A COMPARATIVE ANALYSIS OF LIQUID-BASED PREPARATIONS AND CONVENTIONAL PAP SMEARS WITH COLPOSCOPIC BIOPSY AT KENYATTA NATIONAL HOSPITAL

RESEARCH DISSERTATION SUBMITTED AS PARTIAL FULFILMENT FOR MASTER OF MEDICINE IN OBSTETRICS AND GYNAECOLOGY, UNIVERSITY OF NAIROBI.

PRINCIPAL INVESTIGATOR:

Dr. Macharia H. Chege, MBChB

MMED Student, Department of Obstetrics and Gynecology,

University of Nairobi.

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DECLARATION

This dissertation is my original work and has not been presented for research for a degree at any other university.

Dr. Macharia H. Chege

Signed: ……. Date: ……

APPROVAL:

This is to certify that the commentary in this dissertation was researched upon by Dr. Macharia H. Chege under our guidance and supervision, and that it is submitted with our approval.

Dr. Eunice Cheserem

Senior lecturer

Consultant Obstetrician / Gynecologist

Department of Obstetrics and Gynecology

University of Nairobi.

Signed: ……. Date: .....

Professor Elizabeth Bukusi

Honorary Lecturer

Consultant Obstetrician / Gynecologist

Department of Obstetrics and gynecology

University of Nairobi.

Signed: …… Date: ……

Dr. Lucy Muchiri

Senior Lecturer

Department of Human Pathology

School of Medicine

College of Health sciences

University of Nairobi.

Signed: …….. Date: …….

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CERTIFICATE OF AUTHENTICITY

This is to certify that this dissertation is the original work of Dr Macharia H. Chege, Master of Medicine student in the Department of Obstetrics and Gynaecology, Registration number H58/70861/07 University of Nairobi (2007-2012). The research was carried out in the department of Obstetrics and Gynaecology, School of Medicine, College of Health Sciences. It has not been presented in any other University for the award of a degree.

Signature : .....

Date : .....

Prof. Koigi Kamau,

Associate Professor of Obstetrics and Gynaecology

Consultant Obstetrician and Gynaecologist,

Chairman, Department of Obstetrics and Gynaecology,

University of Nairobi.

LIST OF ABBREVIATIONS

AHRQ	Agency for Healthcare Research and Quality
AGUS	Atypical Glandular cells of Undetermined Significance
ASCUS	Atypical Squamous Cells of Undetermined Significance
ALTS	ASCUS/LSIL Triage Study
BTL	Bilateral Tubal Ligation
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma Insitu
CPAP	Conventional Pap smear
DES	In utero Diethylstilbestrol
DMPA	Depomedroxy Progesterone
DNA	Deoxyribonucleic Acid
ERC	Ethics Research Committee
FDA	Food and Drug Administration
FWC	Family welfare Clinic
GOPC	Gynecology out Patient Clinic
HGSIL	High Grade Squamous Intraepithelial Lesion
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
IUCD	Intrauterine Contraceptive Device
KNH	Kenyatta National Hospital
LBC	Liquid Based Cytology
LGSIL	Low Grade Squamous Intraepithelial Lesion
LMP	Last Menstrual Period
M.MED	Masters in Medicine
OCPS	Oral Combined Pills
Pap	Papanicolaou
SCC	Squamous Cell Carcinoma
SCJ	Squamocolumnar junction
Thin Prep	Thin Film Preparation
UON	University Of Nairobi

DEDICATION

To my late Dad and Mom for the sacrifices you made to ensure I got my education to this level. And to my lovely wife Lilian and wonderful sons Sammy and Sean Chege for your love, support, encouragement and perseverance.

ACKNOWLEDGEMENT

I am grateful to the Ministry of Medical Services for awarding me a scholarship for my postgraduate studies.

Special gratitude to my supervisors Prof Elizabeth Bukusi, Dr Eunice Cheserem and Dr. Lucy Muchiri for their useful critique and guidance offered throughout the process of writing this dissertation. I am also grateful for the support from the department of Obstetrics and Gynaecology, foremost the Chairman, Prof Koigi Kamau for guidance and encouragement, and the entire academic staff; your dedication and wise counsel were always helpful. I wish to appreciate the administration of Kenyatta National Hospital for allowing me to use their facilities to collect and process the samples for this study.

I greatly appreciate the services of Dr. Mungania of KNH Pathology Department for ensuring timely reporting of conventional pap smears and Dr Lucy Muchiri for reporting the liquid based preparation slides, Josephine and her colleagues of KNH cytology Lab, Wangechi for processing the LBC samples, sister Mutemi and her colleagues of clinic 66, and Francis Njiri the statistician.

Finally, to all my colleagues thank you for the interaction and assistance.

LIST OF FIGURES AND TABLES:

ABSTRACT:

BACKGROUND: Cervical cancer is one of the most common female malignancies worldwide. Since the introduction of conventional Papanicolaou smear mortality from cervical cancer has reduced considerably. Despite its success, it has sensitivity of only 51% and false negative rate of 5-10%. Approved LBC products by FDA claim a 65-percent increased detection rate of HSIL compared with conventional smears, as well as decreased unsatisfactory sample rates. Evidence shows that Liquid Based Preparation is more sensitive and accurate for the detection of both squamous and glandular lesions of the cervix. Studies of the accuracy of liquid based preparations reports sensitivity of 61-66% and specificity of 82-91%.

OBJECTIVE: To compare liquid based preparation and conventional pap smears with Colposcopic biopsy results at Kenyatta National Hospital.

STUDY DESIGN: This was a hospital-based comparative cross-sectional study. Eligible clients were recruited into the study by convenient sampling over a period of 4 months between August and November 2011.

METHODOLOGY: Clients referred to colposcopy clinic with abnormal Pap smear results who met inclusion criteria were recruited into the study by convenient sampling. Socio-demographic data was collected. Before colposcopy/biopsy a split sample technique was used to collect conventional Pap smear and liquid based Pap smear.

SETTING: The study was conducted at the Kenyatta National Hospital's colposcopy clinic.

RESULTS: A total of 73 patients referred with abnormal pap smears who met the inclusion criteria were interviewed and samples taken. The mean age of the patients was 38 yrs (SD +-10). More than 50% of the patients interviewed were aware of Pap smear. Less than 20% of these had had a pap smear before referral while 34% despite being aware about a Pap smear had not done any before. About 45% of the patients were not aware about a Pap smear prior to referral.

Both the results of referral pap smear and repeat pap smear were predominantly squamous lesions i.e. LSIL or HSIL.

There were more unsatisfactory smears with liquid based cytology 14(19%) compared to 4(6%) for conventional pap smears.

Liquid based cytology had higher sensitivity in detection of both squamous and glandular lesions than conventional Pap smear.

There was significant increase of false negatives with repeat CPAP (P <0.0001) compared to LBC.

There was increased detection of low grade lesions with LBC 22% compared to conventional pap smears 11% of which 29% of the samples were true LSIL on histology. This was a significant difference for CPAP (P 0.007).

Both tests performed well in detection of HSIL/CIS with no significant difference when compared with histological diagnosis.

There was significant difference with repeat CPAP for detection of SCC (P 0.013).

However, there was no significant difference in the detection of glandular lesions for both tests. In general, the results of repeat CPAP and LBC were in agreement with a kappa of 0.84.

CONCLUSION: Liquid based cytology had a higher sensitivity of 92% as compared with conventional pap smears' 57%. However, it had low specificity of 13% as compared with 50% for conventional Pap smear. The positive predictive values for the two tests were the same. The negative predictive value for liquid based cytology was higher compared to that of conventional Pap smear. Overall, liquid based cytology is more accurate than conventional Pap smear in detection of cervical intraepithelial lesions.

RECOMMENDATION:

1. In patients with abnormal pap smears requiring a repeat pap smear, liquid based cytology is recommended due to its higher sensitivity compared to conventional Pap smear. . However, the cost of this new technology in cervical cancer screening is more than of conventional pap smears. In normal population, conventional Pap smear remains the screening test of choice.

CHAPTER 1: INTRODUCTION, LITERATURE REVIEW, RATIONALE AND OBJECTIVES.

1.1 INTRODUCTION

Cervical cytologic screening is one of modern medicine's greatest success stories. In 1943, George Papanicolaou at Cornell University Medical College laboratories initiated what would become valuable as a screening test world over. This has had a tremendous impact in limiting the development of invasive squamous carcinoma of the uterine cervix, therefore reducing the mortality from cervical cancer.

Use of Pap Smears

A useful screening test should identify asymptomatic patients at high risk for a disease of significant morbidity and mortality at a point in the disease course where intervention can alter the outcome. Cervical cytologic study does this well. At the same time, an optimal screening test meets these objectives at low cost and with acceptable sensitivity and specificity. Precise definition of the sensitivity and specificity of cervical cytologic study is impossible because the gold standard against which it must be compared is histologic evaluation of the entire cervical transformation zone; the performance of random cone biopsies on cytologically normal women solely to determine these rates are not be justified ethically.

Test description: The Pap test aims to identify abnormal cells sampled from the transformation zone, the junction of the ecto- and endocervix, where cervical dysplasia and cancers arise. For conventional Pap smears, cervical samples obtained by brush and spatula are plated on a microscope slide and preserved with fixative.

