THE UTILITY OF A MANUAL LIQUID BASED CYTOLOGY IN SCREENING FOR PRE-CANCEROUS LESION AND CERVICAL CANCER

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The utility of a Manual Liquid Based Cytology in Screening for Pre-cancerous Lesion and Cervical Cancer

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DECLARATION

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

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DEDICATION

I dedicate this work to my Parents Mr. and Mrs. Mutuku and my siblings; Stephen, Daniel and Gladys.

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LIST OF ABBREVIATIONS

AIDS:	Aquired Immuno Deficiency Syndrome
ACCP:	Alliance for Cervical Cancer Prevention
ACS:	American Cancer Society
AGC:	Atypical Glandular Cells
ALBC:	Automated Liquid Based Cytology
ASCUS:	Atypical Squamous Cells of Undetermined Significance
ASCH :	Atypical Squamous Cells cannot exclude HSIL
CCC:	Comprehensive Care Centre
CIN:	Cervical Intraepithelial Neoplasia
CIN:	Cervical Intraepithelial Neoplasia
CPS:	Conventional Pap Smear
DNA:	Deoxyribonucleic acid
HIV:	Human Immuno-deficiency Virus
HPV:	Human Papiloma Virus
HSIL:	High-grade squamous intraepithelial lesion
IARC:	International Agency for Research on Cancer
IDR:	Increased Detection Rate
LBC:	Liquid Based Cytology
LEEP:	Loop Electrosurgical Excision Procedure
LSIL:	Low Squamous Intraepithelial Lesions

MLBC:	Manual Liquid Based Cytology
NCCPP:	National Cervical Cancer Prevention Program
Pap:	Papanicolaou
SCC:	Squamous cell carcinoma
SCJ:	Squamocolumnar Junction
SIL:	Squamous Intraepithelial Lesion
VIA:	Visual Inspection with Acetic Acid
VILI:	Visual Inspection with Lugol's Iodine
WHO:	World Health Organization

OPERATIONAL DEFINITIONS OF TERMS

Carcinoma: A type of cancer that starts in the cells that make up the skin or the tissue lining of the internal organs.

Cervical Cancer: a type of cancer that occurs in the cells of the cervix, the lower part of the uterus that connects to the vagina. Various strains of the human papillomavirus (HPV), a sexually transmitted infection, play a role in causing most cervical cancer.

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

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

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

Dysplasia: The presence of cells of an abnormal type within a tissue, which may signify a stage preceding the development of cancer.

Hysterectomy: A surgical operation to remove all or part of the uterus.

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

Squamocolumnar Junction: A Junction between the stratified squamous epithelium of the ectocervix and the columnar epithelium of the endocervix where majority of cervical cancers and precancerous lesions occur

ABSTRACT

Liquid-based cytology is a technique that enables cells to be suspended in a liquid medium and spread in a monolayer, thereby enabling a better morphological assessment. Automated techniques have been widely used especially in the developed countries but limited in the developing countries due to cost and availability. Conventional Pap smear (CPS) examination has been the mainstay for early detection of cervical cancer. However, its widespread use has not been possible due to the inherent limitations, like presence of obscuring blood and inflammation, reducing its sensitivity considerably. On the other hand, manual liquid based cytology (MLBC) is a technique that is cost effective and improves detection of precursor lesions and specimen adequacy. The general objective of this study was to evaluate the utility of a manual liquid based cytology in screening for pre-cancerous lesion and cervical cancer. A prospective study of 295 cases was assessed for pre-cancerous lesions and cervical cancer using MLBC and CPS at Machakos County Hospital among women who were 18 years and above attending the Comprehensive Care Centre in the hospital. Convenience sampling was used to obtain the study population. Cohen Kappa test was run to determine the level of agreement between CPS and the MLBC results. There was moderate agreement between the two methods (k=0.673, 95% CI, p=0.065). Specimen adequacy was found to be better with MLBC than CPS with 12 unsatisfactory smears in MLBC and 22 in CPS. The prevalence of abnormal cervical cytology with Conventional Pap smear technique was 5.2%. There was increased detection rate of abnormal cervical cytology smears with MLBC of 85.7%. Manual liquid based cytology was found to give better results than the conventional Pap smear method in terms of specimen adequacy, clear back ground and reduced cellular overlapping with increased detection of abnormalities and preservation of specimen for future testing. Therefore, it can be used as an alternative liquid based cytology technique for cervical cancer screening in limited resource settings.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Cervical cancer is increasingly becoming a major threat to health among women in the world particularly in developing countries where screening programs are not well established. Worldwide, cancer of the cervix encompasses approximately 12% of all cancers in women (Siegel *et al*, 2015). It is the second most common cancer in women worldwide after breast cancer but the commonest in developing countries. In 2015, there were 270 000 deaths from cervical cancer worldwide with 90% of these deaths occurring in developing countries (WHO, 2015).

Cervical cancer can be prevented. Success in prevention reflects three factors: First, it takes time for cancer to develop from early cellular abnormalities, termed low-grade dysplasia, through more severe dysplasia, to carcinoma in situ and invasive cancer thus allowing time for detection; secondly, the associated cellular abnormalities can be identified; and thirdly effective treatment is available for precancerous lesions. Invasive squamous cell carcinoma of the cervix is therefore a highly preventable disease (Vesco *et al*., 2011).

Inspite of major success in cervical cancer screening in the developed world, cervical cancer mortality has remained quite high in the developing world (Sherris *et al*., 2001).

Given poor prognosis of cervical cancer diagnosed at late stages and the high cost related to treatment, early detection remains the most feasible way in improving long-term survival of cervical cancer patients. Cervical cancer is preventable if screened and diagnosed in its early stages (Mishra, Pimple & Shastri, 2011).

The introduction of cytologic screening for cervical cancer using the Papanicolaou (Pap) test in the 1950 has led to a reduction in the incidence of invasive cervical cancer in the developed countries. This has been attributed to the effective screening and treatment programs in these countries (Sankaranarayanan, Budhuk & Rajkumar, 2001). However, the establishment and implementation of Pap smear programs have not yet been possible in the developing countries and thus cancer of the cervix continues to threaten the lives of women from these countries up to date (Gatune & Nyamongo, 2005).

Several etiological associations and possible risk factors for cervical carcinoma have been identified. The main risk factors reported are early sexual debut, number of lifetime sexual partners, nonuse of condoms, and infection with human papillomavirus and cigarette smoking which facilitates development of cervical (Reid, 2001).

1.2 Statement of the Problem

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. Although conventional Pap smear (CPS) examination has been the mainstay for early detection of cervical cancer, its widespread use has not been possible due to the

inherent limitations which include: Majority of cells not captured as only a portion of the sample is smeared onto a microscope slide after collection. Furthermore, there is no representative transfer of the sample as the collection device is discarded, sometimes with more than 80% of the patient's sample still on the device. (Sherwani et al., 2007; Kavatkar *et al.,* 2008)

Another problem associated with conventional Pap smear is clumping and overlapping with more than one layer of cells formed leading to a poor visualization of the cells. The conventional Pap smear specimen may often be clouded with debris such as blood and mucus, which obscure cell visibility. 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 incase needed. These limitations have been shown to reduce the sensitivity of CPS to less than 50% (Sherwani et al., 2007; Kavatkar *et al.,* 2008).

An automated liquid-based cytology (ALBC) for cervical cancer screening was developed to improve the sensitivity, but the very high cost related to automated devices has hampered its implementation in the setup of developing countries, like Kenya.

1.3 Rationale of the study

A screening technology that matches the limited resources we have in Kenya will be of benefit both to physician and the patient. It is in this regard that the current study was done to develop and evaluate the utility of a manual liquid based cytology method in screening of pre-cancerous and cervical cancer. This study made use of locally available

reagents and equipment. The fixative and polymer solutions used in this study were formulated from reagents available in the setup of resource limited facilities such as the ones in Kenya and other developing countries.

Manual Liquid Based Cytology (MLBC) is a technique that enables cells to be suspended in a monolayer and thus improves detection of precursor lesions and improvement of specimen adequacy. Studies have shown that MLBC improves the effectiveness of cervical cancer screening in a population by increasing the detection of histologically confirmed neoplastic and pre-neoplastic disease while simultaneously decreasing over diagnosis of benign processes (Baker, 2002). Also, in case of MLBC, the residual sample can be used for other tests like detection of HPV, DNA and immunocytochemistry thereby increasing the utility of MLBC (Sherwani *et al.*, 2007; Kavatkar *et al.*, 2008). Thus a low cost manual liquid based cytology (MLBC) is a reliable method in detecting precancerous lesion and cervical cancer when compared to conventional cervical cytology and it is a cheap technology in terms of cost.

1.4 Research questions

I. Can a low cost manual liquid based cytology be used to make a cytodiagnosis for precancerous lesion and cervical cancer?

II. How does the cytodiagnosis of abnormal smears between manual liquid based cytology and conventional Pap smear compare?

III. What is the percentage agreement between the conventional Pap smear and the low cost manual liquid based cytology for abnormal smears?

1.5 Objectives of the study

1.5.1 General objective

To evaluate the utility of a low cost manual liquid based cytology in screening for pre-cancerous lesion and cervical cancer.

1.5.2 Specific objectives

i). To make cytodiagnosis using both the conventional Pap smear and the low cost manual liquid based cytology

ii). To compare the cytodiagnosis of abnormal smears between manual liquid based cytology and conventional Pap smear.

iii). To determine the percentage agreement between the conventional pap smear and the low cost manual liquid based cytology

1.6 Limitations of the study

In this study, cytology results were not correlated with histology which acts as the gold standard and thus the validity of both methods was not assessed. This was not done due limited financial resources.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cervical Cancer

Cervical cancer is a type of cancer that occurs in the cells of the cervix; located in the lower part of the uterus also called uterine cervix. The cervix is covered by both columnar and stratified non-keratinizing squamous epithelia. Cells in the columnar epithelium are constantly changing into squamous cells in an area of the cervix called the transformation zone. It is in this zone where abnormal cells are likely to develop (ACS, 2014)

In between the stratified squamous epithelium of the ectocervix and the columnar epithelium of the endocervix is a junction called squamocolumnar junction (SCJ) and it is in this region where majority of cervical cancers and precancerous lesions occur (Peel KR, 1995)

Cervical cancer is a malignant disease of the cervix usually occurring in the 5th or 6^{th} decade of life at a mean age of 54 years. The disease has a pre-malignant stage which usually occurs in younger women under the age of 40. It may form in the interior lining of the cervix, junction of the vagina and the uterus (Anorlu RI, 2006)

Cervical cancer begins to develop in the cells around the cervix. Pre-cancerous cells which are described as cervical intraepithelial neoplasia (CIN), squamous intraepithelial lesion (SIL) and dysplasia can fully grow into cancer. There are two main forms of

cervical cancer namely squamous cell carcinoma and adenocarcinoma, of these types 80% to 90% of the cervical cancers are due to the squamous cell carcinoma which begin where the exocervix joins the endocervix. Cervical adenocarcinoma develops from the mucus-producing gland cells of the endocervix (ACS, 2014).

In some cases, some of the cancers can be as a result of a combination of both squamous cell carcinoma and adenocarcinoma; the carcinoma is known as adenosquamous carcinoma or mixed carcinoma. In some women precancerous cells go away with no treatment whatsoever while others turn into true invasive cancers (ACS, 2014)

2.2 Epidemiology and risk factors of cervical cancer

Cervical cancer is a great threat to the health of women in the world, with nearly 500,000 new cases occurring each year worldwide (WHO, 2010). In 2012, it is estimated that 528,000 new cases occurred globally, with 266,000 of the women (50% of cases) dying (Torre *et a*l., 2015). This type of cancer is the second most common malignancy in women worldwide after breast cancer (WHO, 2007). Of the total new cases each year, about 86% occur in developing countries, where 80-90% of cervical cancer related deaths occur (NCCPP, 2012). This high incidence in the third world is attributed to inadequacy of screening programs as well as the unawareness of the disease in those countries. Cervical cancer is a major reproductive health problem for women particularly in the developing countries where screening services are lacking or inaccessible for the majority (Gatune & Nyamongo, 2005).

