistence in immunosuppressed individuals pose increased risk of developing cervical dysplasia and invasive cervical carcinoma. Previous studies have indicated that women who are HIV positive are at increased risk of acquiring Human Papilloma Virus which is the causative agent of cervical cancer [2-3]. The natural history of HPV infection has a slow, 10–20 year progression to pre-cancer in immunocompetent women; however, women living with HIV progress more frequently and quickly to pre-cancer and cancer [4].

In the developing countries, cervical cancer rates are still high with the highest rates recorded in Eastern Africa. Kenya was ranked position 20 among the top 20 countries globally with the highest rates of cervical cancer in 2018 [1]. Current estimates indicate that every year 5250 women are diagnosed with cervical cancer in Kenya and 3286 die from the disease. Cervical cancer ranks as the second most frequent cancer among women in Kenya and the first most frequent cancer among women of the reproductive age [5].

2. MATERIALS AND METHODS

2.1 Study Site and Study Population

This study was carried out at Machakos county hospital at the Comprehensive Care Centre and Machakos Cancer Care and Research Centre. The population of the present study was composed of 400 HIV-positive women of 18 years and above attending Machakos county hospital Comprehensive Care Centre.

2.2 Data Collection and Laboratory Procedures

A total of four hundred HIV-positive women attending comprehensive care centre at the selected facility were recruited in the study between August - December 2020. Convenience sampling was used to recruit the study participants. A structured questionnaire was used to collect the demographic data including; age, marital status, history of tobacco smoking and

method of family planning used. Samples were collected using a cytobrush and the cytological material transferred with brushes into a formulated liquid fixative. Samples were then taken to Machakos Cancer care and research centre cytology laboratory and processed following manual liquid based cytology technique [6].

All the smears were stained using the Papanicolaou staining method. Screening of the Pap smears was done by the principal investigator followed by a review by a certified Clinical cytologist. All abnormal smears were reviewed by a board certified pathologist. The Bethesda system 2014 for reporting cervical cytology was used for reporting all the cytological abnormalities observed during examination and reporting.

2.3 Statistical Analysis

Statistical analysis was performed with the statistical package for the social science system (SPSS version 18). Variables were presented as absolute numbers and percentages. Nominal categorical data between the groups was compared using Chi-squared test as appropriate with a *P*-value < 0.05 considered statistically significant at 95% confidence interval.

3. RESULTS

A total of 400 women were enrolled in this study. Out of the 400 participants, 355 (88.8%) were Negative for intraepithelial lesion or malignancy, 30 (7.5%) had abnormal Pap smear findings while 15 (3.8) had unsatisfactory smears for evaluation as shown in Table 1.

The 15 participants whose smears were unsatisfactory for evaluation were excluded from the analysis in calculating the prevalence. The prevalence of cervical cytology lesions in this study was 30 out of 385 (7.8%) as shown in Fig. 1.

Of those with abnormal Pap smear findings, 9 (30%) women had Atypical Squamous Cells of Undetermined Significance (ASCUS), 8 (26.7%) had High Grade Squamous Intraepithelial lesion (HSIL), 6 (20%) had Low Grade Squamous Intraepithelial Lesion (LSIL), 5 (16.7%) had Atypical Glandular Cells (AGC) and 2 (6.6%) had Atypical Squamous Cells cannot exclude HSIL (ASC-H). Figure 2 shows the Bethesda classification of the cervical cytological lesions.

Table 1. Pap smear findings

Pap smear findings	Frequency	Percent
NILM	355	88.8
Abnormal Pap smear	30	7.5
Unsatisfactory smear	15	3.8
Total	400	100

The age bracket 40-49 had the highest cases of abnormal cytological findings (n=15, 15%) while those aged 60 years and above had the smallest number (n=1, 3.3%). There was no statistical significant association between age and abnormal Pap smear findings (p value = 0.732). Table 2 presents a cross-tabulation of age vs cervical cytological lesions.

According to the marital status, single women had the highest number of abnormal Pap smear findings (n=9, 30%) while those divorced had the smallest number (n=1, 3.3%). Table 3 shows a cross-tabulation of marital status vs cervical cytological lesions.