Thin layer (or liquid-based) cytology, an alternative to conventional cytologic sampling, has been widely implemented in the US. Testing involves transferring samples from the brush and spatula into a liquid fixative solution; the cytology lab subsequently traps the loose cells onto a filter from which they are plated in a monolayer onto a glass slide. The collected specimen can also be used for other diagnostic assessments (i.e., testing for gonorrhea, Chlamydia, and HPV).

Cytology report: Components of a cytology report include the following:

- A description of specimen type — conventional Pap smear, liquid based cytology, or other
- A description of specimen adequacy
- A general categorization (optional) — negative, epithelial cell abnormality, or other
- An interpretation/result — either the specimen is negative for intraepithelial lesions and malignancy (although organisms or reactive changes may be present), or there is an epithelial cell abnormality as defined by the Bethesda 2001 classification, or there is another finding. This latter category may indicate some increased risk, as an example: endometrial cells in a woman over 40 years of age
- A description of any ancillary testing or automated review that was performed (e.g., HPV, AutoPap)
- Educational notes and suggestions by the pathologist (optional)

Pap smear results are now widely classified according to the Bethesda system, first introduced in 1988, and revised in 2001, to standardize and improve the clinical usefulness of Pap smear reports[1] (Appendix 6). The intent of the Bethesda system is to distinguish between abnormalities which are unlikely to progress to cancer and those which are more likely to indicate a precancerous or cancerous lesion. The Bethesda system also includes guidelines for determination of specimen accuracy. Cellular atypia is categorized in the Bethesda system as low grade squamous intraepithelial lesion (LSIL) or high grade squamous intraepithelial lesion (HSIL).

Limitations: The Pap smear is designed as a screening test (to be administered to asymptomatic patients), rather than a diagnostic test (to confirm or refute the suspicion of disease). Reports of test sensitivity and specificity vary significantly, and the screening test is far from perfectly accurate.[2]Considerable interobserver variability in smear interpretation is seen, although variability decreases for smears with more severe abnormalities.[3]

Sources of potential error in sampling and evaluating the Pap smear include:

- The clinician may not sample the area of cervical abnormality.
- The abnormal cells may not be plated on the slide or transferred to the liquid medium.
- The cells may not be adequately preserved with fixative.
- The cytopathologist may not identify the abnormal cells.
- The cytologist may inaccurately report the findings.

Effectiveness: Cytologic screening for cervical cancer has never been evaluated in a randomized controlled trial. Evidence of its effectiveness in reducing the incidence of and mortality from cervical cancer comes exclusively from observational studies. Such studies provide consistent and compelling evidence that has led to the adoption of cervical cytology screening in all developed and many developing nations worldwide. Despite the absence of clinical trials, cervical cytologic study remains the standard tool for cervical cancer screening. Several reports correlate significant declines in the incidence of cervical cancer with the institution of widespread screening programs.[4-7] It has been proposed that cervical cancer could be eradicated if all women complied strictly with screening guidelines.[8]Nevertheless, despite widespread incorporation of cytologic screening into clinical practice, cervical cancer remains a significant public health problem.

The reasons for this are many and have been reviewed.[8] Unfortunately, many women fail to comply with screening recommendations, and many patients with cervical cancer acknowledge that they have not been screened adequately.[9] The reasons for this are not clear, but studies suggest that significant reasons for inadequate screening include patient's ignorance of guidelines, dislike of pelvic examination, lack of access to the medical care system, fear of cancer, fear of pain from diagnostic procedures, and mistrust of medical authorities.[10] Women who fail to receive adequate screening tend to be older and less educated and belong to minority ethnic groups.[10,11] Compliance with screening guidelines has been shown to be improved by education and physician-initiated reminders.[12,13]

The false-negative rate falls significantly when serial smears are taken. Using published data and statistical analysis, Eddy estimated that the risk of cervical cancer fell 64% when screening was performed at 10-year intervals, a result that improved to 91% with 3-year screening intervals.[14]Increasing the frequency of sampling to annually results in only a small further decrease in risk. Epidemiologic risk factors that favor annual screening include early initiation of sexual activity, a history of multiple lifetime sexual partners, tobacco use, and immunosuppression.

Unfortunately, high-risk women may lack sufficient sophistication to respond correctly to queries about screening.[15]Across socioeconomic classes, the reliability of women's recollection of the interval since the last smear and of their history of abnormal findings may be unreliable.[12] Without proper documentation, a lifetime history of normal smear results cannot be assumed.

Alternative Methods for Cervical Cancer Screening

Because the sensitivity of cytologic study as a screening tool for cervical cancer and its precursors is imperfect, alternative techniques for the identification of these abnormalities have been proposed. However, none has yet supplanted cytologic study, and all are currently experimental or limited to certain populations. Most have been tested as adjuncts to cytologic study, improving the sensitivity of Pap smear screening rather than replacing it.

HPV Typing: Women with positive screen results for HPV are at increased risk for CIN, and women infected with HPV types 16, 18, 31, 33, and 35 are more likely to have high-grade CIN.[16]However, although the sensitivity of HPV typing appears to be excellent, its specificity is limited.[17, 18] HPV typing is not indicated for women with smears read as HSIL or cancer, for whom the prevalence of high-grade CIN justifies immediate colposcopy. The high rate of positivity in women with LSIL smears negates its use.[19] The added expense of HPV testing does not appear to be a cost-effective improvement over serial cytologic study for women with atypical smears, [20] although it may have a role in clinical settings where access to colposcopy is restricted.[21]

The utility of HPV testing may change as new assays are tested, and more definitive recommendations are likely to follow completion of a multicenter trial of HPV testing and other strategies for the management of ASC-US and LSIL smears.[19] The use of HPV testing as a primary screen has shown promise in developing countries but remains experimental.[22]

Colpophotography: Most often used as an adjunct rather than a replacement for Pap smears. It involves the use of special photographic equipment to capture an image of the cervix after staining with acetic acid. Slides then are sent to a diagnostic center, where they are projected for magnified viewing by expert colposcopists who assess the transformation zone for abnormalities. Patients with abnormal or equivocal results can be referred for formal colposcopy.

Colposcopy: Used in parts of Europe as a component of the standard gynecologic examination and as part of a screen for cervical cancer and its precursors. However, costly equipment and the need for intensive training make colposcopy prohibitively expensive as an initial screen. It may have a role in populations with a high incidence of CIN, such as women infected with the HIV. HIV-infected women often have multiple risk factors for the development of cervical cancer, and their immunosuppression appears to allow CIN and cancer to develop at an accelerated rate.[23] In some studies, the prevalence of CIN in HIV-infected women exceeds 30% – sufficient frequency to consider colposcopy screening.[24]However, the Centers for Disease Control and Prevention recommend that women with HIV be screened cytologically; if initial screening results are normal or if initial smear shows reactive, inflammatory findings, screening should be repeated after 6 months.[25]The Bethesda guidelines for management do not apply to this high-risk group, and colposcopy appears to be indicated for all HIV-infected women with other abnormal smears, including ASCUS.[25, 26]

Visual inspection: In areas where Pap smear screening is not available or affordable, other methods of testing have been evaluated. Visual inspection of the cervix, using acetic acid (white vinegar; VIA) or Lugol's iodine (VILI) to highlight precancerous lesions so they can be viewed with the "naked eye", shifts the identification of precancer from the laboratory to the clinic. Such procedures eliminate the need for laboratories and transport of specimens, require very little equipment and provide women with immediate test results. A range of medical professionals—doctors, nurses, or professional midwives—can effectively perform the procedure, provided they receive adequate training and supervision. As a screening test, VIA may perform as well as or better than cervical cytology in accurately identifying pre-cancerous lesions.[27] This has been demonstrated in various studies where trained physicians and mid-level providers correctly identified between 45% and 79% of women at high risk of developing cervical cancer.[28]

By comparison, the sensitivity of cytology has been shown to be between 47 and 62%. Cytology provides higher specificity (fewer false positives) than VIA. Like cytology, one of the limitations of VIA is that results are highly dependent on the accuracy of an individual's interpretation.

This means that initial training and on-going quality control are of paramount importance. Increased false positives are particularly important in a screen-and-treat setting, since over-treatment and resulting impairment of fertility is more likely.

VIA can offer significant advantages over Pap in low-resource settings, particularly in terms of increased screening coverage, improved follow-up care and overall program quality. Due to the need for fewer specialized personnel and less infrastructure, training, and equipment, with VIA public health systems can offer cervical cancer screening in more remote (and less equipped) health care settings and can achieve higher coverage. Furthermore, providers can share the results of VIA with patients immediately, making it possible to screen and treat women during the same visit. This helps ensure that follow-up care can be provided on the spot and reduces the number of women who may miss out on treatment because they are not able to return to the clinic at another time. VIA has successfully been paired with cryotherapy, a relatively simple and inexpensive method of treating cervical lesions that can be performed by primary care physicians and mid-level providers.[29]

Speculoscopy: Speculoscopy is similar to VIA, with the addition of a blue-white chemiluminescent light source attached to the upper speculum blade. The examiner can assess the cervix for acetowhite lesions directly or with the use of limited magnification (4 to 6x).

Cervicography: Cervicography refers to standardized photography of the cervix after the application of acetic acid. The 35 mm magnified images can then be interpreted by qualified evaluators anywhere in the world, and the patient triaged accordingly. In some clinical settings, Cervicography is considered to be an adjunct to cervical cytology rather than a primary screening method.