In Kenya, cervical cancer is the leading cause of cancer deaths in women of reproductive age. Each year a total of 4802 cervical cancer cases occur with 2451 cases of deaths. It is the first most common female cancer in women aged 15 to 44 years in Kenya (Bruni *et al*, 2017). It is projected that by the year 2025, the number of new cervical cancer cases annually in Kenya will reach 4261. Data from hospital-based registries in Kenya indicated that cancer of the cervix accounted for 70-80% of all cancers of the genital tract. It has been reported that there are 10 to 15 new cases of cervical cancer in Nairobi each week (NCCPP, 2012)

Cervical cancer is also recognized as an AIDS-defining illness in HIV infection and therefore a disease that affects the quality of life among the HIV positive women. The prevalence of HIV in invasive cervical cancer patients in Kenya is 15%. According to a study conducted among HIV-positive women attending HIV care clinics in Kenya, 43% of the women had abnormal cervical cytological results. The presence of abnormal cervical cytological results in HIV-positive women is also much higher than what is found in the general population and thus cervical cancer is a major cause of morbidity and mortality among HIV-positive women (NCCPP, 2012).

Several risk factors have been associated with cervical cancer. This include; Smoking, having multiple sex partners, immunosuppression, chlamydia infection, being overweight, long term use of contraceptives, Intrauterine device use, having a family history of cervical cancer and poverty (ACS, 2014)

2.3 Natural history and Etiology of cervical cancer

There is probably no one single cause of cervical cancer or pre-cancer. However, early epidemiological studies of cervical neoplasia suggested a causal relation with sexual activity and human papillomaviruses (HPVs) as prime suspects (Muñoz *et al*., 1988).

HPV plays a central role in the development of cervical cancer. HPVs fall into two broad categories: low risk types, associated with cervical condylomas and CIN 1; and high risk types (mostly 16 and 18), found in 50-80% of CIN 2 and CIN 3 lesions, and 90% of cancers. This association with cancer is very strong, with odds ratios of > 15 (often much higher) in case-control studies that are methodologically sound (Arends *et al*., 1990).

More than 70 strains or types of HPV have been classified. For instance, HPV Types 6 and 11 cause warts with other types having oncogenic properties. The best characterized types associated with cervical cancer are Types 16 and 18 (Schiffman M & Kjaer SK, 2003).

Many epidemiological studies have consistently shown that over 90% of cervical cancers can be attributed to specific HPV types. The small proportion of cancers apparently negative for HPV may be either false negatives, reflecting failure to detect new HPV types or very low levels of known HPV genomes, or true HPV negative cancers and thus comprise a separate group (Schiffman *et al*., 2007)

Transmission of HPV occurs through skin to skin or mucosa to mucosa contact during sex. If there are tears in the mucosa the HPV infecting viral particles penetrate the

epithelium and reach the germinal cells in the basal layer. The HPV infecting viral particles integrate into the host genome. Integration induces an overexpression of viral E6 and E7 oncoproteins. HPV DNA breakage occurs in the E2 gene region that leads to up regulation of E6 and E7. E6 interferes with host p53 gene function; E7 interferes with host tumor suppressor genes. Interference with p53 gene means there is no inhibition of apoptosis. E7 causes cell cycle inhibition. Without cell cycle regulation, tumor cells keep replicating and proliferating (Schiff *et al*., 2007)

The major steps known to be necessary in cervical carcinogenesis include HPV infection, HPV persistence over a certain period of time, progression to precancerous, and invasion. Backward steps are possible, including clearance of HPV infection and regression of precancerous lesions (Schiffman M & Kjaer SK, 2003) (Figure 2.1 below)

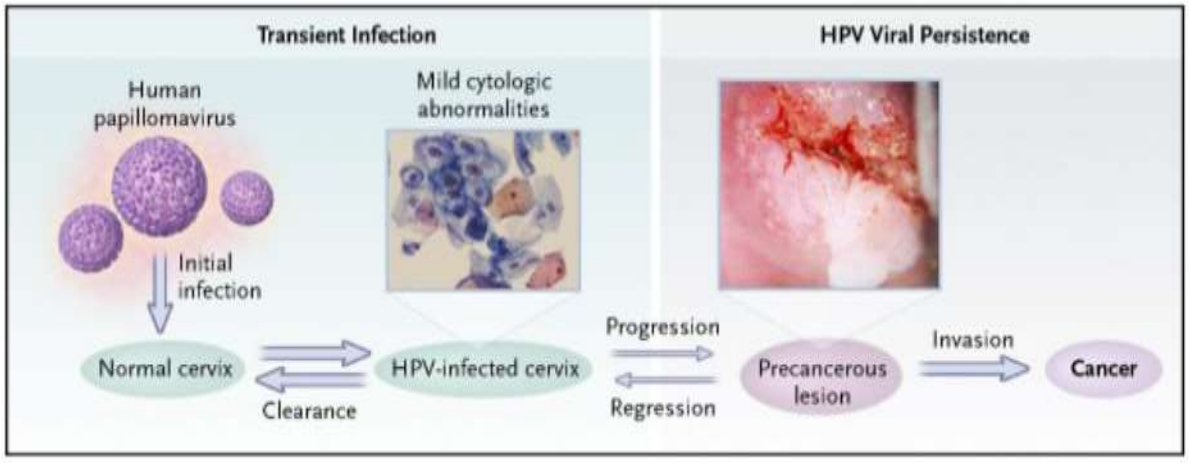

Fig. 2.1: Stages of cancer progression borrowed from *Wright & Schifmann NEJM 2003*

2.4 Pathogenesis

Cervical cancer is a progressive disease that moves from normal cervical epithelial cells to dysplasia to CIS to invasive cancer. It is now known that human papillomavirus (HPV) infection plays an essential role in the pathogenesis of cervical cancer (Arends *et al*., 1990).

The normal cervix is covered on its outer surface by a non-keratinizing, stratified squamous epithelium, which is continuous below with the squamous epithelium lining the vagina, and above the mucus secreting columnar epithelium lining the endocervical canal and its associated crypts (Arends *et al*., 1998)

The junction between the two epithelia normally coincides with the external os but this is not a constant relation. At puberty, in pregnancy (particularly the first one), and in some steroid contraceptive users, changes in the size and shape of the cervix result in the squamocolumnar junction being carried out on to the anatomical ectocervix. This exposes the tissues previously found in the lower endocervical canal to the vagina. This is a physiological process and the exposed tissue forms the "cervical ectopy" (Arends *et al*., 1998).

It is from the epithelium that covers the ectopy that most CINs and invasive carcinomas develop. Under physiological conditions, the columnar epithelium of the ectopy undergoes metaplasia to a stratified squamous epithelium, and it is during this metaplastic process that the epithelium seems to be particularly vulnerable to oncogenic viruses, and perhaps to other factors resulting in the development of an intraepithelial

neoplasm rather than a normal epithelium. The intraepithelial neoplasm may be of squamous or glandular cell type.

The target cells for an initial HPV infection are the immature basal cells of the epithelium, and HPV is thought to reach these cells through micro abrasions or cracks within the epithelium (Stoler *et al*., 1992)

Viral replication is tightly linked to the differentiation state of the virally infected epithelial cells. Replication of the HPV genome is tightly controlled by cellular mechanisms within the basal cells, and appears to be linked to cellular replication so that the viral DNA replicates with the host's genome (Durst *et al*., 1992)

The number of copies of the HPV genome, as a circular or episomal form, is thus low in the basal cell's nucleus, and virally encoded proteins are expressed at very low levels. As a result, HPV-infected basal cells show no specific cytologic or histologic changes and cannot be distinguished from uninfected cells. This stage of an HPV infection is referred to as a "latent" or "clinically unapparent" infection since the woman is HPV DNA positive, but no lesions can be detected, even by microscopy (Arends *et al*., 1998).

As HPV-infected epithelial cells differentiate and move upwards in the epithelium, viral transcription of the early region of the HPV genome dramatically increases (Stoler et al.., 1992)

The early region encodes for a number of proteins, including E1, E2, E6, and E7, which are important for viral replication. The HPV genome is relatively small (approximately 8000 base pairs) and does not encode the enzymes required for viral DNA replication. The only proteins provided by the viral genome that are directly involved in viral

replication are 2 regulatory proteins, E1 and E2, and HPV must therefore rely on the host's DNA replication machinery for viral DNA synthesis However, as the epithelial cells differentiate, the cellular DNA replication machinery is normally inactivated (Chow LT & Broker TR, 1994).

In order to undergo vegetative DNA amplification in the differentiating epithelial cells, the virus needs to reactivate the cellular DNA replication machinery. Studies of cultured human keratinocytes have shown that the viral E7 protein is capable of reactivating the cellular DNA replication machinery in differentiated cells (Munger K & Howley PM, 2002).

The viral E6 protein also appears to play an important role by blocking the apoptosis that would normally occur in the differentiated cells (Horner *et al*., 2004)

Together, these changes provide in the cell the synthetic phase environment necessary for vegetative viral DNA replication and complete virion formation. Infectious virus is eventually released as the differentiated cells are shed from the epithelium. In most women immunity develops against HPV after a period of months or years, and productive viral infection ceases. These women eventually become HPV DNA negative (Arends *et al*., 1998

In some HPV-infected women viral gene expression becomes unlinked to the state of differentiation of the infected epithelial cells, resulting in a change in the topography of viral gene expression within the epithelium (Durst *et al*., 1992)

One of the results of this other mode of viral infection is the dramatic increase in the expression of E6 and E7 HPV in the lower layers of the epithelium due to the proteins' deregulated expression (Stoler *et al*., 1992)

The deregulated expression of HPV proteins E6 and E7 results in the disruption of normal cell cycle regulation; abrogation of apoptosis mechanisms; and genetic instability. Genetic instability, which is a characteristic feature of most malignant neoplasms, occurs early in the development of precancers, thereby allowing for the stepwise acquisition of multiple mutations. Produced by alterations in the mitotic spindle apparatus, genetic instability permits aberrant mitotic events that can produce disequilibrium in the distribution of chromosomes, leading to changes in the number and structure of chromosomes. It can eventually produce a change in the overall DNA content, referred to as aneuploidy. Genetic instability is thought to play a critical role in the development of cervical cancer (Snijders *et al*., 2006)

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 method of cervical screening used to detect potentially pre-cancerous and cancerous cells in the endocervical canal (transformation zone) of the female reproductive system. It is considered by many to be the most cost effective cancer reduction program ever devised (Cibas & Ducatman, 2008).

The test was invented by and named after the prominent Greek Doctor George N. Papanicolaou and it aims to detect potentially pre-cancerous changes (called cervical intraepithelial neoplasia (CIN) or cervical dysplasia), which are caused by sexually transmitted human papilloma virus.

Cervical cytology consists of spreading and staining a smear of collected cervical cells and analyzing them under the microscope to detect lesions. This approach can contribute to early detection, thereby decreasing the incidence of advanced cervical cancer and associated mortality. Screening programs for cervical cancer using the Pap smear have been instituted in developed countries for decades and over a period of time have been shown to be effective in reducing the overall mortality from this disease. However, PAP smears are challenging to perform in developing countries because the process requires trained personnel and certified laboratories that are often unavailable (Mukakalisa *et al*., 2014)

Cervical cytology can be performed by use of either; the conventional Pap smear cytology or Liquid-based cervical cytology (Sherwani et al., 2007)

2.5.1.1 Conventional Pap smear cytology

This technique involves collection of a smear from the cervix and the endocervical canal by the use of an Ayres spatula and cytobrush. Samples taken are then smeared on a slide which is then fixed with cytology fixative. The slide must first be labelled with the woman's name or number. Each laboratory should have a written protocol specifying

what is considered adequate labelling and should not accept inadequately labelled specimens. The person collecting the specimen should ensure that a test requisition is accurately and legibly filled out before collecting the specimen (IARC 2004)

Good visualization of the cervix is important for obtaining an adequate specimen. Sterilized or single-use bivalve speculum of appropriate size is inserted into the vagina in such a manner as to allow complete visualization of the cervix and as much of the transformation zone as possible. Gentle removal of excess mucus and discharge from the cervix with a large cotton-tipped applicator can produce a better-quality smear (Kotaska & Matisic, 2003), but vigorous cleansing may remove many of the most easily exfoliated cells.

Before the specimen is collected, the cervix should be carefully inspected with the naked eye for grossly visible masses or ulcerations that may indicate an invasive cervical cancer. If a grossly visible lesion is identified, the woman should be referred for further confirmation. In many cases, the lesion can be directly sampled and the cellular sample obtained can be submitted separately for cytological assessment.

The procedure for collecting cells from the cervix varies depending on the type of device used and the number of slides to be prepared. If a spatula and conical cervical brush are utilized, the first step is to place the spatula firmly against the ectocervix with the long projection extending into the endocervical canal. The spatula should then be rotated at least 360° around the portion and removed. It is important to ensure that the entire

squamocolumnar junction is sampled, since this is the site where most CIN lesions develop.