Out of the 400 participants included in this study, 9 (2.3%) had history of tobacco smoking while 391 (97.7%) didn't have any history of tobacco smoking in their life time. There was no statistical significant association between tobacco smoking and cervical cytological lesions (P value = 0.106). Table 4 shows a cross-tabulation of tobacco smoking vs cervical cytological lesions.

In regards to the method of family planning, condom was the most common used method (n=179, 44.8%) while Intra uterine contraceptive device (IUCD) was the least used (n=14, 3.5%) as shown in Table 5. Of all the factors studied, none was found to have any statistical significant association with abnormal Pap smear findings.

4. DISCUSSION

The current study has shown that the prevalence of cervical cytological lesions among HIV positive women in Machakos county hospital, Kenya is 7.8% which is comparable to 6% as documented in a study done by Ononogbu et al in Nigeria [7]. This prevalence is also comparable to 6.3% documented in another study done in Northern Uganda in 2016 [8]. The prevalence documented in the current study is slightly higher than 4.3% as documented in a previous study done by Mutuku et al. in 2017 [9].

However, this prevalence is lower than the one documented in previous studies conducted in

Kenya and Africa. In a study done by Memiah et al to determine the prevalence and risk factors associated with precancerous cervical cancer lesions among HIV-infected women, the prevalence of abnormal cervical cytology was documented to be 26.7% [10]. In another study by McKenzie et al, the prevalence of cervical squamous intraepithelial lesions among HIV-positive women on antiretroviral therapy in Kenya was found to be 46% [11].

In a study done in Rwanda by Kayumba et al, the prevalence of abnormal cervical cytology among HIV positive women was found to be 20% [12]. A study done in Southern Ethiopia to determine the prevalence of precancerous cervical cancer lesion among HIV-infected women documented a prevalence of 22.1% [13]. In another study by Liu et al, the prevalence of cervical squamous intraepithelial lesions among HIV-infected women in Dar es Salaam, Tanzania was reported to be 8.7% [14]. In other studies done in North-Central Nigeria, Swaziland and South Africa, the prevalence of abnormal cervical cytology among HIV positive women was found to be 12.2%, 22.9% and 19.6% respectively [15-17]. Early initiation and adherence to antiretroviral therapy may have contributed to the low prevalence of abnormal cervical cytology recorded in this study as all women enrolled in this study were on antiretroviral therapy.

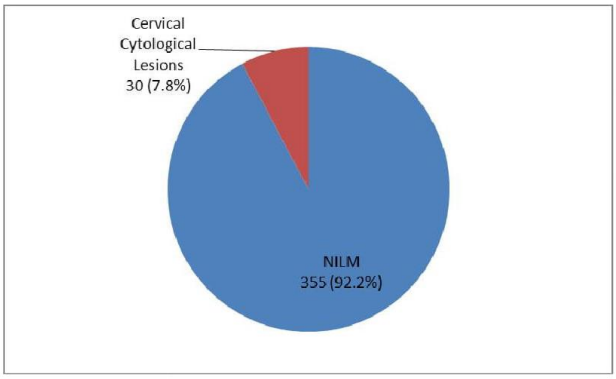

Fig. 1. Prevalence of cervical cytological lesions

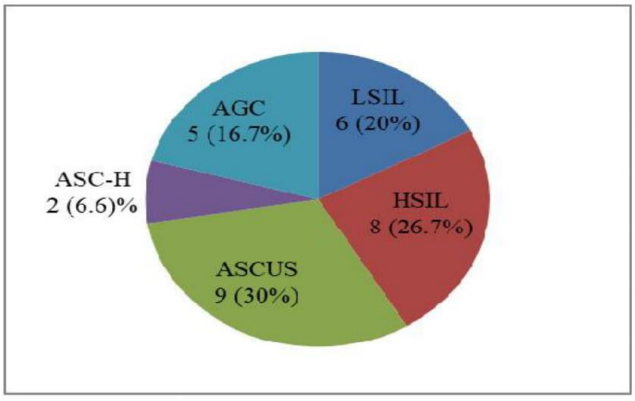

Fig. 2. Bethesda classification of the cervical cytological lesions

Table 2. Comparison of age and pap smear findings

	Pap smear findings				
Age (years)	**Total number (%)**	**NILM (%)**	**Abnormal smear (%)**	**Unsatisfactory smear (%)**	***P-value***
20-29	32(8)	29 (8.2)	2(6.7)	1(6.7)	0.732
30-39	111 (27.8)	102 (28.7)	7 (23.3)	2 (13.3)	
40-49	148 (37)	126 (35.5)	15 (50)	7 (46.7)	
50-59	98 (24.5)	88 (24.8)	5 (16.7)	5 (33.3)	
60 and above	11 (2.8)	10 (2.8)	1 (3.3)	0 (0)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	