Computerized Pap screening or rescreening: Available technologies include AutoPap, PapNet, and Thin Prep. The Thin Prep Pap test has addressed the limitations of conventional pap smear with liquid based preparations, improving specimen adequacy and significantly increasing the test sensitivity. Approved by FDA in 1996, it was the first liquid based pap test and today is the 'gold standard' of pap tests. Since its introduction, it has contributed to further 30% reduction in invasive cervical cancers in the United States.[30]

It is a liquid-based pap test that employs a fluid transport medium to preserve cells and an automated process to eliminate debris and distribute a representative portion of cells on a slide in a uniform, even layer; and is reported to be more effective than conventional Pap smear in detection of squamous intraepithelial lesions of the cervix.[31-35] In addition to its improved slide quality, proprietary technology eliminates common errors associated with preparing a specimen. Also, thin layer liquid-based cervical cytology technology has the advantage over conventional techniques of being able to co-test for HPV and other sexually transmitted diseases.[36] Thin Prep has also been approved by the United States FDA for Chlamydia and gonorrhea testing. Thus the thin layer cytology may save the patient an additional test or visit.

Liquid-based methods are currently used by many physicians to collect and preserve the material collected from the cervix and vagina. Cytyc Corporation (Thin Prep System) and TriPath imaging (SurePath) are the two vendors currently offering Food and Drug Administration (FDA)- approved liquid-based technology for cervicovaginal specimens. Liquid-based preparations may decrease preanalytic errors by fixating the sample more rapidly (preventing air drying and better preservation), decreasing artifacts and obscuring cells, such as mucus, cellular debris and red blood cells (RBCs)/white blood cells [WBCs], and homogeneously mixing the sample. There are data to suggest that utilization of liquid based technology can increase the sensitivity for detection of preneoplastic/neoplastic lesions of the cervix.[31-35] However, regular screening remains the most effective means of cancer prevention.

The goal of this study was to compare the accuracy of the two tests in detection of cervical intraepithelial lesions with Colposcopic biopsies at Kenyatta National Hospital.

1.2: LITERATURE REVIEW

Hippocrates and Galen described invasive cancers of the cervix, but the existence of asymptomatic neoplasms within the cervical epithelium was not recognized until early in the last century. The pre-invasive nature of these lesions has been clarified only in the last few decades. With the development of techniques that allow molecular biologists to explore genomic changes in dysplastic cells the fundamental biology of cervical intraepithelial neoplasia (CIN) has begun to emerge.[37]

Cervical CIS was described in the early 1900s. However, the clinical importance of these lesions was not appreciated until useful means for detecting these asymptomatic, invisible lesions were developed. Beforehand, cervical cancer detection relied on inspection and palpation, with biopsy of obvious invasive cancers. Schiller developed a technique for iodine staining as a gross means for detecting areas of abnormal epithelium, but this test could not distinguish metaplastic from neoplastic areas of the cervix and could not distinguish small areas of invasion present in a field of diffuse nonstaining epithelium. In this era, the nature of intraepithelial lesions was controversial, often described at the margin of invasive lesions but at times noted as a precursor to invasion. Nevertheless, the description in the 1920s and 1930s of what came to be known as CIN provided the foundation for the development of cytologic study.[37]Cytologic examination of exfoliated cervical epithelial cells was first described by Babes in 1928 in the French literature, only with the appearance in 1941 of the findings of Papanicolaou and Traut did this technique enter clinical practice as a means for the early diagnosis of cervical cancer. Papanicolaou described a technique for aspiration of cells from the posterior vaginal pool, fixation, and cytologic staining that remains the foundation of current screening strategies.[37]

A 70% decrease in cervical cancer deaths over the past 50 years has been credited in large part to regular screening with Pap smear testing. Available data indicates that in the late 1930`s and early 1940`s, cervical cancer accounted for 25-30 deaths per 100,000 women. In 1992, the death rate for cervical cancer was 300 per 100,000 women. Organizations including the National Cancer Institute and the Centers for Disease Control and Prevention, state that the death rate for cervical cancer would be even lower if all women of reproductive age who are sexually active received periodic pap tests.[38, 39] Only second to breast cancer, cervical carcinoma is the most common malignancy among women worldwide.[40]Its high mortality makes cervical cancer an important public health problem.

In the United States (US) in 2008, there were estimated to be 11,070 new cases of invasive cervical cancer, and 3870 cancer-related deaths are expected; this represents approximately 1 percent of cancer deaths in women. Incidence and mortality associated with cervical cancer are higher among minorities, as illustrated by 2006 to 2008 American Cancer Society Statistics.[41]

Global incidence and mortality rates are even more disparate. There has been a 75 percent decrease in the incidence and mortality of cervical cancer over the past 50 years in developed countries. In contrast, cervical cancer is the second most common cause of cancer-related morbidity and mortality among women in developing countries. In 2002, in developing countries, 493,243 new cases were observed and 273,606 deaths, corresponding to a 55 percent mortality rate. Eighty-three percent of all cases of cervical cancer worldwide occur in developing countries; this results in a cumulative risk of 1.5 percent for developing cervical cancer by age 65 years.[42]

This discrepancy is largely due to the widespread institution of cervical cancer prevention programs in developed countries, which are essentially non-existent in many developing countries. Based on a recent meta-analysis of process of care failures in the prevention of cervical cancer, poor screening history was the primary factor: 54 percent of invasive cervical cancer patients had inadequate screening histories and 42 percent were never screened.[43] In addition, while cervical cytology tests are excellent screening tools for Preinvasive disease, the false negative rate for patients with invasive cancer is relatively high: 11 to 33 percent in a series of Northern European and US studies.[43] Thus, a negative cervical cytology smear cannot be relied upon to exclude disease in a patient with signs or symptoms of cervical cancer.

In Kenya, the true incidence of cervical cancer is not known, but it is the most common gynecological malignancy. It is estimated to be 2,454 women per year with annual number of deaths estimated at 1,676 women. In the absence of accelerated interventions for screening, detection and early treatment, the incidence of cervical cancer is projected to rise to 4,261 resulting in 2955 deaths in 2025.[29]

From the cancer registry, Kaguta found that malignant tumors of the cervix accounted for 71.5% of all gynecological malignant tumors seen at the Kenyatta National Hospital between 1974 and 1981.[44]

The occurrence of invasive cervical cancer is related to age, with a mean age at diagnosis of 47 years in the United States.[41] The probability of developing cervical cancer by age is: 1 in 638 for women age 39 years and younger; 1 in 359 for women age 40 to 59 years; 1 in 750 for women 60 to 69 years; and 1 in 523 for women age 70 years and older; with a lifetime probability of 1 in 142.[41]

In Africa, cancer of cervix is known to occur at an earlier age compared to more developed countries. Ojwang et al found the mean age to be 42 years in Kenya.[45]This study was supported by a study by Rogo et al which also found a mean of 42 years at presentation.[46]

The incidence of abnormal cervical cytology is not known especially in developing countries where screening for cervical cancer and its pre-malignant precursors is poor. Local prevalence rates of abnormal cervical cytology ranges from 20.4/1000 to 26.4/1000. Kirima, in a retrospective study of 4909 smear results at KNH found a prevalence rate of 20.4/1000.[47]Ndavi in a separate study found the prevalence rate in a rural Kenyan population to be 25.6/1000 [48]. Oguntayo O et al in a retrospective study done in Nigeria found the mean age of CIN to be 37.6 years with a combined prevalence of 48/1000. [49] In another separate study, Okewole et al found the mean age of CIN to be 39.6+/- 9.6 SD.[50]

Generally, the progression to invasive cancer is a slow, predictable pattern. Longitudinal studies indicate that 30-70% of untreated patients with cervical intraepithelial lesions will develop invasive carcinoma in 10-12 years. In about 10% of the patients, lesions progress to invasive carcinoma in less than one year.[37]Prognosis for the disease is influenced by the stage, size and grade of the tumor at the time of detection, the tumor histological type, and whether or not it has spread via the blood or lymphatic systems.[51]

The risk of cervical cancer is increased for women who have had their first sexual intercourse at a young age, multiple sexual partners, high parity, recurrent vaginal infections or sexually transmitted diseases including genital herpes, genital warts and HIV infection, prolonged use of oral contraceptives, smoking, low socio-economic status, and previous history of vulval or vaginal squamous dysplasia.[52-60]

Infection with HPV is a major risk factor development of cervical cancer. Various types of HPV infection are associated with different levels of risk of development of malignant and pre-malignant lesions. Epidemiological and molecular biology studies have shown that persistent infection with high risk Human papilloma virus types 16,18,45,31 and 33 being the most frequently identified viruses in these lesions[61]. The two most common ones, HPV 16 and 18 are found in over 70 percent of all cervical cancers. Most HPV infections are transient and the virus alone is not sufficient to cause cervical neoplasia. When HPV infection persists, the time from initial infection to development of HGSIL and finally invasive cancer takes an average of 15 years, although more rapid causes have been reported.[61]

Basically, there are 4 major steps in cervical cancer development:

- Oncogenic HPV infection of metaplastic epithelium at the transformation zone.
- Persistence of the HPV infection.
- Progression of a clone of epithelial cells from persistent viral infection to precancer.
- Development of carcinoma and invasion through the basement membrane.[13]

Epidemiological data show that organized screening with pap-smear has had a major impact on both morbidity and mortality from cervical cancer: subsequently leading to a reduction of approximately 75% in incidence of carcinoma.[51]Although studies are currently underway to determine how HPV typing can be used to help determine therapy patterns for women infected with the virus, no therapy or follow up protocols have been established.