The endocervical canal is then sampled, using a conical cervical brush, which is placed in the endocervical canal so that the last few bristles remain visible and then gently rotated 90° to 180° once. One such rotation will adequately sample the endocervical canal and generally does not produce bleeding. Material from both sides of the spatula should be spread onto the slide.

Cell fixation must be performed within a few seconds of specimen collection in order to prevent air-drying, which obscures cellular detail and hinders interpretation (Somrak *et al.,* 1990). Immersing the slide in alcohol or spraying it with a specially formulated spray fixative can prevent air-drying. With immersion fixation, the slide is either immersed in alcohol and transferred to the laboratory in the container of alcohol or allowed to fix for 20 to 30 minutes in the alcohol, removed and allowed to air-dry.

Various different spray fixatives are available. Only spray fixatives specifically designed for cytological specimens should be used and the manufacturer's instructions for a given product must be followed. The fixative should be liberally applied such that the slide appears moist over its entire surface. In order to prevent disruption of the cellular layer on the slide, the container of spray fixative should generally be held 15–25 cm from the slide during application (Arbyn *et al*., 2010).

Although the Conventional Pap smear (CPS) examination has been the mainstay for early detection of cervical cancer, its widespread use has not been possible due to the inherent limitations, like presence of obscuring blood and inflammation, reducing its sensitivity considerably. Studies have shown that its sensitivity reduces to less than 50% due to these inherent limitations (Sherwani *et al.,* 2007; Kavatkar *et al*., 2008)

2.5.1.2 Liquid-based cervical cytology

Liquid-based cytology was introduced to solve the problems associated with the conventional Pap smear. Samples for liquid-based cytology (LBC) are collected the same way as those for the conventional Pap smear but instead of smearing the cells on a slide, the collection device is rinsed in a vial containing a liquid fixative and then transported to the laboratory, where the slide is prepared.

Numerous studies have evaluated the comparative performance of the two most commonly used LBC methods (ThinPrep and SurePath) and conventional cytology with respect to their sensitivity and specificity for identification of CIN, the time required for evaluation of the specimens, and specimen adequacy. Beerman *et al*. (2009) showed that the liquid based cytology had superior performance compared to conventional cytology. The study indicated that LBC has a decreased number of unsatisfactory samples, an overall decrease in the false –negative rate and increased sensitivity compared with the conventional Pap smear.

The diagnostic accuracy of cervical cytology has been shown to improve with LBC as the results show enhanced cyto-histological correlation rate and positive predicative value in comparison with conventional Pap smear (Schledermann *et al*., 2006).

There are three techniques used in LBC; a) ThinPrep Pap test, b) SurePath Pap test and c) MonoPrep Pap test

2.5.1.2.1Thin Prep Pap test

In the ThinPrep Pap test, sample is obtained with either a broom-type device or a plastic spatula/endocervical brush combination. The sampling device is swirled or rinsed in a methanol-based preservative solution and transported to the cytology laboratory then discarded. Red blood cells are lysed by the transport medium. The vials are placed one at a time on the ThinPrep automated instrument. The entire procedure takes about 70 seconds per slide and results in a thin deposit of cells in a circle 20 mm in diameter. In most cases, only a fraction of the sample is used to prepare the slide used for diagnosis. If needed, the residual sample is available for additional ThinPrep slide preparation, cell block preparation, or molecular diagnostic testing (e.g., High risk HPV & chlamydia) (Cibas & Ducatman, 2008)

Joseph *et al*. (1991) found that the ThinPrep detected 18% more cases of LSILs and more serious lesions as compared to conventional smears, with no significant difference in the detection of organisms. Studies have shown that the ThinPrep is equivalent to the conventional smear in the detection of endocervical AIS (Collins *et al*., 1996) and also

comparable with the conventional smear for the detection of endometrial pathology (Hall *et al*., 1994)

2.5.1.2.2 Sure Path Pap test

In contrast to the ThinPrep and MonoPrep methods, the practitioner snips off the tip of the collection device which is included in the sample vial. The equipment to prepare slides includes a centrifuge and a PrepStain sample processer with computer and monitor. There is automated mixing of the sample and dispensing it onto the density reagent. Red blood cells and some leukocytes are eliminated by density centrifugation. In addition to preparing an evenly distributed deposit of cells in a circle 13 mm in diameter, the method incorporates a final staining step that discretely stains each individual slide (Cibas & Ducatman, 2008).

Tritz *et al*. (1995) showed that there is a 7.2% increase in the detection of LSILs and more serious lesions and a significant decrease in the percentage of unsatisfactory specimens.

2.5.1.2.3 MonoPrep Pap test

The practitioner obtains the MonoPrep sample with standard collection devices that are swirled or rinsed in a preservative-filled collection vial, after which the sampling device is discarded. As with the ThinPrep, red blood cells are lysed by the transport medium. The vials are delivered to the laboratory where slides are prepared using the MonoPrep Processor, a fully automated, batch processing instrument capable of processing 40

samples per hour, with a throughput capacity of 324 samples per 8-hour run (Cibas & Ducatman, 2008).

In a split-sample clinical trial, slides prepared by the MonoPrep method showed a 26% increase in the detection of LSIL and more serious lesions, with no significant difference in relative specificity. MonoPrep also provided a significant reduction in unsatisfactory slides, and there was no difference in the presentation of endocervical or transformation zone component or the detection of benign conditions (Krieger *et al*., 1994)

2.5.2 HPV-DNA Testing

A common cause of cervical cancer is HPV. HPV- DNA approach is a molecular based test for cervical cancer screening which consists of screening for high-risk strains of HPV. HPV testing has been shown to reduce mortality in high grade lesions in advanced invasive cervical cancer and even in women with human immunodeficiency (HIV) (Louie *et al*., 2009). The HPV-DNA test has shown promising results with high sensitivity and specificity to detect high grade lesions, and therefore is used as a primary screening test in women aged 30 years or older (Mukakalisa *et al*., 2014)

There are various techniques available for HPV-DNA testing of which Southern Blot hybridization is regarded as a laboratory gold standard. This is however unsuitable for clinical use as it is laborious, tedious and requires fresh tissue. The specimen for HPV-DNA testing can be obtained in two ways, either by using a cell suspension from liquid based cytology or by using the endocervical cytobrush (Krishnakumar *et al*., 2011)

Although suitable for low resource settings, HPV testing is expensive and it requires a sophisticated laboratory to read the samples. Unfortunately, most developing countries do not have reliable laboratory facilities (Maine *et al*., 2011)

2.5.3 Visual Inspection with Acetic Acid (VIA)

VIA screening is the simplest method of screening with the lowest cost and relative ease of use as it does not require high technology and has been demonstrated to reduce the deaths of women in developing countries (Wright & Kuhn, 2012).

After obtaining the clinical history and performing a general examination, the cervix is exposed using a speculum. A 5% acetic acid or vinegar is applied to the cervical mucosa. Normal tissue is unaffected by vinegar wash, but abnormal cells including dysplastic and cancerous cells turn acetowhite. Those with dull white plaques and faint borders are considered low grade VIA while those with sharp borders are considered high grade VIA (Duraisamy, Jaganathan & Bose, 2011)

The screening method allows the practitioner to diagnose and treat abnormal cells almost immediately in a health center, typically using cryotherapy which is the application of liquid nitrogen or carbon nitrogen to the dysplastic area. Studies have shown that VIA is a reliable, sensitive and cost effective and alternative to conventional Pap smear testing, particularly in low resource settings in the developing countries (Duraisamy, Jaganathan & Bose, 2011)

2.5.4 Visual Inspection with Lugol's Iodine (VILI)

VILI is also promising as a visual method to screen for cervical cancer. After the woman's cervix is examined using VIA, the cervix is painted with Lugol's iodine solution and examined again with the naked eye. The small high-grade lesion is easier to see within the larger low-grade area. Normal squamous epithelial cells have substantial stores of glycogen.

Glycogen stains mahogany-brown with iodine solution. Abnormal areas of squamous epithelium (CIN or inflammation) do not contain glycogen to the same extent and do not stain brown (Sarian et al., 2007). VILI is more accurate and more reproducible than VIA and better than a Pap smear for identifying CIN. VILI is simple to perform and provides an immediate result without expensive equipment (Duraisamy, Jaganathan & Bose, 2011)

2.5.5 Colposcopy

Colposcopy is a procedure that allows illuminated stereoscopic and magnified viewing of the cervix and the vagina (Camilleri & Blundell, 2009). This procedure is carried out to examine the transformation zone where metaplastic squamous epithelium develops, the medial or internal border being defined by the new squamo-columnar junction. The squamous metaplastic process may become abnormal and when such abnormalities happen, they are usually graded based on the morphological features namely: acetowhiteness, margins, blood vessels and iodine uptake (Urasa & Darj, 2011).

The modern colposcope is a binocular microscope with a variable intensity light source providing a stereoscopic view of the cervix, with a field of view and depth of focus that vary inversely with the magnification selected (Camilleri & Blundell, 2009)

The colposcope can also be used to assess the remainder of the lower genital tract (vagina, vulva and perianal skin), especially if no cervical lesion is found in a woman with abnormal cytology. Women who are HIV positive tend to have multifocal disease involving the vagina, vulva and perianal areas, and therefore these regions need to be examined (Camilleri & Blundell, 2009)

2.6 Treatment of cervical cancer

The treatment of invasive cervical cancer continues to be a major challenge in many sub- Saharan African countries, including Kenya due to the lack of surgical facilities, skilled providers and radiotherapy services. Management of women with invasive cervical cancer requires a multidisciplinary approach, including: gynaecologists, radiation oncologists, medical oncologists, pathologists, medical physicists, technicians, nurses and counsellors. These specialists are lacking in many places across the continent, and where they exist they tend to work in isolation rather than in teams (Ntekim, 2012)

Treatment of cervical cancer is dependent on the stage of the disease, age and medical state of the patient, tumor characteristics, patients' preferences and resources within the health sector of each country (Urasa & Darj 2011)

The following are the recommended treatment strategies for precancerous lesions for the Kenya program: Cryotherapy, Loop Electrosurgical Excision Procedure (LEEP) and Cold knife conization.

2.6.1 Cryotherapy

Cryotherapy, a freezing treatment, is recognized as the most cost-effective and feasible approach to treating precancerous cervical lesion for low-resource settings. This method involves freezing abnormal tissues with a probe cooled by liquid nitrous oxide or carbon dioxide. It has an overall effectiveness rate of 80-90% in women with suitable lesions. It is simple, safe, and major complications are uncommon. (NCCPP, 2012)

2.6.2 Loop Electrosurgical Excision Procedure (LEEP)

LEEP is a method used to remove abnormal cells from the cervix by using a thin wire loop that acts like a scalpel (surgical knife). An electric current is passed through the loop which cuts away a thin layer of the cervix (Conner *et al*., 2014)

This method has been shown to treat large lesions not amenable to cryotherapy and ones that extend into the cervical canal. It also can provide tissue specimens for histology and it is 90-95% effective in treating high-grade cervical dysplasia. However it requires more expensive equipment than cryotherapy and necessitates a highly skilled provider. It also requires electricity, local anesthesia and has a higher risk of serious complications as therefore emergency backup facilities to deal with complications must be available (NCCPP, 2012)

2.6.3 Cold Knife Colonization

This is used in situations where cryotherapy and LEEP are not available, and there is a doctor competent to do the procedure. It may be offered to clients with high grade cervical lesions. Cold knife conization generally has similar requirements to LEEP and additionally has to be performed in theatre usually by a gynecologist. It carries risk of short and long term complications similar to those of LEEP (NCCPP, 2012)

2.6.4 Treatment of invasive cervical cancer

Cervical cancer is curable if detected and treated at an early stage; about 80% of those detected at the early stage are cured with suitable treatments. In developing countries cervical cancer is often diagnosed at very late stages due to the poor or even lack of good screening and treatment methods as opposed to the developed countries that have continuously been able to detect and treat early stages of cervical cancer mostly in the precancerous stages (ACCP, 2004.)