Table 3. Comparison of Marital status and Pap smear findings

	Pap smear findings				
Marital Status	**Total number (%)**	**NILM (%)**	**Abnormal smear (%)**	**Unsatisfactory smear (%)**	***P-value***
Single	100 (25)	90 (25.4)	9 (30)	1 (6.7)	0.408
Married	197 (49.3)	170 (47.9)	16 (53.3)	11 (73.3)	
Divorced	39 (9.8)	37 (10.4)	1 (3.3)	1 (6.7)	
Widowed	64 (16)	58 (16.3)	4 (13.3)	2 (13.3)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	

Table 4. Comparison of Tobacco Smoking and Pap smear findings

	Pap smear findings				
History of Tobacco smoking	**Total number (%)**	**NILM (%)**	**Abnormal smear(%)**	**Unsatisfactory smear (%)**	***P-value***
Yes	9 (2.3)	6 (1.7)	2 (6.7)	1 (6.7)	0.106
No	391 (97.7)	349 (98.3)	28 (93.3)	14 (93.3)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	

Table 5. Comparison of Family planning Methods and Pap smear findings

	Pap smear findings				
Family Planning Method	**Total number (%)**	**NILM (%)**	**Abnormal smear (%)**	**Unsatisfactory smear (%)**	***P-value***
Natural	68 (17)	61 (17.2)	4 (13.3)	3 (20)	0.921
Condom	179 (44.8)	158 (44.5)	15 (50)	6 (40)	
Injection	70 (17.5)	61 (17.2)	6 (20)	3 (20)	
Pill	30 (7.5)	26 (7.3)	2 (6.7)	2 (13.3)	
IUCD	14 (3.5)	13 (3.7)	0 (0)	1 (6.7)	
Implant	39 (9.7)	36 (10.1)	3 (10)	0 (0)	
Total	400 (100)	355 (100)	30 (100)	15 (100)	

In the current study, age was found not to have any statistical significant association with the prevalence of cervical cytological lesions. This agrees with other previous studies which found age not to have any statistically significant association with abnormal cervical cytology [9,12,13,16]. However, the age bracket 40-49 had the highest cases of abnormal Pap smear findings in this study.

In this study, there was no statistical significant association between method of family planning and abnormal cervical cytology. This contradicts with another study done in Australia which documented that prolonged use of hormonal contraceptive was associated with high risk of developing cervical cancer [18]. McKenzie et al in his study showed that less regular condom use was associated with increased chances of squamous intraepithelial lesions [11]. In a study by Roura et al, use of oral contraceptives for over 15 years was associated with increased risk of developing cervical intraepithelial lesions [19]. In another study by Loopik et al, women who used intrauterine device had an increased risk of developing cervical intraepithelial neoplasia than those who used oral contraceptives [20].

The current study has showed that tobacco smoking did not have any statistical significance association with abnormal Pap smear findings. This contradicts previous studies which have indicated that prolonged cigarette smoking contributed to development of cervical lesions [21-22]. Matsumoto et al showed that prolonged tobacco smoking interferes with regression of cervical precursor lesions and thus increasing the risk of persistent cervical abnormalities among young women [23]. In a study by Jiang et al, there was increased risk of deaths from cervical cancer among women who were smokers compared to non-smokers [24]. However, in this study, only a small number of women (n=9) were identified as smokers and this could be the reason as to why we were not able to find any association between tobacco smoking and abnormal cervical cytology.

5. CONCLUSION

In this study, we have shown that the prevalence of cervical cytological lesions among HIV positive women visiting the comprehensive care center at Machakos county hospital was 7.8%. Of the demographic variables studied in this study; age, marital status, tobacco smoking and family planning methods, none was found to have any statistical significant association with cervical cytological lesions.