Cervical cancer is not age limited, and women should continue to receive periodic pap tests throughout their lives. Invasive cancer is most common in women between the ages of 30-50 years.

Initiation of Screening

Cervical cancer does occur in adolescents, but it is rare. Screening should begin approximately 3 years after coitarche or by age 21, whichever occurs first. Screening by age 21 regardless of sexual history protects those who are unwilling or unable to reveal sexual activity, whether consensual or resulting from abuse or assault. The 3-year window after coitarche recognizes that high-grade cervical neoplasia and cancer usually take several years to develop after exposure to HPV and avoids unnecessary detection of transient HPV infections and low-grade neoplastic lesions in adolescents.[62] Earlier commencement of screening is acceptable at the discretion of the health care provider.

After age 30, women at average risk for cervical cancer can be screened at 2- to 3-year intervals if three consecutive, annual negative Pap tests have been documented. Women at higher risk due to prior treatment for CIN 2, CIN 3, or cervical cancer, in utero DES exposure, or immunosuppressive illness or medications should receive at least annual screening. Specifically, HIV-infected women require a Pap test twice during the first year after diagnosis and annually thereafter.[63]

These guidelines should not preclude or delay other indicated gynecologic care. Supplying contraception and other medical therapies should not be contingent upon compliance with cervical cancer screening recommendations or evaluation of cytologic abnormalities, especially for adolescent.

Discontinuation of Screening

Screening may be stopped at age 65 or 70 in women not at high risk for cervical cancer. Similarly, vaginal cancers are rare, accounting for less than 2 percent of cancers in women, and screening in most women who have undergone total hysterectomy for benign disease may be halted.[64]

METHODS OF SCREENING FOR ABNORMAL CERVICAL CYTOLOGY

CYTOLOGY

There are presently two cervical cytology techniques in use: conventional and liquid-based:

Conventional Pap Collection

The conventional Pap test is a smear of cells made directly from collection device to glass slide at the time of sampling. Goodman and Hutchinson demonstrated that most cellular material remains on the collection device and is discarded after a single conventional smear is prepared. Although examination of the excess material ordinarily discarded did not result in additional diagnoses of HSIL or cancer, the discarding of most cervical material sampled has raised concern with this method.[65]

The Pap test has never been evaluated in a randomized, controlled, or masked trial.[8] However, countries with organized screening programs have consistently realized a dramatic decline, generally 60 to 70 percent, in both cervical cancer incidence and mortality.[66] The Pap test's specificity is consistently high, approximating 98 percent. However, estimates of its sensitivity are lower and more variable.

A recent meta-analysis found a sensitivity of 51 percent (95 percent confidence interval; 0.37 to 0.66) for detection of any grade of CIN by a single Pap test, but a higher sensitivity for high-grade lesions.[67] The Pap test is thought to be less sensitive for the detection of Adenocarcinomas than for squamous lesions. Eighty percent of cervical cancers are squamous, whereas 15 percent are Adenocarcinomas.[68]

Women should be aware of the imperfect sensitivity of the Pap test and the need for periodic screening to compensate. Providers likewise should use the Pap test appropriately as a screening test in asymptomatic women. Physical signs or symptoms suspicious for cervical cancer should be evaluated with diagnostic studies such as colposcopy and biopsy.

Although up to 70 percent of cervical cancer cases in screened populations are associated with either inadequate screening or surveillance of abnormal results, 30 to 40 percent develop in screened women.[69]

False-negative Pap tests may result from sampling error, in which abnormal cells are not present in the Pap test; from screening error, in which the cells are present but missed by the screener; or from interpretation error, in which abnormal cells are misclassified as benign.[8,70]Although quality assurance measures and new computerized screening technologies address the latter two factors, clinicians can favorably impact the sensitivity of the Pap test by obtaining an optimal cytologic specimen

Liquid-Based Pap Collection

The imperfect sensitivity and variable smear quality of conventional Pap collection have driven the development of thin-layer liquid-based cytology (LBC) during the past decade. Several liquid-based systems for evaluating cervical cytology are available (e.g., Thin Prep®, SurePath™, and MonoPrep™).

Liquid-based cytology collects cells in a liquid transport medium that is subsequently processed to produce an even monolayer of cells on a glass slide.

There are currently two LBC products marketed in the U.S.: Thin Prep 2000 (Cytyc Corp., Boxborough, MA) and SurePath (TriPath Imaging, Inc., Burlington, NC). Both products are FDA approved as alternatives to the conventional Pap test.

The number of cells, between 50,000 and 75,000, and the area of the slide covered with cells are less than with a conventional smear. However, obscuring blood, mucus, debris, and cellular overlap are largely eliminated.

Theoretically, abnormal cells that might be few in number, clustered, and obscured on a conventional smear will be randomly and evenly distributed over the area of the LBC slide and thus be more visible for detection. In addition, most or all of the collected cellular material is available for laboratory processing and is not discarded in the sampling process.

Residual LBC specimens can undergo testing for HPV, herpes simplex virus, Neisseria gonorrhea, and Chlamydia trachomatis. Thin Prep is FDA approved for reflex HPV testing and it is possible that SurePath will gain similar approval in the future.

Performing a Pap test

Preparation

Ideally, Pap tests should be scheduled to avoid menstruation. Patients should abstain from vaginal intercourse, douching, and use of vaginal tampons and medicinal or contraceptive cream preparations for a minimum of 24 to 48 hours before a test.

Treatment of cervicitis or vaginitis prior to Pap testing is optimal. However, Pap testing should never be deferred due to unexplained inflammatory conditions or bleeding, as these signs and symptoms may be caused by cervical or other genital tract cancers. Pap screening should be performed on high-risk patients whenever an opportunity arises.

Complete clinical information is essential for the accurate interpretation of a Pap test and includes documenting the date of last menstrual period or pregnancy, exogenous hormone use, menopausal status, and past history of abnormal bleeding or abnormal Pap test results, dysplasia, or cancer. Additionally, intrauterine devices (IUDs) can cause reactive cellular changes and their use should be noted. Important risk factors such as immunosuppression, recent immigration from an underdeveloped country, or prior lack of adequate screening may be helpful.

Adequate visualization of the cervix is essential for detection of gross lesions and identification of the SCJ. Touching the cervix should be avoided prior to performing a Pap, as dysplastic epithelium, particularly high-grade lesions, may be inadvertently removed with minimal trauma. Discharge covering the cervix may be carefully removed with a large swab, preferably without touching the cervix. Vigorous blotting or rubbing may cause scant cellularity or a falsely negative Pap test result. When indicated, additional cervical sampling to detect infection should be performed after Pap testing.[71]

Location

Sampling of the transformation zone is paramount to the sensitivity of the Pap test. Technique should be adapted and sampling devices chosen according to the location of the squamocolumnar junction which varies widely with age, obstetric trauma, and hormonal status (Squamocolumnar Junction). Women known or suspected of in utero DES exposure may also benefit from a separate Pap test of the upper vagina, as these women are at additional risk for vaginal cancers.[72]

Sampling Tools

Three types of devices are commonly used to sample the cervix: the spatula, the broom, and the endocervical brush. A spatula predominantly samples the ectocervix. An endocervical brush samples the endocervical canal and is used in combination with a spatula. A broom samples both endo- and ectocervical epithelia simultaneously.[73]

A spatula is oriented to best fit the cervical contour, straddle the squamocolumnar junction, and sample the distal endocervical canal.

A clinician firmly scrapes the cervical surface completing at least one full rotation. For spatulas, plastic is preferred to wood because cells are more easily released from a plastic surface.[73]

The endocervical brush, with its conical shape and plastic bristles, has largely replaced the moistened cotton swab to sample the endocervical canal because of its superior ability to collect and release endocervical cells.[74] After the spatula sample is obtained; the endocervical brush is inserted into the endocervical canal only until the outermost bristles remain visible. This prevents inadvertent sampling of lower uterine segment cells, which can appear falsely atypical. To avoid excessive bleeding, the brush is rotated only one-quarter to one-half turn. If the cervical canal is wide, as in parous women, the brush is moved to contact all surfaces of the entire endocervical canal.

Broom devices have longer central bristles that are inserted into the endocervical canal. These longer bristles are flanked by shorter bristles that splay out over the ectocervix during multiple clockwise rotations. The recommended number of broom rotations varies by manufacturer. Broom devices are favored for LBC.

Conventional Slide Testing

This cytology method requires special care to avoid air drying of cells, a leading cause of poor slide quality. The spatula sample should be held while the endocervical brush sample is taken. The spatula sample is then quickly spread as evenly as possible over one half to two thirds of a glass slide. The endocervical brush is firmly rolled over the remaining area of the slide, after which fixation is carried out immediately by spray or immersion. After collection, the samples are stained using Papanicolaou stain (Appendix 5).