Treatment options for invasive cervical cancer will depend on the stage of disease. However, other factors that affect the decision on the type of treatment include; the exact location of the cancer within the cervix, the type of cancer (squamous cell or adenocarcinoma), age, overall physical condition, and whether one still wants to have children. Currently in Kenya radiotherapy is only available at level 6 facilities (referral hospitals). Primary level facilities utilize existing structures to refer patients to these tertiary sites where treatment is available. (NCCPP 2012)

Previously early stage cervical cancer was treated radically through radiotherapy or radical hysterectomy, every five years the survival rates were placed at about 80-90%. In 1999 a National Cancer Institution recommended that radiotherapy should be used alongside chemoradiation for women with advanced stages of cervical cancer, this was based on five randomized trials that showed evidence of better survival with progressive cancer free survival (Tierney, 2009).

Surgery may be recommended for small tumors in the cervix especially when the patient has no sign of metastasis in the lymph node. Some of the surgical procedures are like hysterectomy which is a surgical procedure that involves removal of the uterus. There are two types of hysterectomy namely simple hysterectomy that involves removal of the uterus and the cervix. The surgery is done laparoscopically and takes a very short time of recovery with one to two days of hospitalization. Radical hysterectomy involves removal of the uterus and about two centimeters of the upper vagina and soft tissues around the cervix, the procedure may be done laparoscopically therefore taking less recovery time but may cause some effect on the bowel and bladder function. After radical hysterectomy a patient is hospitalized for at least 6 weeks with a urine catheter and an abdominal drainage which is removed three to five days after operation. Pain relief medication can be administered for pain management (Long *et al*., 2007)

The National Cervical Cancer Prevention Plan recommends the following treatment modalities for the various stages of overt cervical cancer: For Micro invasive carcinoma to stage 1a, extended abdominal hysterectomy should be used. Stage1 to 2a, Wertheim's

hysterectomy or Radiotherapy and adjuvant chemotherapy is recommended. Finally for Stage 2b to 4, Radiotherapy, Palliative care and adjuvant chemotherapy should be used.

CHAPTER THREE

Materials and Method

3.1 Study site

Machakos Level 5 Hospital of Machakos County in South - Eastern part of Kenya. This study was done at the comprehensive care Centre which serves around 2000 women infected with HIV aids. The hospital was chosen considering the high number of women attending the comprehensive care clinic as it serves as the referral hospital in the county. Samples were processed and stained at JKUAT cytology laboratory.

3.2 Study Design

This was a Comparative cross-sectional study.

3.3 Target population

The population of the present study was composed of women of 18 years and above who were sexually active and attending Machakos level 5 Comprehensive Care Centre (CCC). The target population was chosen based on the fact that the prevalence of cervical squamous intraepithelial lesions among HIV-positive women is relatively higher compared to the HIV –negative population. According to a study done by Kevin *et al*. (2011), the prevalence of cervical squamous intraepithelial lesions among HIV-positive women in Kenya is 46%.

3.3.1 Inclusion criteria

Women of 18 years and above who were sexually active attending Machakos level 5 Comprehensive Care Centre and completed an informed consent form and accepted to participate were included in this study.

3.3.2 Exclusion criteria

Women who were pregnant or declined to complete an informed consent form were excluded from the study. Women on treatment for precancerous lesion or cervical cancer and a previous hysterectomy were not eligible.

3.3.3 Sample size determination

According to a study done by Peter Memia *et al* (2012), the prevalence and identified associated risk factors for precancerous cervical cancer lesions among HIV-infected women in resource-limited settings in Kenya was found to be 26%

Using Fisher's et al, 1998 formula, to calculate 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 (26%)

E= range of possible random error (5%)

Q= 1 – P or estimated proportion of failure

$$n = \frac{1.96^2 \times 0.26(1-0.26)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.26 \times 0.74}{0.05 \times 0.05}$$

= 295 participants.

3.4 Sampling method

Convenience sampling method was used, with this method, patients available during the study period and willing to participate were considered until the sample size was reached.

3.5 Recruitment of research participants and data collection

This study was carried out at Comprehensive Care Clinic (CCC) in Machakos County Hospital during June to December 2016. The Principal investigator (PI) contacted the manager of CCC after the ethics committee approved the study. The manager then introduced the PI to a trained clinician who assisted in the study.

The PI recruited two hundred and ninety five (295) study participants who met the inclusion criteria and who gave a written consent to participate in the study. Every day, recruited study participants were grouped and a short education session given by the principal investigator 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 (by signing the consent form); a structured questionnaire was used by the principal investigator to collect the demographic data after which sample collection procedures and processing proceeded.

3.6 Laboratory methods

Study samples were collected as whole sample. This was done so as to avoid sampling errors. Processing of the samples was done in two phases.

Phase 1: collection of samples for conventional Pap smear

After obtaining a signed consent from the patient, the clinician explained the procedure, assured and placed the patient in a comfortable and convenient position for sample collection. First sample was collected using cytobrush for conventional Pap smear. The cytological material obtained was spread on glass slides directly and fixed immediately in 95% ethanol for at least 15 minutes. Staining was done

Phase 2: collection of samples for manual liquid based cytology

A second sample was collected using a different cytobrush. The cytological material was transferred with brushes into a formulated liquid fixative (containing sodium chloride, sodium citrate, 10% formalin and isopropyl alcohol). Brushes were broken off into the container of collection fluid. The collected samples were vortex-mixed, and then about 10ml transferred into a formulated alcoholic-agar (polymer solution containing agarose, polyethylene glycol and alcohol) in nipple-bottom test tubes. Test tubes were centrifuged for 10 minutes at 2000rpm. The supernatant was discarded and from the deposit smear made on a clean glass slide using a Pasteur pipette. The prepared slides

were fixed by drying them in a hot air oven for 15 minutes at 50˚C. The slides were further fixed by dipping them in 95% alcohol for 15 minutes. All the smears were stained using the Papanicolaou staining method.

All the Pap smears were screened by the principal investigator and signed out together with a pathologist. The Bethesda system 2014 for reporting cervical cytology was used for reporting all the cytological abnormalities observed during examination and reporting. All abnormal cytological smears of the study patients were blindly and independently re-evaluated by a board certified pathologist. The abnormal smears were triaged for further investigation**.**

3.7 Quality assurance

Specimen collection was carried out by experienced health professionals who perform Pap smears regularly. Pap smears were stained in line with the Jomo Kenyatta University of Agriculture and Technology cytology laboratory standard operating procedures (SOPs) used for staining gynecological specimens. The stains and reagents were prepared using relevant standard operating procedures (SOPs) and filtered before each use to ensure good quality staining. Deteriorated stains were discarded and new stains prepared. The principal investigator examined all the smears followed by a review with a qualified Clinical Cytologist. All abnormal smears were reviewed by a board certified pathologist.

3.8 Data presentation and analysis

All data was stored & analyzed using SPSS version 18. Statistical tests were performed at 5% level of significance. A P-value < 0.05 was considered statistically significant. Cohen's kappa test was done to determine the agreement level between Conventional Pap smear results and Manual liquid based cytology results. Chi square test was done to determine association between age and Pap smear findings. Descriptive statistics were presented as proportions and percentages in the form of tabulation charts and graphs. Increased detection rate (IDR) was calculated as follows: IDR= ((Pm-Pc)/Pc)*100, where Pm is the number of positive cases through MLBC and Pc is number of positive cases for conventional Pap smear.

3.9 Ethical considerations

Ethical approval was obtained from the Kenyatta University Research and Ethical Review committee. An informed, written and voluntary consent was sought from the patients before obtaining samples for screening. All procedures were explained to the patients and clarifications made in a language they understood. No woman was coerced into taking the cervical smear test without their permission. The anxiety or trauma that one may undergo during the cervical smear test can be unsettling but assurances was offered to calm the women. In this study, a qualified and experienced clinician collected Pap smears from the patients. Patient privacy and confidentiality was strictly observed. 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 sent to the patient's file and communicated in the usual manner by the doctor or nurse counselor taking care of the patient during their next visit. Names of the patients were not used but instead study numbers.

3.10 Limitations of the study

In this study, cytology results were not correlated with histology which acts as the gold standard and thus the validity of both methods was not assessed. This was not done due limited financial resources.

CHAPTER FOUR

Results

A total of 295 study participants were recruited into the study.

4.1 Social Demographic characteristics of the study population

4.1.1 Age

Out of the 295 women recruited in the study, 30 (10.2%) were between 20 – 30 years, 80 (27.1%) between 30 – 39 years, 99 (33.6%) between 40– 49, 65 (22.0%) between 50 – 59 and 21 (7.1%) were aged 60 and above. (Table 4.1)

Table 4.1 Distribution of the age groups

Age in years	Frequency	Percent
20 – 29	30	10.2
30 – 39	80	27.1
40 – 49	99	33.6
50 – 59	65	22.0
60 and above	21	7.1
Total	**295**	**100.0**

4.1.2 Marital Status

Out of the 295 women recruited in the study, 134 (45.4%) were married, 75 (25.4%) widowed, 44 (14.9%) single, while 42 (14.2%) were divorced. (Table 4.2)

Table 4.2 marital status

Marital Status	Frequency	Percent
Single	44	14.9
Married	134	45.4
Divorced	42	14.2
Widowed	75	25.4
Total	295	100.0

4.1.3 History of Tobacco smoking

Only 3 (1%) out of 295 women had history of tobacco smoking (Table 4.3)

Table 4.3 History of tobacco smoking

Tobacco Smoking	Frequency	Percent
Yes	3	1.0
No	292	99.0
Total	295	100.0

4.1.4 History of Pap smear screening prior to this study

Only 12 (4.1%) out of 295 women had had cervical cancer screening done prior to this research (Table 4.4)

Table 4.4 History of Pap smear screening prior to this study

Pap smear screening before	Frequency	Percent
Yes	12	4.1
No	283	95.9
Total	295	100.0

4.1.5 Education level

Majority of the women had primary education 137 (46.4%). Only 43 (14.6%) women had college/university education (Table 4.5)

Table 4.5 Education level

Education Level	Frequency	Percent
Primary	137	46.4
Secondary	115	39.0
College/University	43	14.6
Total	295	100.0

4.1.6 Contraceptive use

The most used family planning methods were Condoms and Injection with 175 (59%) and 48 (16%) respectively (Figure 4.1)

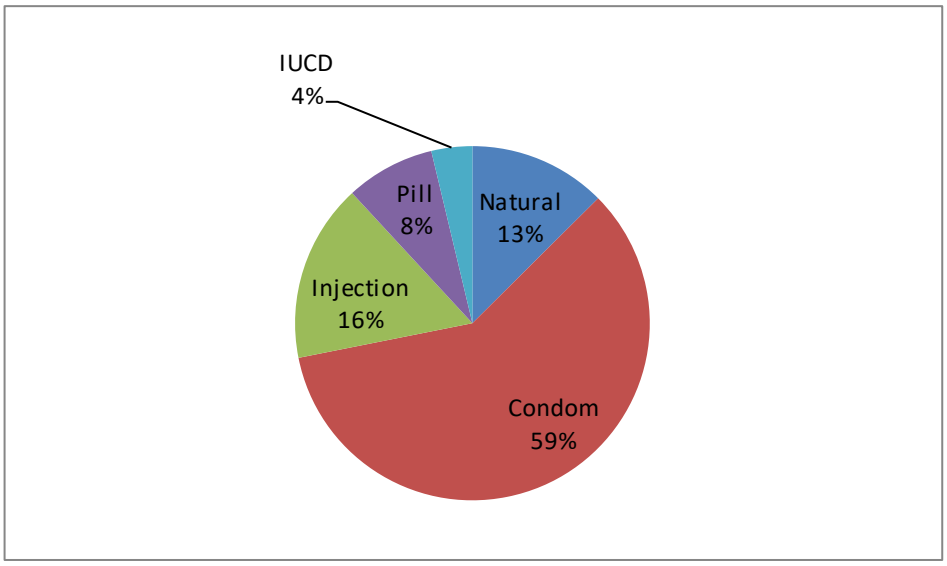

Figure 4.1: Contraceptive use in the HIV infected women

4.2 Pap Smear Findings using the Conventional Pap smear method

Out of the total women recruited in this study, 259 (87.8%) were Negative for intraepithelial lesion or malignancy, 14 (4.7%) had abnormal smears while 22 (7.5%) had unsatisfactory smears for evaluation. A total of 295 women were enrolled in the study. Twenty two were excluded from the analysis due to inadequate smear or missing cervical cells at all. The prevalence of cervical cytology abnormalities as seen by the conventional Pap smear method in this research was 14 out of 273 (5.1%) with HSIL being the most prevalent at 5 out of 273 (1.8%), ASC-H 4 out of 273 (1.46%), LSIL 3 out of 273 (1.05%), SCC was seen in 1 out of 273 (0.36%) and lastly Adenocarcinoma was also seen in 1 out of 273 (0.36%) (Table 4.6)