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We are grateful to Machakos County Hospital CCC Staff led by Peter Mukenya and Andrew Mului for their support during collection of Pap smear samples. We wish also to thank the medical laboratory officers at the Machakos cancer care and research centre cytology laboratory; specifically Simon Gachau and Lawrence Kavivya for helping us in staining the cytological samples.

CONSENT AND ETHICAL

The study was approved by the Kenyatta University Research and Ethical Review Committee (Protocol Number: PKU/2066/I1213). Witten informed consent was sought from all patients who agreed to participate in this study before obtaining samples for screening. All procedures were explained to the patients and clarifications made in a language they could understand.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Appendix VII: Publication Two

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Prevalence of Bacterial Vaginosis among HIV-Positive Women in Machakos County Hospital, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MOM and KCM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WGN and SGM managed the analyses of the study. Author TKK managed the literature searches and reporting of the slides. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Bacterial vaginosis (BV) is a common infection in women during their reproductive age. However, the burden of BV among HIV-positive women in Kenya is not clear. This study aimed to determine the prevalence of BV in HIV-positive women visiting the comprehensive care center in Machakos county hospital, Kenya.
Study Design: Cross-sectional study.

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Place and Duration of Study: Machakos County Hospital Comprehensive Care Centre and Machakos Cancer Care and Research Centre, between August 2020 and December 2020.
Methodology: We conducted a cross-sectional study at Machakos county hospital in Kenya by enrolling 400 women who were HIV-positive and attending the comprehensive care center at the facility. Convenience sampling was used to select the study participants. Cytological samples obtained using a cytobrush were processed using manual liquid based cytology technique. All smears were stained using the Papanicolaou staining method and examined microscopically for the presence of clue cells. A Chi-square test was performed to evaluate the association between BV and the demographic variables.
Results: Out of the 400 participants enrolled in this study, 41 (10.3%) had BV. The age groups 30-39 years (n=14, 34.1%) and 40-49 years (n=14, 34.1%) had the highest BV burden. Based on marital status, married women had the highest cases of BV (n=18, 43.9%) while divorced women had the least cases of BV (n=4, 9.8%). Women who used condom as the method of family planning showed to have the highest cases of BV (n=18, 43.9%). Of all the variables studied, only age had a statistically significant association with BV (*P*=0.002).
Conclusion: The prevalence of BV among HIV-positive women in Machakos county hospital, Kenya in this study was 10.3%. Age and BV were significantly associated.

Keywords: Age; bacterial vaginosis; cervical cytology; HIV-positive women; pap smear.

1. INTRODUCTION

Bacterial vaginosis (BV) is a prevalent vaginal infection, particularly among women of childbearing age, and is associated with a myriad of health problems. The prevalence of BV varies across countries and regions. A recent systematic review reported a global burden of 23%-29% among the general population, with Europe and Central Asia and South Asia reporting the highest and lowest prevalence, respectively [1]. However, the burden of BV may also differ according to the population's characteristics such as race, hospitalization, number of sexual partners, pregnancy, and marital status [1,2]. It arises when there is a decline in the count of helpful bacteria such as lactobacilli and an overgrowth of harmful bacteria, including Enterobacteriaceae, *Streptococcus spp*, *Veillonella spp*, *Bacteroides fragilis*, *Ureaplasma urealyticum*, and *Gardnerella vaginalis* [3]. The harmful bacteria are opportunistic microbes that grow singly or in combination with other organisms.

BV is a non-inflammatory condition and therefore in most cases lacks the typical symptoms such as vaginal discharge, odor, irritation, itching, erythema and dysuria [4]. According to the Centers for Disease Control and Prevention (CDC), about 84% of the patients with BV do not report any symptoms [5]; hence, the infection may be undetected, exposing the patients to health complications. Despite the absence of symptoms in most patients with BV, pregnant women are exposed to a high risk of endometritis and low birth weight [6]. These complications adversely affect the wellbeing and safety of both the mother and the neonate. Besides, BV has been linked with a high risk of pelvic inflammatory disease that leads to chronic pelvic pain and infertility [6,7]. Patients with BV are also vulnerable to sexually transmitted diseases and Human Immunodeficiency Virus (HIV) [6]. Due to the absence and non-specificity of symptoms, many BV cases are undiagnosed, hindering the initiation of timely treatment and raising the risk of adverse health events.