Liquid-Based Testing

Sampling and cell transfer to a liquid medium should be performed according to manufacturer specifications. SurePath allows for the use of all three device types, but with modified device tips that can be broken off and sent to the laboratory in the liquid medium. Thin Prep requires immediate and vigorous agitation of the chosen collection device in the liquid medium, after which the device is discarded.

The vials are placed in the Thin prep processor machine, and hollow cylinder with a 20mm diameter filter bonded to its lower surface is inserted into the vial. After a rotary motion to disperse loose cell clusters and mucin, a vacuum is applied to the cylinder and cells are trapped onto the filter.

The cylinder with the filter are removed when a set percentage of pores on the filter are occluded. The cylinder is then inverted 180 degrees and the filter is gently pressed onto a glass slide, which transfers the cells from the filter to slide. The slide is then fixed and stained the same way as in conventional pap smear. This technique results in a monolayer of cells on the slide, which can be read more easily than the conventional cytology slides. Thin prep is less likely to be affected by blood than traditional pap smear, but the manufacturer does not recommend obtaining during menstruation.

Comparison of Conventional and Liquid-Based Cytology

Liquid-based cytology now accounts for most of Pap tests performed in the U.S. Both LBC products are FDA-approved to claim a 65-percent increased detection rate of HSIL compared with conventional smears, as well as decreased unsatisfactory sample rates. Furthermore, there is evidence that LBC is more sensitive and accurate for the detection of both squamous lesions and Adenocarcinoma of the cervix.[33] Comparison studies show variable results with respect to atypical squamous cell detection rates.

Although the preponderance of numerous studies show an increase in sensitivity by LBC technology, controversy exists about data significance because of study methodologies.[75-77] Ronco and colleagues (2006) have published the first randomized controlled trial that compares conventional Pap testing to LBC in a screening population. Although LBC decreased the unsatisfactory Pap rate, its sensitivity was similar to that of conventional Pap tests, but with a lower positive predictive value.

The deficiencies of comparative data, uncertainties of cost effectiveness, and potential adverse consequences of decreased specificity with LBC have been reviewed elsewhere.[78-80]

When evaluating a new screening test, cost is important. The AHRQ review and a modeled cost and outcomes analysis concluded that liquid-based cytology falls within the accepted ranges of cost-effectiveness if used at 3-year screening intervals.[81, 82] Another computer-based model evaluated different triage strategies for ASCUS Pap smears and found that reflex HPV testing provides the same or greater life expectancy benefits and is more cost-effective.[83] This strategy requires the use of liquid-based cytology. The large ALTS trial supports the use of liquid-based cytology because it has shown HPV testing in patients with ASCUS decreases the need for colposcopy.[84]Ultimately, when deciding which Pap test is better, other factors in addition to sensitivity must be considered.

1.3: RESEARCH QUESTION

What is the accuracy of conventional pap smear as compared to liquid based cytology in the detection of abnormal cervical cytology when compared to Colposcopic biopsy?

1.4: RATIONALE/JUSTIFICATION

Cervical cancer is the second most common malignancy among women worldwide after breast cancer. Theoretically, it is almost entirely preventable with effective screening tests and programmers. The Pap smear is a model for cervical cancer screening against which other tests are measured. Cervical cancer screening remains an evolving field with ongoing re-evaluation of well-established Pap Smear Screening practices and development of new screening technologies. The conventional Pap smear is the standard screening test for cervical neoplasia. Despite success, the Pap smear has high false-negative rates due to poor sensitivity (51%; 95% confidence interval [CI], 0.37–0.66). The false positive rate of the Pap smear is thought to be in the range of 1% to 10%. False-positive test results lead to needless follow-up procedures. At a minimum, the patient will probably be subjected to more screening (thereby increasing her risk of still more false-positive results!). The workups for an abnormal Pap smear typically include colposcopy and biopsy-expensive, time-consuming tests that are unpleasant or painful and create anxiety about possible findings. Moreover, these tests, like all other tests, are imperfect, and further follow-up, including cone biopsy or even hysterectomy, may be undertaken, with the risks of anesthesia and surgery, as well as infertility, not to mention still more time, money, pain, and anxiety. In a study done in Kenya on impact of colposcopy on management outcomes of patients with abnormal cervical cytology by Koigi Kamau et al showed that a substantial proportion of women had normal Colposcopic findings (42, 26.7, 18.6 and 11.1% for cytologic abnormalities- CIN I,II, III and invasive carcinoma respectively) which were significant proportions. Concordance rate between cytological and Colposcopic findings were 38.6, 32.5 and 60% for CIN I, II, III respectively.

1.5: OBJECTIVES

1.5:1 BROAD OBJECTIVE:

To compare liquid based preparation and conventional pap smears with Colposcopic biopsy at Kenyatta National Hospital.

1.5:2 SPECIFIC OBJECTIVES:

- **Primary**

1. To compare and determine the degree of agreement of the liquid based preparation results and concurrent conventional Pap smear results.
2. To compare the results and determine the sensitivity and specificity of conventional Pap smears and liquid based preparations using Colposcopic biopsy as the gold standard.

- **Secondary**

1. To document the socio-demographic characteristics of the patients who have pap smears done at KNH.

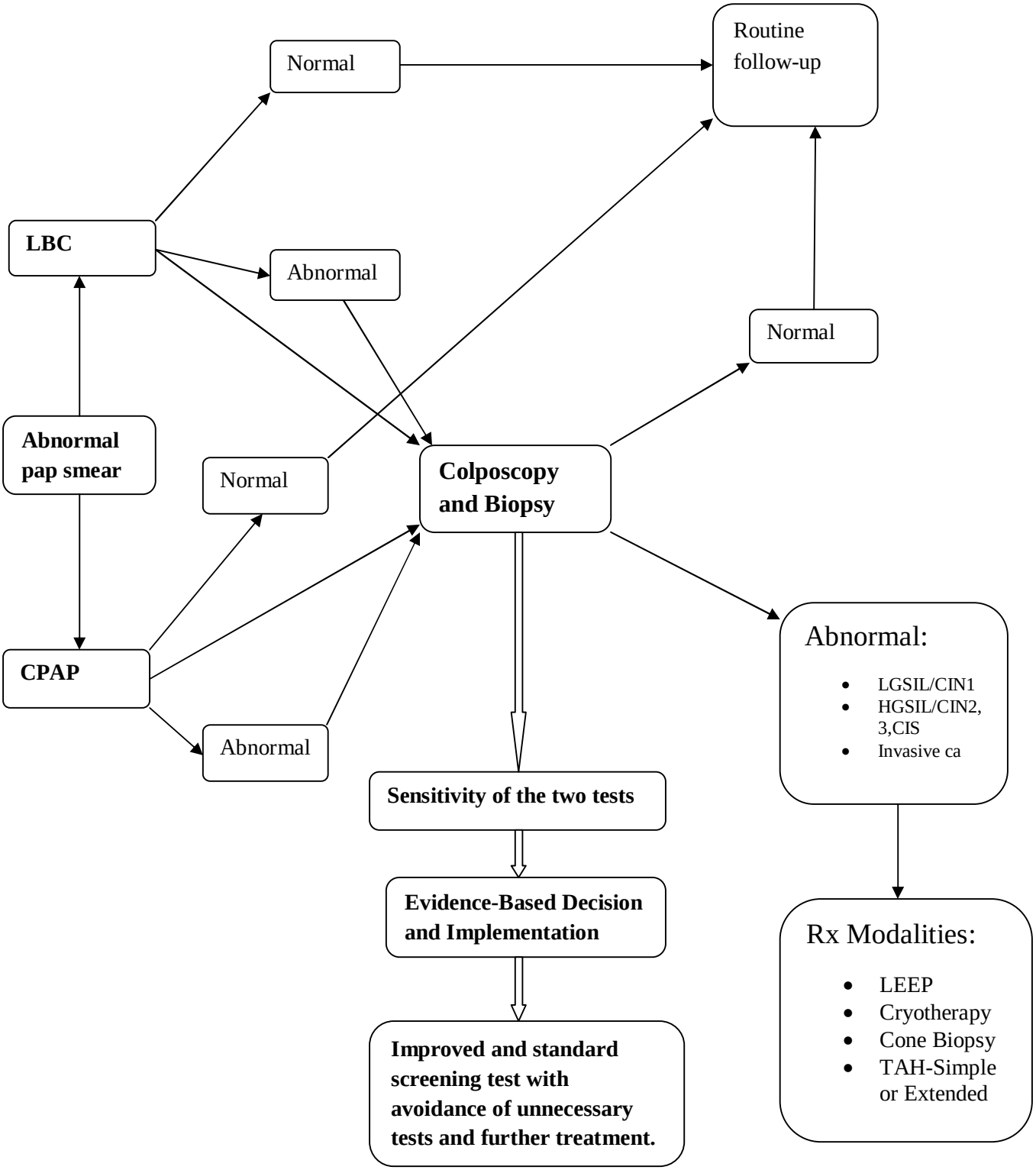
1.6: CONCEPTUAL FRAMEWORK

Narrative:

Conventional Pap smear is the standard screening test for cervical neoplasia. Despite its success, it has poor sensitivity. The false positive rate of the Pap smear is thought to be in the range of 1% to 10%. False positives results may lead to needless follow-up procedures and further treatment.