Table 4.6 Pap smear findings as seen in the conventional Pap smear method

Pap smear Findings	Frequency	Percent
NILM	259	87.8
Abnormal smear	14	4.7
Unsatisfactory smear	22	7.5
Total	295	100.0

4.3 Cross tabulation of Age groups and Pap smear findings

There was no statistically significant association between age and abnormal Pap smear findings (Table 4.7)

Table 4.7 Cross tabulation of Age groups and Pap smear findings

Age	NILM	Abnormal	Unsatisfactory	Total	Pearson Value	P-Value
20 – 29	27(10.4%)	1(7.1%)	2(9.1%)	30(10.2%)	6.262	0.618
30 – 39	72(27.8%)	3(21.4%)	5(22.7%)	80(27.1%)		
40 – 49	90(34.7%)	3(21.4%)	6(27.3%)	99(33.6%)		
50 – 59	53(20.5%)	6(42.9%)	6(27.3%)	65(22.0%)		
60 and above	17(6.6%)	1(7.1%)	3(13.6%)	21(7.1%)		
Total	259(100.0%)	14(100.0%)	22(100.0%)	295(100.0%)		

4.4 Photomicrographs of cytomorphological patterns of abnormal Pap smears as seen on the conventional Pap smear method

With the CPS method, majority of the smears showed overlapping of cells, dirty background and cervical cells obscured by mucus, debris or blood (Figure 4.2, Figure 4.3 and Figure 4.4)

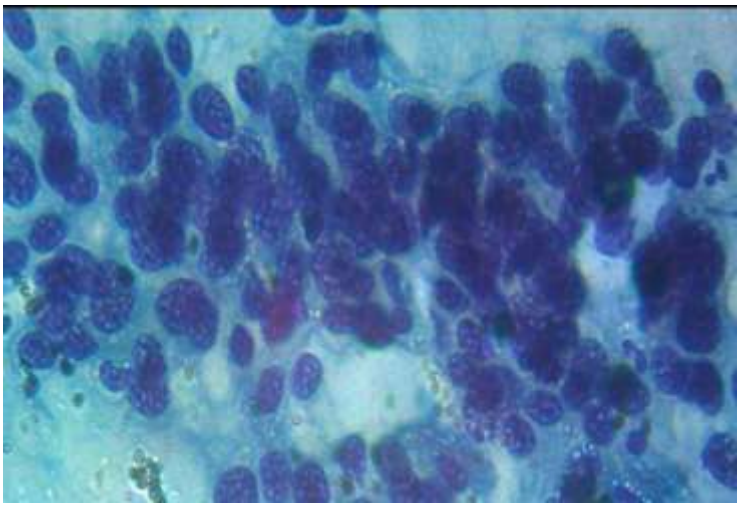

Figure 4.2 High grade squamous intraepithelial Lesion on CPS at x 400 field

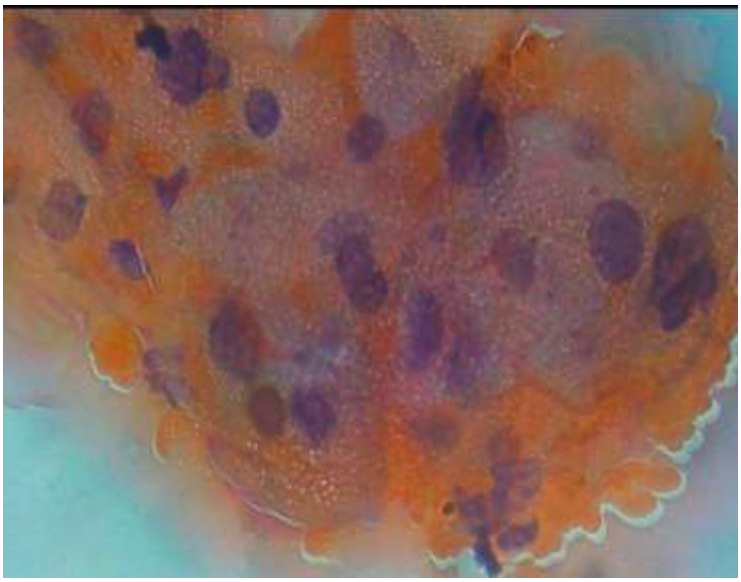

Figure 4.3 Atypical squamous cells of undetermined significance on CPS at x 400 field

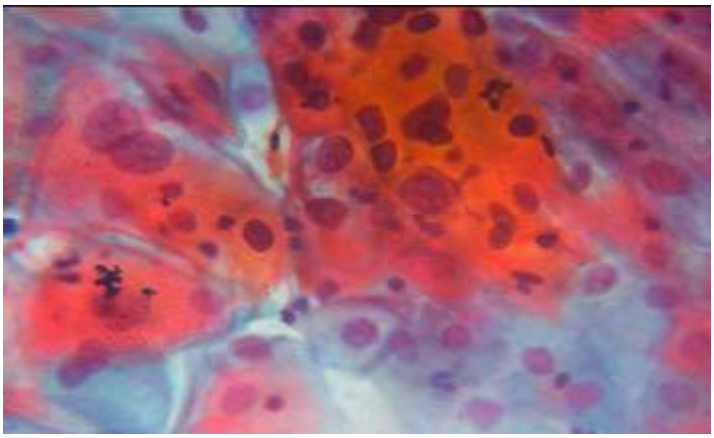

Figure 4.4 Low grade squamous intraepithelial Lesion on CPS at x 400 field

4.5 Pap Smear Findings using the Manual Liquid based cytology Method

Out of the 295 Pap smear samples screened using the manual liquid based cytology technique, 257 (87.1%) were Negative for intraepithelial lesion or malignancy, 26 (8.8%) had abnormal smears while 12 (4.1%) had unsatisfactory smears for evaluation and therefore excluded from analysis. The prevalence of cervical cytology abnormalities as seen by the manual liquid based cytology method in this research was 26 out of 283 (9.2%). LSIL was the most prevalent at 9 (2.7%), HSIL 7 (2%), ASC-H 5 (1.6%), ASCUS 3 (1%), Adenocarcinoma 1 (0.3%) and squamous cell carcinoma 1(0.3%) (Table 4.8)

Table 4.8 Pap smear findings as seen in the MLBC method

Pap smear Findings	Frequency	Percent
NILM	257	87.1
Abnormal smear	26	8.8
Unsatisfactory smear	12	4.1
Total	295	100.0

4.6 Photomicrographs of cytomorphological patterns of abnormal Pap smears as seen on MLBC Technique

With MLBC, cells were seen in monolayer with minimal obstruction by debris, mucus or red blood cells (Figures 4.5, 4.6 and 4.7)

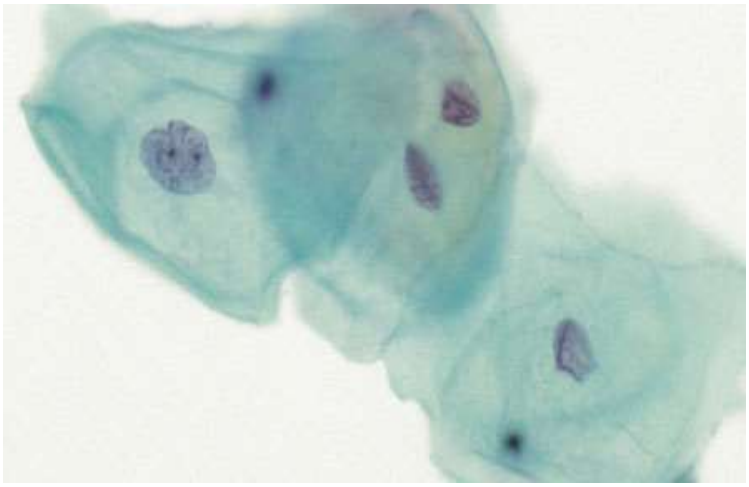

Figure 4.5 Atypical Squamous Cells – Undetermined Significance on MLBC at x 400 field

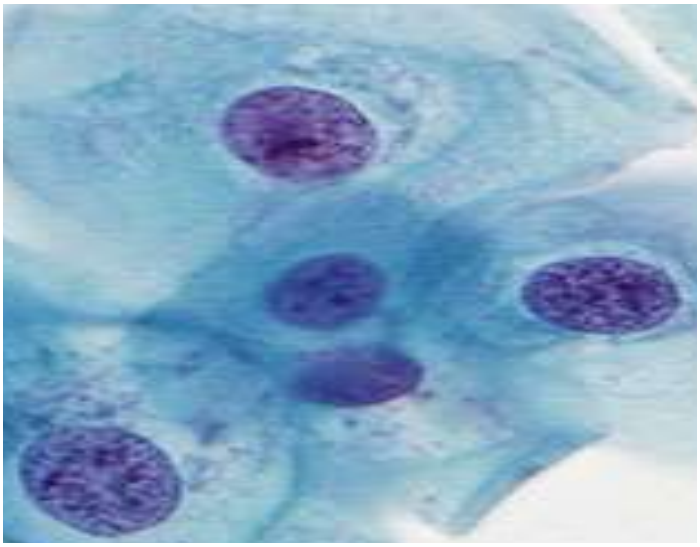

Figure 4.6 Low-Grade Squamous Intraepithelial Lesion on MLBC at x 400 field

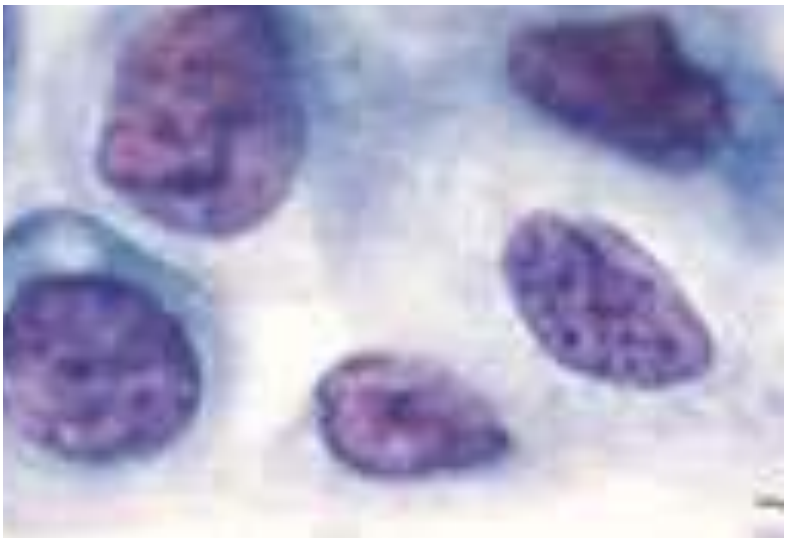

Figure 4.7 High-Grade Squamous Intraepithelial Lesion on MLBC at x 400 field

4.7 Comparison of Manual liquid based cytology and conventional Pap smear Results

Cohen Kappa test was run to determine the level of agreement between conventional Pap smear and the MLBC results. There was moderate agreement between the two methods (k=0.673, 95% CI, p=0.065). (Table 4.9)

Table 4.9 Cross tabulation of Conventional Pap smear and Manual Liquid based cytology results.