Women who visit gynecologic clinics are likely to be tested for BV, especially when undergoing other examinations such as Pap smear to diagnose cervical cancer [8]. These patients may be undergoing routine screening or may have genital complaints. Notably, a recent systematic review and meta-analysis have found a significant positive association between BV and cervical cancer [8]. A disturbance in the vaginal flora has been linked to cervical cytological abnormalities [9]. Therefore, during cervical cancer screening, concurrent testing for BV is performed to inform the clinical decision-making of the healthcare provider. In Kenya, research on the diagnosis of BV during cervical cytological examination is limited. In this study, we aimed to determine the prevalence of BV among women undergoing cervical cancer screening using a liquid-based cytological technique.

2. MATERIALS AND METHODS

2.1 Study Population

We conducted a cross-sectional study at Machakos county hospital in Kenya and included HIV-positive women who were sexually active and attending the comprehensive care center at the facility. Convenience sampling was used to select the study participants. Women who were pregnant or declined to complete an informed consent form were excluded from the study.

2.2 Data Collection and Laboratory Procedures

A questionnaire was used to collect demographic data, including participants' sexual and reproductive history. A qualified clinician collected a cervical cytology sample using a cytobrush and the cytological material was transferred with brushes into a formulated liquid fixative. Samples were then taken to Machakos cancer care and research centre cytology laboratory and processed following manual liquid-based cytology technique [10].

All the smears were stained using the Papanicolaou method and examined microscopically for the presence of clue cells. All smears were examined by the principal investigator and reviewed by a qualified experienced clinical cytologist.

2.3 Statistical Analysis

All statistical analyses were performed using the statistical package for the social science system (SPSS version 18). We performed a Chi-square test to determine association between BV and the demographic variables studied. A P-value <0.05 was considered statistically significant.

3. RESULTS

A total of 400 participants were enrolled in the study. Of the 400 participants, 41 (10.3%) had BV as shown in Fig. 1. Table 1 presents a cross-tabulation of age vs BV. The age range of the participants was 21 – 74 years. The highest cases of BV were observed in the age groups (30-39) years (34.1%) and (40-49) years (34.1%) while among those aged 60 years and above there was no any case of BV. Age was statistically associated with BV (*P*=0.002).

Table 2 shows a cross-tabulation of marital status, Education, and Family planning methods vs BV. Married women had the highest cases of BV (43.9%) while divorced women had the least cases (9.8%). There was no statistical significant association between marital status and BV (*P*=0.53). Those with secondary education as the maximum qualification had the highest cases of BV (61.0%) while those who had never gone to school had the least cases of BV (2.4%). Education level had no statistical association with BV cases (*P*=0.12). Based on the family planning method, women who used condoms had the highest cases of BV (43.9%) followed by injection (19.5%) with those using IUCD having zero cases of BV.

4. DISCUSSION

In this study, we have shown that the prevalence of BV among HIV-positive women in Machakos county hospital, Kenya was 10.3%. This prevalence is lower than that of India which was 20.9% in a study done in 2020 [11]. Another study conducted in the United States found a 17.3% prevalence of BV among HIV-positive women [12]. This variation between our research and other studies could be due to methodological differences since we relied on cervical specimens only, while the other two studies used both cervical and vaginal specimens. In our study, BV diagnosis was based on observation of clue cells only, but this approach is considered insufficient [13].

An accurate diagnosis of BV should be based on a combination of tests, including clinical manifestation (Amsel Criteria); Gram stain (Nugent score); wet mount microscopy and polymerase chain reactions (PCR) [13]. According to Amsel criteria, BV should be considered present if the patient presents with three of the following conditions: white, thin, and homogenous vaginal discharge; vaginal fluid pH >4.5; fishy odor of the vaginal discharge; and clue cells [14,15]. The Nugent score is obtained by Gram staining the vaginal smear to evaluate the dominant bacteria based on morphology [13]. Following diagnostic advancement, PCR can identify and quantify different bacteria present in the vaginal samples [13]. In another study, when gram stain was taken as the standard diagnostic method, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic value of Pap smear for BV diagnosis were 43.1%, 93.6%, 73.8%, 79.8%, and 78.8%, respectively [16]. Due to its low sensitivity, Pap smear may not be the ideal test for screening of BV.