Cervical cancer screening remains an evolving field with development of new screening technologies. With this in mind, the study sought to determine how the new technology- LBC perform compared to conventional Pap smear in previously abnormal pap smears with Colposcopic biopsy as the gold standard. As shown in the figure below, all clients referred to colposcopy clinic with abnormal pap smears i.e. persistent LGSIL, ASCUS, and LGSIL with HPV changes, HGSIL were recruited in the study by convenient sampling if they met the inclusion criteria. The samples were taken and results analyzed to determine the accuracy of the two tests.

Figure 1: Diagrammatic



CHAPTER 2: STUDY DESIGN AND METHODOLOGY

2.1: STUDY DESIGN

This was a hospital-based comparative cross-sectional study. Eligible clients were recruited into the study by convenient sampling over a period of 4 months between August and November 2011.

2.2: STUDY SITE

The study was conducted at Kenyatta National Hospital's colposcopy clinic. Kenyatta National Hospital is main national referral and teaching hospital in Kenya. It is a teaching hospital for the College of Health Sciences of the University of Nairobi and for the Kenya Medical Training College, Nairobi. The colposcopy clinic is conducted on Thursday and Friday at the family planning outpatient clinic and manages patients referred to the clinic with abnormal pap smears from within the hospital, from peripheral health facilities around Nairobi as well as from hospitals outside Nairobi. The clinic is run by a consultant gynaecologist, gynaecology resident and a nurse assigned duty on particular days of the week. On average, 111 patients are reviewed and management decided per month with an average of 14 patients per day. The abnormal pap smears are first reviewed by the gynaecology resident on duty in consultation with gynaecologist on duty and the management decided. Services offered at the clinic include Colposcopic examination with directed biopsies and specific modes of management such as loop electrosurgical excision (LEEP) and cryotherapy. A minimum of 4 colposcopy/biopsies and 2 LEEPs are done each day. Follow-up of patients after management is also done at the clinic.

2.3: STUDY POPULATION

The study subjects consisted of women referred to colposcopy clinic with abnormal pap smears and were able to give written informed consent (18-60yrs).

Inclusion Criteria

1. Age, 18-60 years with abnormal pap smears.
2. Able to give voluntary informed consent.

Exclusion criteria

1. Clients who did not wish to be included in the study.
2. Clients previously treated for any cervical abnormality.

2.4: SAMPLE SIZE AND SAMPLING PROCEDURE

2.4:1 Sample size calculation

The false positive rate of the Pap smear is thought to be in the range of 1% to 10% (*RM DeMay - Should we abandon Pap smear testing; Am J Clin Pathol, 2000 - ajcp.ascpjournals.org)* .Taking the average 5% as the estimated rate of false positives;

The following formula was used to estimate the desired sample size.

$n = [z^2 \times p \times q] / [ME^2]$

Where;

z is the critical z score at 95% confidence level = 1.96

p is the estimated population proportion of false positives among patients who have abnormal Pap results ~=5%

q = 1-p = 95% the proportion of true positives among patients who have abnormal Pap results

ME is the margin of error set at 5%.

$n = [1.96^2 \times 0.05 \times 0.95] / [0.05^2]$

$n = [0.182476] / [0.0025]$

$n = 73$

This means 73 patients were the calculated sample size.

2.4:2 Sampling Procedures

Client recruitment was done by convenient sampling. All eligible clients were enrolled during the study period until the desired sample size of 73 was attained. This minimized selection bias by consecutively recruiting any client who met the inclusion criteria.

2.5: DATA COLLECTION AND MANAGEMENT METHODS

2.5:1 Data collection

This was by use of a structured questionnaire administered at the time of recruitment, Pap smear and Colposcopic biopsy collection at the colposcopy clinic. Pretesting of the questionnaire for data collection was done at the KNH colposcopy clinic before commencement of the study. Recruitment was done by the research assistants who are gynaecology residents and nurses assigned duties in the colposcopy clinic on various days. They were trained first on recruitment of participants and sample collection especially the liquid based preparation Patient recruitment was by convenient sampling i.e. no randomization; those willing signed the informed consent form, their socio-demographic data collected. Pap smears collection was done by either the principal investigator or the consultant gynaecologist, then immediately colposcopy and biopsy were done by the same consultant gynaecologist on duty in the procedure room. Specimen collection was by use of a split sample technique. The Pap smear was collected by a cytobrush, smeared on the slide and the remainder collected in a vial containing preservative for liquid based preparation processing .Then, colposcopy was done and biopsy taken. The smears and Colposcopic biopsies were read at the Department of Human pathology U.O.N. Because these were biased samples, the pathologists reading the specimens were blinded both at the level of smears and biopsies reading. Smear results were availed to patients in 4 weeks, while biopsies report in 6 weeks.

2.5:2 DATA MANAGEMENT

After data collection, the questionnaire with raw data was verified for errors or omissions daily then kept under lock and key by the principal investigator. Pap smears, liquid based preparations biopsies collected were labeled and outpatient numbers indicated to coincide with request forms to avoid mix up of results. Results of the pap smears for both techniques were typed on two labeled laboratory request forms and also entered into the database. The principal investigator was responsible for availing the collected data to the statistician for computer entry, and also distributing the collected pap smears, LBC and biopsy specimens to the cytology laboratory in the U.O.N. Pathology department. Because these were biased samples, quality control was ensured by blinding of the pathologists reading the samples.

The conventional pap smears and the biopsies were processed routinely in the pathology department without notification they were study samples. For CPAP no indication of diagnosis was made on the laboratory request form while for biopsies indication was done as is recommended.

Liquid based preparations were processed and read by a pathologist with blinding of earlier results of Pap smear. Random numbers of all samples were re-read, every 5^{th} CPAP and LBC sample by a 2^{nd} pathologist.

2.6: DATA ANALYSIS AND PRESENTATION OF RESULTS

Data analysis was performed using Statistical Package for Social Scientists (SPSS Version 19.0). Nominal variables were summarized using frequencies and percentages whereas continuous variables were summarized using measures of central tendency (mean median, minimum, maximum and standard deviation). Tests for statistical association for were done using Chi-squared tests and Fishers Exact tests nominal variables and analysis of variance for continuous variables. Sensitivity analysis was carried out to determine sensitivity, specificity, positive predictive value, negative predictive value were estimated for each of the tests.

2.7: ETHICAL CONSIDERATIONS

This was a comparative cross-sectional study of liquid based preparations and conventional pap smears with Colposcopic biopsy. Split sample was used thus there was no double collection of samples. The results of both pap smears were compared to the gold standard – biopsy.

Approval was sought from the Ethics Committee of Kenyatta National Hospital and U.O.N. The study was self funded.

On accepting to participate in the study, the participants signed a consent form. There was no financial inducement to participate in the study. Patients were not required to pay more than the test they were referred for.

The patients were assured of confidentiality i.e. only the OP numbers and not names, and the filled questionnaire will be kept in the custody of the principal investigator.

Those who declined to participate in the study however had colposcopy and biopsy collected as earlier booked and results availed when ready.

Possibility of discrepancy in the two Pap smear results was explained before consenting to the study that the results may differ. Assurance was however made that any discrepancies confirmed by colposcopy and biopsy will be treated appropriately as per the guidelines.

The contact address of the principal investigator and supervisor was given to the client in case she may have required further details about the study or may have wished to withdraw from the study.

The research assistants were gynaecology residents and nurses assigned to the colposcopy clinic on various days. Training of research assistants on data and Pap smear collection procedure was done before commencement of the study.

2.8: STUDY LIMITATIONS

- Sampling errors during smear collection.
- Interobserver variability in smear interpretation for specimens not read by the same cytopathologist.

CHAPTER 3: RESULTS

Socio – Demographic characteristics:

Table 1: Age distribution

		n	%	Cumulative frequency
Age groups	<20 years	1	1.4%	1.4
	20-24 years	4	5.5%	6.9
	25-29 years	12	16.4%	23.3
	30-34 years	13	17.8%	41.1
	35-39 years	10	13.7%	54.8
	40-44 years	16	21.9%	76.7
	45-49 years	7	9.6%	86.3
	50-54 years	7	9.6%	95.9
	>=55 years	3	4.1%	100
	Total	73	100.0%	

The mean age of the study population was 38 years (SD +/-10) with 76.7% of the patients below 44 years with a peak age range of 40-44yrs (21.9%) followed by those in the 30-34years age group (17.8%)(Table 1). The 25-29years age group accounted for 16.4% of the study population. Only 1.4% was < 20years. Those above 49years were 24.7% of which only 4.1% were >55years. Mean age at menarche was 16years (SD+/-2) with a minimum of 12years and a maximum of 20years. Two of the patients could not remember their age at menarche. The mean parity of the patients was 3 (SD+/- 2).