		MLBC			Total	kappa	P value
		NILM	Abnormal	Unsatisfactory			
CP	NILM	247	12	0	259	0.673	0.065
	Abnormal	0	14	0	14		
	Unsatisfactory	10	0	12	22		
Total		257	26	12	295		

4.8 Comparison of cytomorphological patterns between Conventional Pap smear and Manual Liquid based cytology

With the MLBC technique, majority of the smears showed cells suspended in a monolayer thus improved detection of precursor lesions and improvement of specimen adequacy. A clear background was also observed for the MLBC smears with minimal obstruction of cells by debris, mucus or blood further improving the specimen adequacy (Figures 4.8 and 4.9)

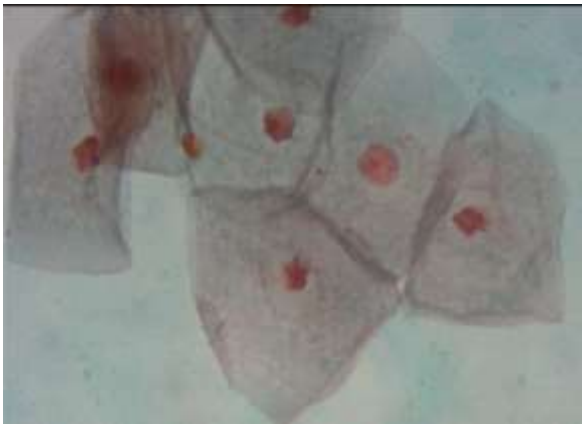

Figure 4.8 Monolayer of cells Negative for intraepithelial lesion or malignancy on MLBC technique at x 400 field

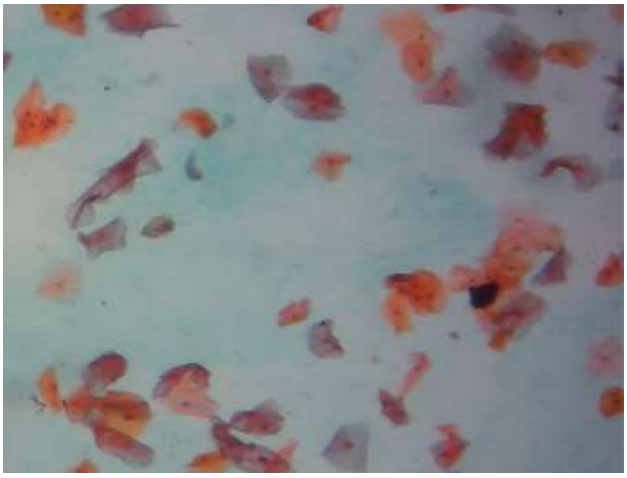

Figure 4.9 Monolayer of cells negative for intraepithelial lesion or malignancy on MLBC technique at x 100 field

On the other hand, with conventional Pap smear method, majority of the smears showed overlapping of cells, dirty background and cervical cells obscured by mucus, debris or blood.

4.9 Increased detection Rate

Increased Detection Rate with (IDR) was calculated as follows;

IDR= ((Pm-Pc)/Pc)*100, where Pm is the number of positive cases through MLBC and Pc is number of positive cases for conventional Pap smear.

$$IDR = \frac{(26-14)100}{14}$$

$$= 85.7\%$$

Thus Increased detection rate with MLBC in this study was 85.7%

CHAPTER 5

Discussions

5.1 Liquid Based Cytology versus Conventional Pap smear

Although conventional Pap smear (CPS) examination has been the mainstay for early detection of cervical cancer, its widespread use has not been possible due to its inherent limitations. On the other hand, liquid-based cytology is a technique that enables cells to be suspended in a liquid medium and spread in a monolayer, making better morphological assessment. Automated techniques have been widely used especially in the developed countries but limited in the developing countries due to high cost and unavailability of advanced technology (Kavatkar *et al.,* 2008). Several reports have discussed the benefits of LBC technique in cytologic diagnosis and found it to be superior to CPS in diagnosis of cervical cancer and precancerous lesions (Obwegeser, 2001; Nanda *et al*., 2000)

Although conventional Pap smear screening leads to reduced rate of invasive cancer of the uterine cervix, studies have shown that its sensitivity reduces to less than 50% when there is presence of obscuring blood, inflammation or thick areas of overlapping cells (Kavatkar *et al.,* 2008). Specimen may often be clouded with debris such as blood and mucus, which obscure cell visibility. Drying artifacts may also be formed if the cells are not immediately sprayed with a fixative solution. Furthermore, the collection device is discarded and thus a repeat sample is not available incase needed. These among other

limitations have been shown to reduce the sensitivity of CPS to less than 50% (Sherwani et al., 2007; Kavatkar *et al.,* 2008).

An automated liquid-based cytology (ALBC) for cervical cancer screening was developed to improve the sensitivity, but the very high cost related to automated devices have hampered its implementation in the setup of developing countries, like Kenya. In liquid-based methods, the specimen is collected in a preservative solution rather than being directly spread on the slide. Cellular structure is better preserved because the cells are immediately fixed. The process prevents drying artefacts and removes most contaminating mucus, red blood cells bacteria and yeast (Burd, 2003). On the other hand, manual liquid based cytology (MLBC) is a technique that is cost effective and improves detection of precursor lesions and specimen adequacy.

5.2 Comparison of MLBC with CPS in screening for pre-cancerous lesions and cervical cancer

In this study, specimen adequacy was found to be better with MLBC than CPS with 12 unsatisfactory smears in MLBC and 22 in CPS. Many studies have reported LBC to be better in terms of specimen adequacy compared to CPS. Majority of studies comparing LBC and CPS found that the quality of slides improved in LBC, which is consistent with the results obtained in our study that MLBC has higher satisfactory specimen rates as compared to CPS.

With the MLBC technique, majority of the smears showed cells suspended in a monolayer thus improved detection of precursor lesions and improvement of specimen adequacy. A clear background was also observed for the MLBC smears with minimal obstruction of cells by debris, mucus or blood further improving the specimen adequacy. In a study done by Kavatkar *et al*. (2008), MLBC was found to give a clear background in more smears than in conventional Pap smear.

In CPS, there was higher rate of unsatisfactory smears due to the presence of obscuring blood, inflammation and dirty background which obscured the epithelial cells thus affecting the screening process. Also, in CPS only 20% of the cells collected on the brush are smeared on to the slide leading to lesser cells being transferred to the smear for screening thus unsatisfactory smears for evaluation (Nandini et al., 2012). Specimen adequacy in MLBC was also better compared to CPS due to the fact that the entire specimen collected from the cervix was transferred to the fixative solution for processing without any wastage.

In the current study 7.5% cases were unsatisfactory with conventional Pap smear method while with MLBC, 4.1% cases were found unsatisfactory due to inadequate cells for examination. A study done in Pakistan to compare cervical cell morphology using MLBC and CPS recorded 27% cases of unsatisfactory smears for conventional Pap smear method and 24% with the MLBC technique (Moosa *et al* 2014). Other studies have documented lower percentages for unsatisfactory smears with LBC technique. Bergeron *et al*. (2003) reported (0.14%) while Garbar *et al*. (2005) found (0.9%). In

these studies, automated liquid based cytology technique was used thus the lower percentages of unsatisfactory smears compared to the current study.

In LBC, the sample is first placed in fixative solution followed by further processing instead of making slides directly as in CPS. This makes cellular structure better preserved with reduced drying artifacts as cells are immediately fixed (Brud, 2003). In MLBC there is marked decrease in artifacts, contaminating mucus and blood. Cells are evenly distributed on slides and centrifugation in this method offers a proper mixing (Elnashar *et al*., 2012). Similarly, in the current study cellular overlapping is reduced with majority of the cells forming a monolayer with a clear background. Thus manual liquid based cytology gives more clear results with clear background, less artifacts and lesser degree of cellular overlapping when compared with conventional Pap smear.

5.3 Level of Agreement between MLBC and CPS Results

In this study, the level of agreement between MLBC and the conventional Pap smear method was 67.3%. This is comparable to 68% as documented in a study done in India by Nandini et al. (2012). In another study done in India on manual method of liquid-based cervical cytology by Kavatkar *et al.* (2008), the level of agreement between MLBC and the conventional Pap smear method was found to be 88%. However, in another study done by Moosa et al. (2008), conventional Pap smear method was found to be comparable to the MLBC technique in terms of specimen adequacy and detection of abnormal cervical cytology. In the current study, there was increased detection rate of

abnormal cervical cytology smears with MLBC of 85.7%. However, this is low compared to 150% as documented in a study done by Nandini *et al*. (2012).

5.4 Prevalence of Abnormal Cervical Cytology

In the current study, the prevalence of abnormal cervical cytology with Conventional Pap smear technique was 5.2%. This is comparable to 6% as documented in a study done in Nigeria by Ononogbu *et al*. (2013) to determine cervical cancer risk factors among HIV-infected Nigerian women.

However, this prevalence is lower compared to other studies done in Kenya and Africa. In a study done in Kenya by Memiah *et al.* (2012) to determine the prevalence and risk factors associated with precancerous cervical lesions among HIV infected women, the prevalence of abnormal cervical cytology was 26.7%. In another unpublished study done by Odhiambo at Kenyatta National Hospital in 2016 to determine cervical cytological patterns among HIV infected women on antiretroviral therapy, the prevalence of abnormal cervical cytology was 9.9%.

A study conducted in Ethiopia reported a prevalence of cervical pre-cancer and cancer among HIV-positive women of 22.1% (Gedefaw et al., 2013). Another study in Southern Nigeria to determine effect of low CD4 Cell count on cervical squamous intraepithelial lesions among HIV-positive women reported a 5.7 % prevalence of SIL among the high CD4 group and 10.2% among the low CD4 group. (Enebe et al., 2015)

The national cervical cancer screening guidelines recommends that HIV infected women should be screened every 1 year because of their high risk to development of cervical cytological lesions. At Machakos comprehensive care center, VIA/VILLI are done annually and positive cases referred for further management. Most of the women in this study assented to having the VIA/VILLI done in 2015 at the Comprehensive Care Center.

Early initiation and duration of combined antiretroviral therapy may have contributed to less prevalence rate as all the women in this study were on antiretroviral therapy. A study done by Adler *et al*. (2012) showed that women on HAART with a normal baseline smear were 38% less likely to have abnormal Pap smear on follow-up.

There was no statistically significant association between age and abnormal Pap smear findings. This is consistent with another study done in Rwanda by Kayumba in 2013 which reported lack of statistically significant association between age and abnormal Pap smear findings.

CHAPTER 6

Conclusions and Recommendations

6.2 Conclusion

1. Manual liquid based cytology was found to give better results than conventional Pap smear method in terms of specimen adequacy, clear back ground and reduced cellular overlapping with increased detection of abnormalities and preservation of specimen for future testing.

2. There was moderate level of agreement between the two methods (k=0.673, 95% CI, p=0.065). MLBC had increased detection rate of abnormal cervical cytology smears of 85.7%.

3. The prevalence of abnormal cervical cytology with Conventional Pap smear technique was 5.2% with no statistically significant association between age and Pap smear findings.

6.3 Recommendations

Manual Liquid based cytology should be introduced as alternative strategy for cervical cancer screening in limited resource settings since it shows better results than the Conventional Pap smear method.

Periodic Pap smear screening for all HIV infected women should be introduced at Machakos County Hospital Critical care Centre for early detection of pre-cancerous lesions.

Future studies to assess the validity of MLBC in screening for precancerous lesion and cervical cancer by correlating it with histology.

Future studies should be done to assess the cost benefit for manual liquid based cytology technique compared to conventional Pap smear method.

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APPENDICES

APPENDIX I: CLIENT CONSENT INFORMATION FORM

TITLE: THE UTILITY OF A LOW COST MANUAL LIQUID BASED CYTOLOGY IN SCREENING FOR PRE-CANCEROUS LESION AND CERVICAL CANCER

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

BENEFITS

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

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

Pap collection explanation for study participants

You will be required to lie on an examination couch.

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

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

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

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

CONFIDENTIALITY

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

Who to Contact

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

1. Onesmus Muia Mutuku (Principal investigator) on mobile number 0712652085, Faith Wangui Ruria (Research assistant) on mobile number 0725675795, and my supervisors: Dr Mutinda Kyama (mobile number 0711169526), Dr. Kavoi M. Boniface (mobile number 0720895968) and Dr. Michael K. Ngugi (mobile number 0722921248) or Kenyatta University Ethics Review committee, P.O. Box 43844 – 00100, Telephone No; 0208710901/12

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

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

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

Principal investigator……Date……

Clinician (Research assistant)…… Date……

APPENDIX II: QUESTIONNAIRE

THE UTILITY OF A LOW COST MANUAL LIQUID BASED CYTOLOGY IN SCREENING FOR PRE-CANCEROUS LESION AND CERVICAL CANCER

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

Section A: Socio demographic information

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 of the stain

The nucleus is stained with hematoxylin to the intensity desired. The bluing agent, following the hematoxylin, sets the nuclear dye in place. The cytoplasm is barely tinted. Scott's tap water substitute (STWS), is commonly used as a bluing agent. Eosin Azure solution being acidic stains the cytoplasm which is basic so that the eosin has affinity for the mature cells while light green has affinity for the young cells. Orange G is also an acidic dye and consequently has an affinity or the cytoplasm and stains keratin.