In the present study, we have reported a statistically significant association between age and BV with the highest cases observed among women aged 30-49 years. Our findings are consistent with those of Ranjit et al., who found a higher prevalence of BV in women aged 30-40 years and lower prevalence in those aged 10-20 years and 50-60 years in Nepal [4]. However, in the study by Ranjit et al., the association between BV and age was not statistically significant [4]. Studies by Kamga et al. in Cameroon [17] and Ibrahim et al. in Nigeria [18] found a higher prevalence of BV in those aged 18-24 years. The age bracket 18-49 years corresponds to the reproductive age of women and is characterized by increased sexual activity.

The increased sexual activity, one of the transmission methods of BV, could also explain the high prevalence of BV among married women and the lower rate in divorced and widowed women in this study. Our findings are consistent with those of Gad et al., who also found a higher prevalence of BV among married women [19]. Frequent sexual intercourse disrupts the normal flora of the vagina and prevents its restoration [20]. Bacteria located in the perianal area are likely to be translocated to the vagina [20]. Cases of BV in sexually inactive women and virgin girls have been reported before [21,22]. Therefore, sexual activity is not the only cause of BV as other factors such as improper menstrual hygiene, tight clothing, food habits, and lifestyle changes can alter the vaginal flora [21,22].

Moreover, the family planning method has been linked to the risk of BV. While condom use is considered protective against BV due to the physical barrier provided [23], the prevalence of BV was higher in women who used condoms than other contraceptive methods. However, the results for contraceptives use in our study were not statistically significant. Studies have shown that the effectiveness of condoms depends on consistent use [23]. In our study, we did not evaluate the consistency of condom use, and this affects the accuracy of our findings. Women who used pills, implants, and injections had the lowest prevalence of BV. Singh et al. [24] and Vodstrcil et al. [25] associate the low prevalence of BV among women who use the aforementioned contraceptives with the protective effects of hormones against bacterial overgrowth in the vagina.

Another factor that is likely to influence the occurrence of BV is literacy level. Low literacy is associated with a higher likelihood of getting BV due to poor knowledge about preventive measures [4]. In our study, the educational level had no significant association with BV, and those with secondary educational levels had the highest burden of BV. The effect of education could have been modified by other factors such as sexual activity, access to healthcare, and age.

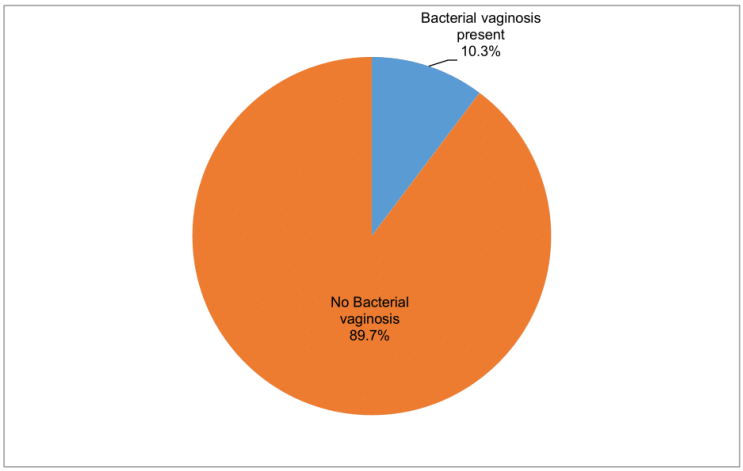

Fig. 1. Prevalence of bacterial vaginosis

Table 1. Comparison of age and cases of bacterial vaginosis

Age (years)	Bacterial Vaginosis			
	Total number (%)	**Positive number n=41 (%)**	**Negative number n=359(%)**	***P-value***
20-29	32(8)	9 (22.0)	23 (6.4)	0.002
30-39	111 (27.8)	14 (34.1)	97 (27.0)	
40-49	148 (37)	14 (34.1)	134 (37.3)	
50-59	98 (24.5)	4 (9.8)	94 (26.2)	
60 and above	11 (2.8)	0 (0)	11 (3.1)	
Total number	400 (100)	41 (100)	359 (100)	

Table 2. Comparison of marital status, education, family planning method and cases of bacterial vaginosis