Table 2: Socio-Demographic characteristics

Socio-Demographic Characteristic		n	%	Cum.Freq. %
Age of sexual debut	<15 years	11	15.3%	15.3
	15-20 years	49	68.1%	83.4
	20-25 years	10	13.9%	97.3
	25-30 years	2	2.7%	100
	Total	72	100%	
Marital status	Single	10	13.7%	13.7
	Monogamous	44	60.3%	74
	Polygamous	6	8.2%	82.2
	Separated/Divorced/Widowed	13	17.8%	100
	Total	73	100%	
No. of recent sexual partners	<2	63	86.3%	86.3
	2-4	9	12.3%	98.6
	>5	1	1.4%	100
	Total	73	100.0%	
Education level	None	5	6.8%	6.8
	Primary	28	38.4%	45.2
	Secondary	32	43.8%	89
	Tertiary	8	11.0%	100
	Total	73	100.0%	

More than 83.4% of the patients had had their sexual debut before the age of 20 of which 68.1% had their first sexual experience between 15-20years and 15.3% before 15years (Table 2 overleaf). More than 60% were married and 60.3% stated that they were in a monogamous relationship while 8.2% were in a polygamous relationship. About 17.8% were separated, divorced or widowed while 13.7% were single. About 98.6% reported having had < 4 recent sexual partners with most, 86.3% having <2 sexual partners.

Only 1 patient (1.4%) had had > 5 recent partners (Table 2). More than 80% of the patients had formal education with the majority (43.8%) having completed secondary education while 38.4% had completed primary education. Only 6.8% had had no formal education.

Contraceptives Use

Figure 2: Contraceptives use

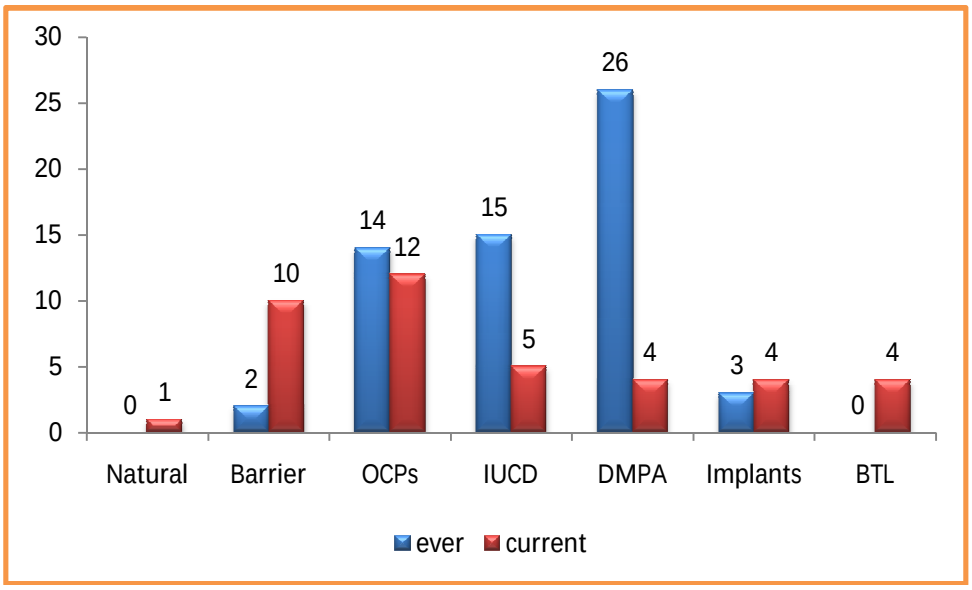
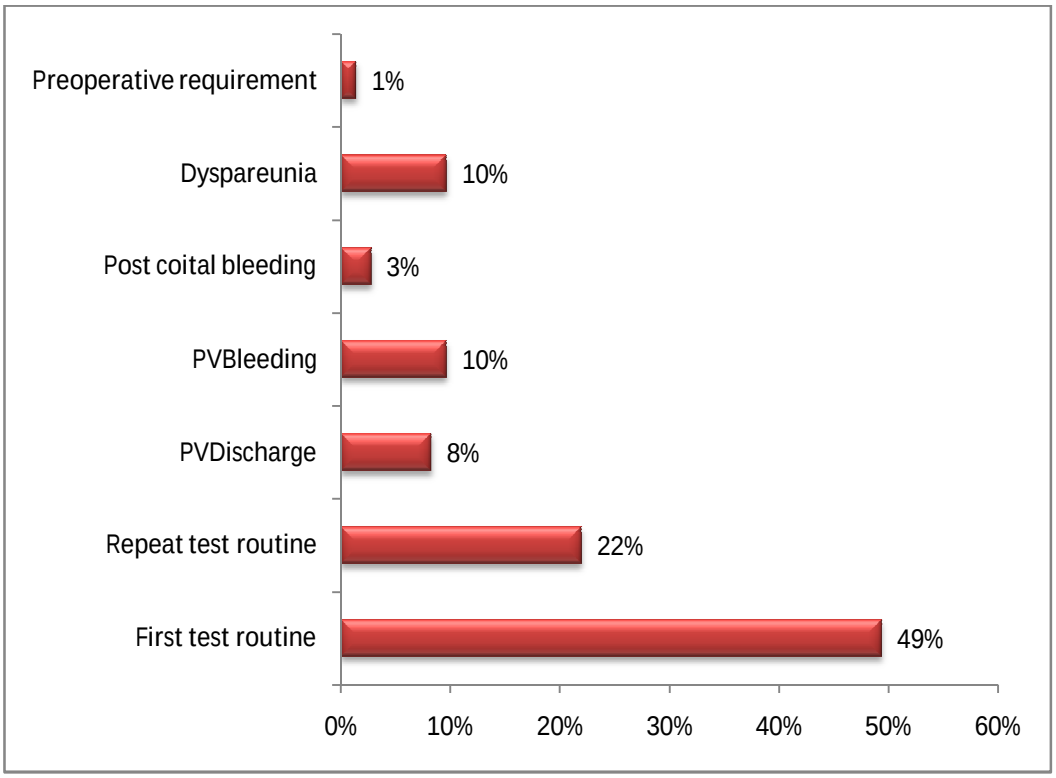


Figure 2 shows the contraceptive use reported by women in the study population. The most common ever used methods of contraception were DMPA and IUCD, 26(35.6%) and 15(20%) patients respectively as shown in Fig.2 below. Of those who had ever used DMPA 13(50%) had used it for <2years and only 2 (7.7%) for >10years, while for those ever used IUCD 5(33.3%) had used for <2years and 4 (26.7%) for >10years. About 19.2% of the patients had used OCPS while 6.8% had either used barrier method or implants.

For Current use, the most widely reported methods of contraception were OCPs and barrier methods, 12(16.4%) and 10(13.7%) patients respectively (Fig.2). Half of the patients had been using them for <2years while only 1 for each had been on either for >10years.

Reasons for Referral Pap smear

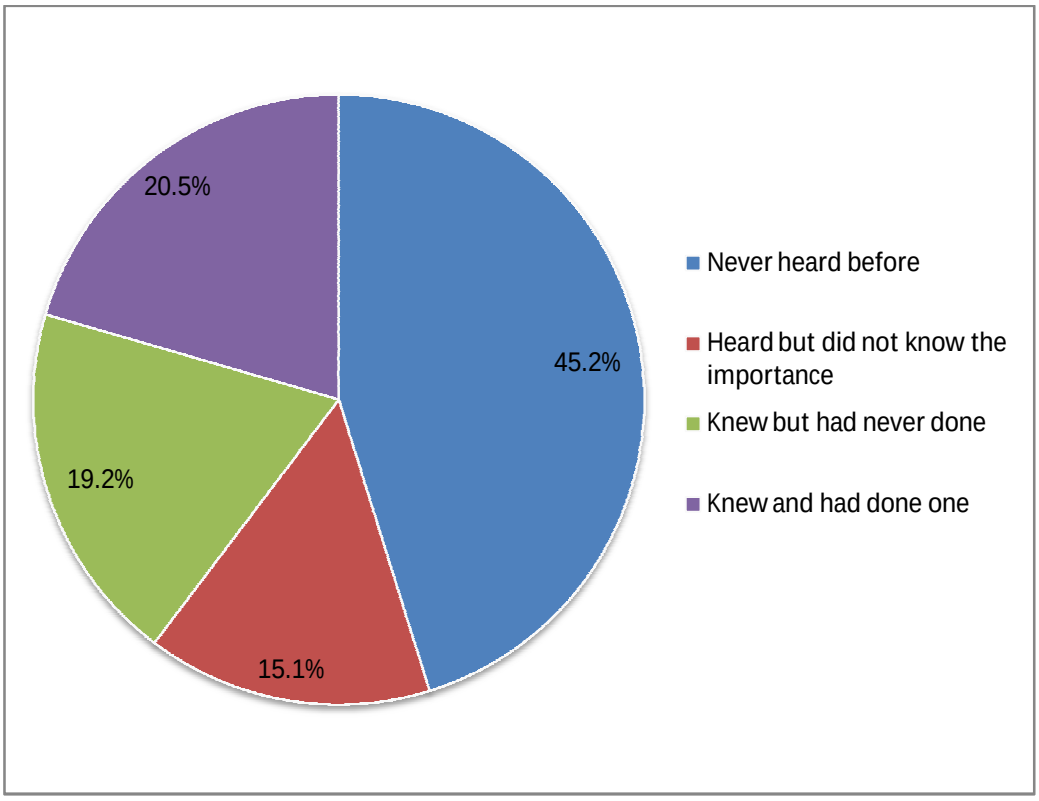
Figure 3: Reasons for Referral Pap smear



The majority of the patients (71%) had come for routine tests before referral with those coming for first Pap smear test being 49% (36) and repeat pap smear test being 22% (16). About 10% each had been referred due to either dyspareunia or vaginal bleeding and 8% due to vaginal discharge. Only 3% of the patients had been referred due to post coital bleeding and 1% as a preoperative requirement. Some patients had more than one complaint for referral.