Staining Technique

1.	95% ethanol (fixative)	15 min
2.	80% ethanol	6-8 dips
3.	70% ethanol	6-8 dips
4.	50% ethanol	6-8 dips
5.	Water, distilled	6-8 dips until glossy look disappears
6.	Harris hematoxylin,* undiluted	45 sec
7.	Water, distilled	Rinse

8. Water, distilled	Rinse
9. Water, distilled	Rinse
10. 50% ethanol	6-8 dips
11. Ammonium hydroxide NH_4OH (1.5% in 70% ethanol)	1 min
12. 70% ethanol	6-8 dips
13. 70% ethanol	6-8 dips
14. 80% ethanol	6-8 dips
15. 95% ethanol	6-8 dips
16. OG-6	1-1/4 min
17. 95% ethanol	Rinse
18. 95% ethanol	Rinse (do not allow slides to remain in alcohol)
19. EA-50 or -65	3 min
20. 95% ethanol	Rinse gently

21. 95% ethanol	Rinse gently
22. 95% ethanol	Rinse gently
23. 100% ethanol	6-8 dips
24. 100% ethanol (if large volume)	6-8 dips
25. 100% ethanol: xylene (1:1)	6-8 dips
26. Xylene	6-8 dips
27. Xylene	6-8 dips
28. Xylene	Until ready to mount

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

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

SPECIMEN ADEQUACY

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

GENERAL CATEGORIZATION (optional)

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

INTERPRETATION/RESULT

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

- Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

Organisms

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

Other

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

Epithelial Cell Abnormalities

Squamous Cell

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

Glandular Cell

- Atypical

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

ADJUNCTIVE TESTING

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

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY

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

EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (optional)

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

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

APPENDIX V: ETHICAL APPROVAL

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
ercku2008@gmail.com
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
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Our Ref: KU/R/COMM/51/668

Date: 18th April, 2016

Onesmus Muia Mutuku
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Mutuku,

APPLICATION NUMBER PKU/464/E42– "THE UTILITY OF A LOW COST MANUAL LIQUID BASED CYTOLOGY IN SCREENING FOR PRE-CANCEROUS AND CERVICAL CANCER" –VERSION 2.

IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic, **"The utility of a low cost manual liquid based cytology in screening for pre-cancerous and cervical cancer"- Version 2**

2. APPLICANT
Onesmus Muia Mutuku
3. SITE
Machakos Level 5 Hospital, Kenya
4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 18th April, 2016.**
5. ADVICE/CONDITIONS
i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I Onesmus Mutuku accept the advice given and will fulfill the conditions therein.

Signature ..... Dated this day of 19th April 2016.

cc. Vice Chancellor
DVC-Research Innovation and Outreach

APPENDIX VI: PUBLICATION 1

East African Medical Journal Vol. 91 No. 4 May 2017
PREVALENCE OF ABNORMAL CERVICAL CYTOLOGY AMONG WOMEN INFECTED WITH HIV IN MACHAKOS COUNTY HOSPITAL KENYA.

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Request for reprints to: O. Mutuku, P.O. BOX 2307- 90100 Machakos, Kenya

PREVALENCE OF ABNORMAL CERVICAL CYTOLOGY AMONG WOMEN INFECTED WITH HIV IN MACHAKOS COUNTY HOSPITAL KENYA.

O.M. MUTUKU, B.M. KAVOI, KAHATO. M, R. CHIBVONGODZE and C.M.KYAMA

ABSTRACT

Background: **Cervical cancer is increasingly becoming a major threat to health among women in the world particularly in developing countries where screening programs are not well established. In Kenya, cervical cancer is the second most frequent cancer among women and the leading cause of cancer deaths in women of reproductive age. Studieshave shown that women infected with HIV especially those with low CD4 counts or decreasing immunity are at a higher risk of developing precancerous cervical lesions and invasive cervical cancer than those in the general population without HIV infection.**

Objective: **To determine the prevalence of abnormal Pap smears in HIVpositive women attending Comprehensive care clinic at Machakos Level 5 Hospital.**

Design: **Cross sectional descriptive study**

Setting: **Machakos County hospital**

Subjects: **Women infected with HIV attending Machakos County Hospital Comprehensive care clinic**

Results: **295 women infected with HIV were enrolled in this study and cervical smear taken for cytology screening. 22 were excluded from the analysis due to unsatisfactory smears. The prevalence of cervical cytology abnormalities was 14 out of 273 (5.1%) with HSIL being the most prevalent at 5 out of 273 (1.8%). Other lesions were ASC-H 4 (1.46%), LSIL 3 (1.05%), SCC and Adenocarcinoma both with 1(0.36%).**

In this study, age and Pap smear findings had no statistically significant association, X^2=6.262, p=0.618.

Conclusion: **This prevalence of abnormal cervical cytology among HIV infected women in this study was 5.2%. There was no statistically significant association betweenage and Pap smear findings.**

INTRODUCTION

Cervical cancer is increasingly becoming a major threat to health among women in the world particularly in developing countries where screening programs are not well established. (1) In 2012, it is estimated that 528,000 new cases occurred globally, with 266,000 of the women (50% of cases) dying (2). In Kenya, cervical cancer is the second most frequent cancer among women and the leading cause of cancer deaths in women of reproductive age. (3)

Cervical cancer is considered to be one of the diseases associated with AIDS (4). Even though .HPV infections are very common in the general population and most women with healthy immune systems will normally clear these infections over time, women with compromised immune systems (such as women living with HIV) are far less likely to clear an HPV infection and thus having high chances to develop pre-invasive lesions that can progress to invasive cervical cancer if not treated. (5, 6)

Studies have shown that in areas where women have been hit hardest by the AIDS epidemic, such as in the developing countries have historically also had a very high prevalence of human papillomavirus (HPV) infections and a high incidence of cervical cancer. (7)

As there was no study that documented the prevalence of cervical cytology abnormalities in HIV-infected women at Machakos county Hospital, this motivated us to carry out the research. This study will be of help to policy makers in developing guidelines for prevention and treatment strategies for cervical cancer among HIV-infected women at Machakos County and Kenya at large.

MATERIALS AND METHODS

Approval to conduct the study was sought from Kenyatta University Research and Ethical Review committee (Protocol Number: PKU/464/E42). A total of 295 HIV positive women attending Comprehensive care clinic at Machakos Level 5 Hospital were recruited in the study between June - November 2016. An informed, written and voluntary consent was sought from the patients before obtaining samples for screening. After the participants had given their written consent (by signing the consent form), a structured questionnaire was used to collect the demographic data after which sample collection procedures and processing proceeded

Samples were collected using Cytobrush and the cells spread on glass slides directly and fixed immediately in 95% ethanol for at least 15 minutes. All the smears were stained using the Papanicolaou staining method. Screening was done by the principal investigator and signing out 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.

Data collected was analyzed using SPSS version 18. In an attempt to find the association between the age and cases of abnormal cervical cytology, a cross-tabulation was made and Chi-square statistics was used for the statistical significance of associations between variables. A p- value below 0.05 was considered as statistically significant

RESULTS

Table 1 shows distribution of the age groups. Out of the 295 women recruited in the study, 30 (10.2%) were between 20 – 30 years, 80 (27.1%) between 30 – 39 years, 99 (33.6%) between 40– 49, 65 (22.0%) between 50 – 59 and 21 (7.1%) were aged 60 and above.

Table 1
Distribution of Age groups

Age in years	Frequency	Percent
20 – 29	30	10.2
30 – 39	80	27.1
40 – 49	99	33.6
50 – 59	65	22.0
60 and above	21	7.1
Total	**295**	**100.0**

Table 2 shows the history of Pap smear screening prior to this study. Only 12 (4.1%) out of 295 women had had cervical cancer screening done prior to this research

Table 2
History of Pap smear screening prior to this study.

Pap smear screening before	Frequency	Percent
Yes	12	4.1
No	283	95.9
Total	295	100.0

Table 3 shows methods of family planning. Condom was the main method of family planning used (59.3%) and IUCD been the least used (3.7%)

Table 3
Methods of family planning.

Method of Family Planning	Frequency	Percent
Natural	37	12.5
Condom	175	59.3
Injection	48	16.3
Pill	24	8.1
IUCD	11	3.7
Total	295	100.0

Out of the total women recruited in this study, 259 (87.8%) were Negative for intraepithelial lesion or malignancy, 14 (4.7%) had abnormal smears while 22 (7.5%) had unsatisfactory smears for evaluation.

Table 4
Pap smear findings

Pap smear Findings	Frequency	Percent
NILM	259	87.8
Abnormal smear	14	4.7
Unsatisfactory smear	22	7.5
Total	295	100.0

A total of 295 women were enrolled in the study. Twenty two were excluded from the analysis due to inadequate smear or missing cervical cells at all. The prevalence of cervical cytology abnormalities in this research was 14 out of 273 (5.1%) with HSIL being the most prevalent at 5 out of 273 (1.8%), ASC-H 4out of 273 (1.46%), LSIL 3 out of 273 (1.05%), SCC was seen in 1 out of 273 (0.36%) and lastly Adenocarcinoma was also seen in 1 out of 273 (0.36%).

Figure 1
shows the Bethesda classification of the cervical cytology abnormalities.

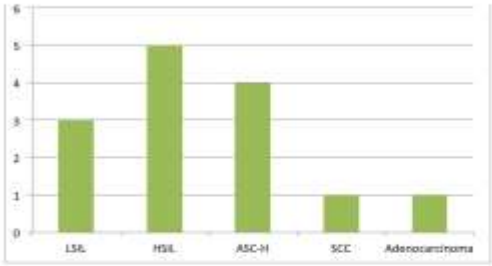

Table 5
Cross tabulation of Age groups and Pap smear findings

Age	NILM Abnormal	Unsatisfactory	Total	Pearson Value	P-Value
20 – 29	27(10.4%)	1(7.1%)	2(9.1%)	30(10.2%)6.262	0.618
30 - 39	72(27.8%)	3(21.4%)	5(22.7%)	80(27.1%)	
40 - 49	90(34.7%)	3(21.4%)	6(27.3%)	99(33.6%)	
50 - 59	53(20.5%)	6(42.9%)	6(27.3%)	65(22.0%)	
60 and above	17(6.6%)	1(7.1%)	3(13.6%)	21(7.1%)	
Total	259(100.0%)	14(100.0%)	22(100.0%)	295(100.0%)	

DISCUSSIONS

In this study, the prevalence of abnormal cervical cytology was 5.2% which is comparable to 6% as documented in a study done in Nigeria by Ononogbu, et al to determine cervical cancer risk factors among HIV-infected Nigerian women in 2013 (8).

However, this prevalence is lower compared to other studies done in Kenya and Africa.In a study done in Kenya by Memiah et alto determine the prevalence and risk factors associated with precancerous cervical lesions among HIV infected women, the prevalence of abnormal cervical cytology was 26.7% (9). Another unpublished study done at Kenyatta National Hospital in 2016 to determine cervical cytological patterns among HIV infected women on antiretroviral therapy, the prevalence of abnormal cervical cytology was 9.9%.

Studies conducted in Rwanda and Ethiopia reported a prevalence of cervical pre-cancer and cancer among HIV-positive women of 20.0% and 22.1% respectively (10, 11). Another study in Southern Nigeria to determine effect of low CD4 Cell count on cervical squamous intraepithelial lesions among HIV-positive women reported a 5.7 % prevalence of SIL among the high CD4 group and 10.2% among the low CD4 group. (12)

The national cervical cancer screening guidelines recommends that HIV infected women should be screened every 1 year because of their high risk to development of cervical cytological lesions. At Machakos comprehensive care center, VIA/VILLI

are done annually and positive cases referred for further management. Most of the women in this study assented to having the VIA/VILLI done in 2015 at the Comprehensive Care Center.