Marital Status	Bacterial Vaginosis			
	Total number (%)	**Positive number n=41 (%)**	**Negative number n=359 (%)**	***P-value***
Single	100 (25.0)	14 (34.1)	86 (24.0)	0.531
Married	197 (49.3)	18 (43.9)	179 (49.9)	
Divorced	39 (9.8)	4 (9.8)	35 (9.7)	
Widowed	64 (16.0)	5 (12.2)	59 (16.4)	
Total number	400 (100.0)	41 (100.0)	359 (100.0)	
Education	**Total number (%)**	**Positive number n=41 (%)**	**Negative number n=359 (%)**	***P-value***
Primary	158 (39.5)	9 (22.0)	149 (41.5)	0.116
Secondary	188 (47.0)	25 (61.0)	163 (45.4)	
College/University	47 (11.8)	6 (14.6)	41 (11.4)	
Never gone to school	7 (1.7)	1 (2.4)	6 (1.7)	
Total number	400 (100.0)	41 (100.0)	359 (100.0)	
Family Planning Method	**Total number (%)**	**Positive number n=41 (%)**	**Negative number n=359 (%)**	***P-value***
Natural	68 (17.0)	6 (14.6)	62 (17.3)	0.784
Condom	179 (44.8)	18 (43.9)	161 (44.9)	
Injection	70 (17.5)	8 (19.5)	62 (17.3)	
Pill	30 (7.5)	4 (9.8)	26 (7.2)	
IUCD	14 (3.5)	0 (0)	14 (3.9)	
Implant	39 (9.7)	5 (12.2)	34 (9.5)	
Total number	400 (100.0)	41 (100.0)	359 (100.0)	

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/66112

Appendix VIII: Ethical Approval Letter

Kenyatta University
P.O Box 43844-00100
Nairobi-Kenya

REF: KU/ERC/APPROVAL/VOL1/1

Date: 2nd March, 2020

Onesmus Muia Mutuku
P.0 Box 43844-00100
NAIROBI

Dear Mr. Mutuku,

RE: APPLICATION NUMBER: PKU/2066/I1213 VALIDATION OF MANUAL LIQUID BASED CYTOLOGY AND DETECTION OF HIGH RISK HUMAN PAPILOMMA VIRUS IN RESIDUAL SAMPLES FROM HIV POSITIVE WOMEN AT MACHAKOS COUNTY HOSPITAL KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2066/I1213.** The approval period is ***2nd March, 2020 – 2nd March, 2021.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Prof. Judith Kimiywe

CHAIRPERSON- KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.

Appendix IX: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **483165**

Date of Issue: **25/March/2020**

RESEARCH LICENSE

This is to Certify that Mr.. Onesmus Muia Mutuku of Kenyatta University, has been licensed to conduct research in Machakos on the topic: VALIDATION OF MANUAL LIQUID BASED CYTOLOGY AND DETECTION OF HIGH RISK HUMAN PAPILOMMA VIRUS IN RESIDUAL SAMPLES FROM HIV POSITIVE WOMEN AT MACHAKOS COUNTY HOSPITAL KENYA for the period ending : 25/March/2021.