Early initiation and duration of combined antiretroviral therapy may have contributed to less prevalence rate as all the women in this study were on antiretroviral therapy.A study done by Adler, et al showed that women on HAART with a normal baseline smear were 38% less likely to have abnormal Pap smear on follow-up. (13)

In this study, there was no statistically significant associationbetween age and abnormal Pap smear findings. This is consistent with another study done in Rwanda by Kayumba in 2013 which reported lack of statistically significant association between age and abnormal Pap smear findings. (10)

ACKNOWLEDGEMENTS

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APPENDIX VII: PUBLICATION 2

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The Utility of a Manual Liquid Based Cytology in Screening for Pre-Cancerous Lesion and Cervical Cancer

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Abstract
Introduction: *Liquid-based cytology is a technique that enables cells to be suspended in a liquid medium and spread in a monolayer, thereby enabling a better morphological assessment. Automated techniques have been widely used especially in the developed countries but limited in the developing countries due to cost and availability.*
Conventional Pap smear (CPS) examination has been the commonly used method for detection of cervical cancer. However, there have been challenges in its use due to the inherent limitations, like presence of obscuring blood and inflammation which has reduced its sensitivity considerably. On the other hand, manual liquid based cytology (MLBC) is a technique that is cost effective and improves detection of precursor lesions and specimen adequacy.
Methodology: *A total of 295 women were assessed for pre-cancerous lesions and cervical cancer using Manual Liquid Based Cytology and Conventional Pap Smear method.Cohen Kappa test was run to determine the level of agreement the two methods.*
Results: *There was moderate agreement between the two methods (k=0.673, 95% CI, p=0.065). Specimen adequacy was found to be better with MLBC than CPS with 12 unsatisfactory smears in MLBC and 22 in CPS. There was increased detection rate of abnormal cervical cytology smears with MLBC of 85.7%.*
Conclusion: *Manual liquid based cytology was found to give better results than conventional Pap smear and therefore it can be used as an alternative liquid based cytology technique for cervical cancer screening in limited resource settings.*
Keywords: *Manual Liquid Based Cytology, Conventional Pap Smear.*

Introduction

The introduction of cytologic screening for cervical cancer using the Papanicolaou (Pap) test in the 1950 has led to a reduction in the incidence of invasive cervical cancer in the developed countries. This has been attributed to the effective

screening and treatment programs in these countries[1]. However, the establishment and implementation of Pap smear programs have not yet been possible in the developing countries and thus cancer of the cervix continues to threaten the lives of women from these countries up to date. 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[2,3].

Conventional Pap smear (CPS) examination has been the commonly used method for detection of cervical cancer. However, there have been challenges in its use due to the inherent limitations which include: Majority of cells not captured as only a portion of the sample is smeared onto a microscope slide after collection. Furthermore, there is no representative transfer of the sample as the collection device is discarded, sometimes with more than 80% of the patient's sample still on the device. [4,5]

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 conventional Pap smear specimen may often be clouded with debris such as blood and mucus, which obscures cell visibility[5] 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 incase needed. These limitations have been shown to reduce the sensitivity of CPS to less than 50% [5, 6]

An automated liquid-based cytology (ALBC) for cervical cancer screening was developed to improve the sensitivity, but the very high cost related to automated devices has hampered its implementation in the setup of developing countries, like Kenya.

A screening technology that matches the limited resources we have in Kenya will be of benefit both to physician and the patient.It is in this regard that the current study was done to develop and evaluate the utility of a low cost manual liquid based cytology method in screening of pre-cancerous and cervical cancer. This was expected to increase the survival of cervical cancer patients and thus improving their health condition and quality of life.

Methodology

The present study was carried out at Machakos Level 5 Hospital of Machakos County in South - Eastern part of Kenya. The study was done at the comprehensive care Centre which serves around 2000 women infected with HIV aids. Ethical approval was obtained from the Kenyatta University Research and Ethical Review committee (Protocol Number: PKU/464/E42). The population of the present study was composed of women of 18 years and above who were sexually active and attending Machakos level 5 Comprehensive Care Centre (CCC).Two hundred and ninety five women were included in this study.

Women who were pregnant or declined to complete an informed consent form were excluded from the study. Women on treatment for precancerous lesion or cervical cancer and a previous hysterectomy were not eligible. Convenience sampling method was used, with this method, patients available during the study period and willing to participate were considered until the sample size was reached. Sampling was done until the intended sample size was obtained. After obtaining a signed consent from the patient, the clinician explained the procedure, assured and placed the patient in a comfortable and convenient position for sample collection. First sample was collected using cytobrush for conventional Pap smear. The cytological material obtained was spread on glass slides directly and fixed immed-iately in 95% ethanol for at least 15 minutes.

A second sample was collected using a different cytobrush. The cytological material was transferred with brushes into a formulated liquid fixative (containing sodium chloride, sodium citrate, 10% formalin and isopropyl alcohol). Brushes were broken off into the container of collection fluid. The collected samples were

vortex-mixed, and then about 10ml transferred into a formulated alcoholic-agar (polymer solution containing agarose, polyethylene glycol, poly-l-lysine and alcohol) in nipple-bottom test tubes. Test tubes were centrifuged for 10 minutes at 2000rpm. The supernatant was discarded and from the deposit smear made on a clean glass slide using a Pasteur pipette. The prepared slides were fixed by drying them in a hot air oven for 15 minutes at 50˚C. The slides were further fixed by dipping them in 95% alcohol for 15 minutes. All the smears were stained using the Papanicolaou staining method.

All the Pap smears were screened by the principal investigator and signed out together with a pathologist. The Bethesda system 2014 for reporting cervical cytology was used for reporting all the cytological abnormalities observed during examination and reporting. All abnormal cytological smears of the study patients were blindly and independently re-evaluated by a board certified pathologist. The abnormal smears were triaged for further investigation.

Cohen's kappa test was done to determine the agreement between CPS and MLBC. Increased detection rate (IDR) was calculated as follows: IDR= ((Pm-Pc)/Pc)*100, where Pm is the number of positive cases through MLBC and Pc is number of positive cases for conventional Pap smear.

Results

Table 1 Pap smear findings as seen in the conventional Pap smear method

Pap smear Findings	Frequency	Percent
NILM	259	87.8
Abnormal smear	14	4.7
Unsatisfactory smear	22	7.5
Total	295	100.0

Table 1 shows the Pap smear findings as seen in the conventional Pap smear method. Out of the total women recruited in this study, 259 (87.8%) were Negative for intraepithelial lesion or malignancy, 14 (4.7%) had abnormal smears while 22 (7.5%) had unsatisfactory smears for evaluation. A total of 295 women were enrolled in the study. Twenty two were excluded from the analysis due to inadequate smear or missing cervical cells at all. The prevalence of cervical cytology abnormalities as seen by the conventional Pap smear method in this research was 14 out of 273 (5.1%) with HSIL being the most prevalent at 5 out of 273 (1.8%), ASC-H 4 out of 273 (1.46%), LSIL 3 out of 273 (1.05%), SCC was seen in 1 out of 273 (0.36%) and lastly Adenocarcinoma was also seen in 1 out of 273 (0.36%).

Table 2 Pap smear findings as seen in the MLBC method

Pap smear Findings	Frequency	Percent
NILM	257	87.1
Abnormal smear	26	8.8
Unsatisfactory smear	12	4.1
Total	295	100.0

Table 2 shows Pap Smear Findings using the Manual Liquid based cytology Method. Out of the 295 Pap smear samples screened using the manual liquid based cytology technique, 257 (87.1%) were Negative for intraepithelial lesion or malignancy, 26 (8.8%) had abnormal smears while 12 (4.1%) had unsatisfactory smears for evaluation. LSIL was the most prevalent at 9 (2.7%), HSIL 7 (2%), ASC-H 5 (1.6%), ASCUS 3 (1%), Adenocarcinoma 1 (0.3%) and squamous cell carcinoma 1(0.3%)

Table 3 Cross tabulation of Conventional Pap smear and Manual Liquid based cytology results

		MLBC			Total	kappa	P value
		NILM	Abnormal	Unsatisfactory			
CP	NILM	247	12	0	259	0.673	0.065
	Abnormal	0	14	0	14		
	Unsatisfactory	10	0	12	22		
Total		257	26	12	295		

Table 3 shows Cross tabulation of Conventional Pap smear and Manual Liquid based cytology results. There was moderate agreement between the two methods (k=0.673, 95% CI, p=0.065).

Comparison of cytomorphological patterns between Conventional Pap smear and Manual Liquid based cytology

Figure 1 High-Grade Squamous Intraepithelial Lesion on MLBC

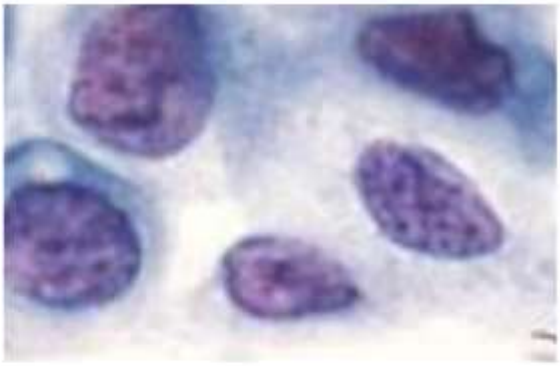
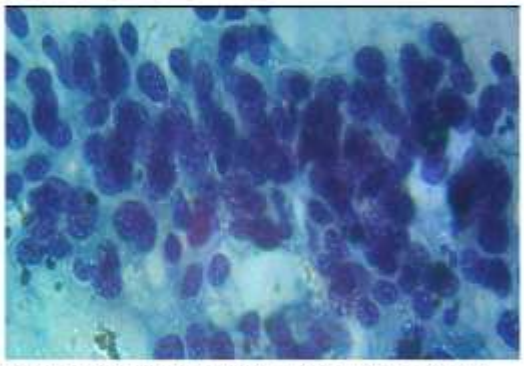


Figure 2 High-Grade Squamous Intraepithelial Lesion on MLBC



With the MLBC technique, majority of the smears showed cells suspended in a monolayer thus improved detection of precursor lesions and improvement of specimen adequacy. A clear background was also observed for the MLBC smears with minimal obstruction of cells by debris, mucus or blood further improving the specimen adequacy (Figures 1 and 2)

Increased detection Rate

Increased Detection Rate with (IDR) was calculated as follows;

IDR= ((Pm-Pc)/Pc)*100, where Pm is the number of positive cases through MLBC and Pc is number of positive cases for conventional Pap smear.

$$IDR = \frac{(26-14)100}{14}$$

$$= 85.7\%$$

Thus Increased detection rate with MLBC in this study was 85.7%

Discussions

In this study, specimen adequacy was found to be better with MLBC than CPS with 12 unsatisfactory smears in MLBC and 22 in CPS. Many studies have reported LBC to be better in terms of specimen adequacy compared to CPS. Majority of studies comparing LBC and CPS found that the quality of slides improved in LBC, which is consistent with the results obtained in our study that MLBC has higher satisfactory specimen rates as compared to CPS.

With the MLBC technique, majority of the smears showed cells suspended in a monolayer thus improved detection of precursor lesions and improvement of specimen adequacy. A clear background was also observed for the MLBC smears with minimal obstruction of cells by debris, mucus or blood further improving the specimen adequacy. In a study done by Kavatkar et al. (2008), MLBC was found to give a clear background in more smears than in conventional Pap smear.[6]

In CPS, there was higher rate of unsatisfactory smears due to the presence of obscuring blood, inflammation and dirty background which obscured the epithelial cells thus affecting the screening process. Also, in CPS only 20% of the cells collected on the brush are smeared on to the slide leading to lesser cells being transferred to the smear for screening thus unsatisfactory smears for evaluation[7]. Specimen adequacy in MLBC was also better compared to CPS due to the fact that the entire specimen collected from the cervix was transferred to the fixative solution for processing without any wastage.

In the current study 7.5% cases were unsatisfactory with conventional Pap smear method while with MLBC, 4.1% cases were found unsatisfactory due to inadequate cells for examination. A study done in Pakistan to compare cervical cell morphology using MLBC and CPS recorded 27% cases of unsatisfactory smears for conventional Pap smear method and 24% with the MLBC technique[9]. Other studies have documented lower percentages for unsatisfactory smears with LBC technique. Bergeron et alreported (0.14%) while Garbar et alfound (0.9%). In these studies, automated liquid based cytology technique was used thus the lower percentages of unsatisfactory smears compared to the current study. [12,13]

In LBC, the sample is first placed in fixative solution followed by further processing instead of making slides directly as in CPS. This makes cellular structure better preserved with reduced drying artifacts as cells are immediately fixed [11, 14]. In MLBC there is marked decrease in artifacts, contaminating mucus and blood. Cells are evenly distributed on slides and centrifugation in this method offers a proper mixing[15]. Similarly, in the current study cellular overlapping is reduced with majority of the cells forming a monolayer with a clear background. Thus manual liquid based cytology gives more clear results with clear background, less artifacts and lesser degree of cellular overlapping when compared with conventional Pap smear.

In the current study, the level of agreement between MLBC and the conventional Pap smear method was 67.3%. This is comparable to 68% as documented in a study done in India by Nandini et al[8]. In another study done in India on manual method of liquid-based cervical cytology by Kavatkar et al, the level of agreement between MLBC and the conventional Pap smear method was found to be 88%[6]. However, in another study done by Moosa et al, conventional Pap smear method was found to be comparable to the MLBC technique in terms of specimen adequacy and detection of abnormal cervical cytology[9]. In the current study, there was increased detection rate of abnormal cervical cytology smears with MLBC of 85.7%. However, this is low compared to 150% as documented in a study done by Nandini et al[8]

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