License No: **NACOSTI/P/20/4328**

483165

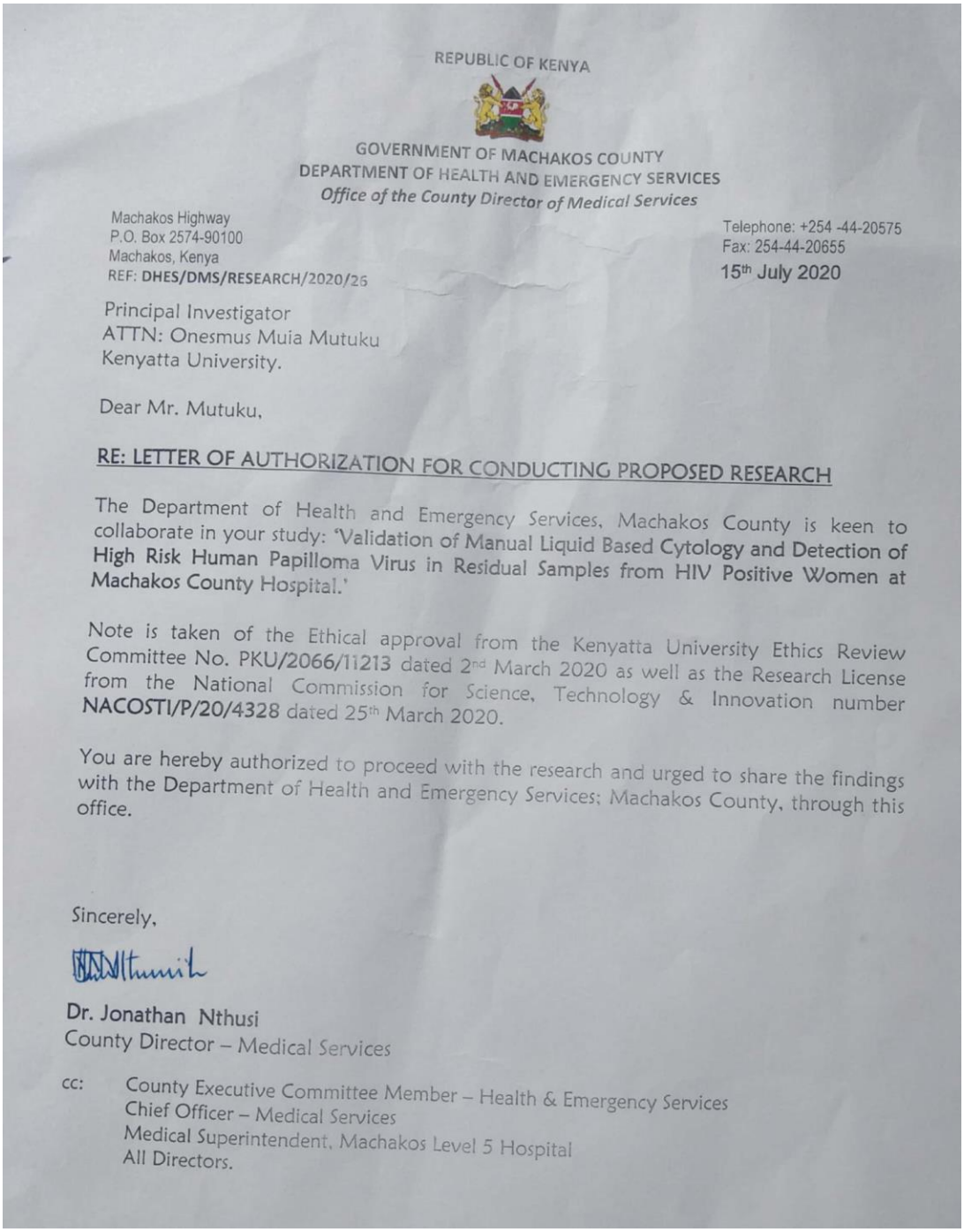
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix X: Machakos County Hospital Authorization Letter



REPUBLIC OF KENYA

GOVERNMENT OF MACHAKOS COUNTY
DEPARTMENT OF HEALTH AND EMERGENCY SERVICES
Office of the County Director of Medical Services

Machakos Highway
P.O. Box 2574-90100
Machakos, Kenya
REF: **DHES/DMS/RESEARCH/2020/26**

Telephone: +254 -44-20575
Fax: 254-44-20655
15th July 2020

Principal Investigator
ATTN: Onesmus Muia Mutuku
Kenyatta University.

Dear Mr. Mutuku,

RE: LETTER OF AUTHORIZATION FOR CONDUCTING PROPOSED RESEARCH

The Department of Health and Emergency Services, Machakos County is keen to collaborate in your study: **'Validation of Manual Liquid Based Cytology and Detection of High Risk Human Papilloma Virus in Residual Samples from HIV Positive Women at Machakos County Hospital.'**

Note is taken of the Ethical approval from the Kenyatta University Ethics Review Committee No. **PKU/2066/11213** dated 2nd March 2020 as well as the Research License from the National Commission for Science, Technology & Innovation number **NACOSTI/P/20/4328** dated 25th March 2020.

You are hereby authorized to proceed with the research and urged to share the findings with the Department of Health and Emergency Services; Machakos County, through this office.

Sincerely,

Dr. Jonathan Nthusi
County Director – Medical Services

cc: County Executive Committee Member – Health & Emergency Services
Chief Officer – Medical Services
Medical Superintendent, Machakos Level 5 Hospital
All Directors.

Appendix XI: A Map of Machakos County