Pap Smear Awareness

Figure 4: Pap Smear Awareness



More than 50% of the patients interviewed had Pap smear awareness. Less than 20% of these had done a pap smear before referral while19.2% knew about Pap smear importance but had not done any before (Fig 4). About 15.1% did not know the importance of a Pap smear but had heard about it while 45.2% were not aware about the Pap smear.

Final Results of Referral CPAP and repeat CPAP

Table 3:

	Referral CPAP		Repeat CPAP	
	N	%	N	%
Squamous Lesions				
Normal	0	0.0%	32	43.8%
Inflammatory	0	0.0%	3	4.1%
ASCUS	7	9.6%	2	2.7%
LSIL	15	20.5%	8	11.0%
HSIL/CIS	45	61.6%	24	32.9%
SCC	2	2.7%	0	0.0%
Unsatisfactory	0	0.0%	4	5.5%
Glandular Lesions				
AGC/Adenocarcinoma	4	5.5%	0	0%
Total	73		73	

Table 3 shows the Pap smear results of the initial and repeat pap smears. Of the 73 women referred with abnormal pap smears 32 (43.8%) were normal on repeat Pap smear for the same patients. There was 5.5% increase in the number of unsatisfactory smears.

More than 90% of the referrals were due to squamous lesions with HSIL/CIS accounting for 61.6% and LSIL for 20.5%. Similar observations were made on the repeat CPAP with HSIL/CIS accounting for 32.9% while 11% were LSIL.

There was no sample reported as inflammatory on referral CPAP compared with 3 (4.1%) reported on repeat CPAP while of the 7 (9.6%) samples which had been reported as ASCUS, only 2 (2.7%) were similarly reported on repeat CPAP.

Only 2 patients had been referred with SCC of which none was detected on repeat CPAP.

Only 4 (5.5%) of the referred patients had pap smears with glandular lesions of which none was detected on repeat CPAP.

LBC and Histology results for the Normal results on repeat Pap smear

Table 4:

	Repeat CPAP		LBC		Biopsy	
	N	%	N	%	N	%
Squamous Lesions						
Normal	32	43.8%	4	5.5%	4	5.5%
Inflammatory			0	-	9	12.3%
ASCUS			3	4.1%	0	-
LSIL			9	12.3%	12	16.4%
HSIL/CIS			10	13.7%	5	6.8%
SCC			0	-	2	2.7%
Unsatisfactory			5	6.8%	0	-
Glandular Lesions						
AGC/Adenocarcinoma			1	1.4%	0	-
Total			32	43.8%	32	43.8%

Out of the 43.8% of the samples reported as normal on repeat Pap smear, 4 (5.5%) agreed with histology. Of the normal LBC results, only one result agreed with histology, one sample was inflammatory and 2 samples were reported as LSIL on histology. While there was no inflammatory sample on LBC, histology confirmed 9 (12.3%) of the samples. Of these, one sample was reported as normal, one each was reported as ASCUS and AGUS, two each as LSIL and HSIL and two were unsatisfactory on LBC. About 12.3% of the normal samples on repeat Pap smear were LSIL on LBC with histology confirming 12 (16.4%%) of the samples. Of these, 3 results agreed with histology results, 4 samples had been reported as HSIL, 2 as normal, 2 were unsatisfactory and 1 as AGUS on LBC.

More than 10% of these normal samples were reported as HSIL on LBC with histology confirming about 7%. Of the 7%, one sample result was in agreement with histology with 3 having been reported as LSIL, and one reported as ASCUS on LBC. While there was no sample reported as SCC on LBC, histology confirmed two of which had been reported as HSIL and LSIL respectively. About 7% of the sample results were unsatisfactory on LBC. Only one of the normal samples was reported as AGUS on LBC with histology detecting none.

Results of cytologic diagnosis of Repeat CPAP, LBC and Colposcopic tissue biopsies

Table 5:

	Repeat CPAP		LBC		Biopsy	
	N	%	N	%	N	%
Squamous Lesions						
Normal	32	43.8%	6	8.2%	8	11.0%
Inflammatory	3	4.1%	0	0.0%	11	15.1%
ASCUS	2	2.7%	5	6.8%	0	0.0%
LSIL	8	11.0%	16	21.9%	21	28.8%
HSIL/CIS	24	32.9%	30	41.1%	20	27.4%
SCC	0	0.0%	1	1.4%	7	9.6%
Unsatisfactory	4	5.5%	14	19.2%	0	0.0%
Glandular Lesions						
AGC/Adenocarcinoma	0	0.0%	1	1.4%	1	1.4%
Total	73		73		73	

Of the 73 patients referred with abnormal pap smears, 32 (43.8%) were negative for cervical intraepithelial lesions on repeat CPAP while 6 (8.2%) were negative on LBC. Histology showed 8 (11%) to be negative.

There were more unsatisfactory smears with LBC 14(19.2%) than for repeat CPAP 4 (5.5%).

About 4.1% of repeat CPAP were inflammatory while for LBC none were reported as inflammatory. Histology however showed inflammatory changes.

The two tests, CPAP and LBC detected ASCUS in 2.7% of the patients for CPAP and 6.8% for LBC.

Repeat CPAP showed 8(11%) of the referred patients with abnormal pap smears as LSIL and 24(32.9%) as HSIL while LBC revealed 16(21.9%) and 30(41.1%) respectively. Histology confirmed 21(28.8%) as LSIL and 20(27.4%) as HSIL.

No sample was reported as SCC on repeat CPAP while on LBC only one case was detected with histology confirming 7(9.6%) cases.

Few glandular lesions were detected, with none by repeat CPAP, one by LBC while histology revealed one case.

Table 6: Degree of Agreement for final cytologic diagnosis of Repeat CPAP and LBC

		LBC							
		Normal	AGC	ASCUS	LSIL	HSIL	CIS	SCC	Unsatisfactory
Repeat CPAP	Normal	4	1	3	9	9	1	0	5
	Inflammatory	0	0	0	2	0	0	0	1
	ASCUS	0	0	1	O	1	0	0	0
	LGSIL	1	0	0	1	5	0	0	1
	HGSIL	1	0	1	4	14	0	0	4
	Unsatisfactory	0	0	0	0	0	0	1	3

Table 6 shows the degree of agreement for the results of repeat pap smears and LBC.

From the table, 4 normal results were in agreement for the two tests. About 9 each of the normal results on repeat pap smears were reported as LSIL and HSIL with 3 reported as ASCUS on LBC while 5 were unsatisfactory. Only one test reported as LSIL agreed for the two tests with 5 reported as LSIL on repeat pap smear being reported as HSIL on LBC. About 14 results reported as HSIL agreed for both tests with 4 results reported as HSIL on repeat CPAP being reported as LSIL while 4 results were unsatisfactory on LBC.

Overall, there was agreement of the results for both tests with a Kappa of 0.84.

Results of Cytologic diagnosis of Repeat CPAP compared With Biopsy Results

Table 7:

	Repeat CPAP		Biopsy		X^2	P value
	N=73	%	N=73	%		
Normal	32	43.8%	8	11%	19.8	<0.0001*
Inflammatory	3	4.1%	11	15.1%	5.06	0.025*
ASCUS	2	2.7%	0	0.0%	2.03	0.496
LSIL	8	11.0%	21	28.8%	7.27	0.007*
HSIL/CIS	24	32.9%	25	34.2%	0.52	0.861
SCC	0	0.0%	7	9.6%	7.35	0.013*
Unsatisfactory	4	5.5%	0	0.0%	4.11	0.119
AGC/Adenocarcinoma	0	0.0%	1	1.4%	1.01	0.999

***Significant results**

Table 7 shows comparison of repeat CPAP results with the gold standard- Colposcopic biopsy. Of the 73 patients referred with abnormal pap smears, about 43.8% of repeat CPAP samples were reported as normal with 11% being confirmed by histology. While repeat CPAP showed 4.1% of abnormal pap smears to be inflammatory, histology revealed 15.1%. This difference was significant (P 0.025).

Of the two samples reported as ASCUS on repeat CPAP, 1 was reported as inflammatory and the other as HSIL on histology.

While histology revealed 28.8% of patients to be having LSIL, repeat CPAP showed only 8% which was a significant difference (P 0.007).

Detection of HSIL/CIS with CPAP (32.9%) agreed well with histology (34.2%) with minimal difference. About 9.6% of the samples were confirmed as SCC on histology although repeat CPAP detected (P 0.013). Only one of the samples had a glandular lesion on histology which had been undetected on repeat CPAP.

Comparison of Cytologic diagnosis on LBC and Colposcopic tissue Biopsy Results

Table 8:

	LBC		Biopsy		P value
	N=73	%	N=73	%	
Normal	6	8.2%	8	11%	0.574
Inflammatory	0	0.0%	11	15.1%	0.001*
ASCUS	5	6.8%	0	0.0%	0.058
LSIL	16	21.9%	21	28.8%	0.341
HSIL/CIS	30	39.7%	25	27.4%	0.393
SCC	1	1.4%	7	9.6%	0.063
Unsatisfactory	14	19.2%	0	0.0%	<0.0001*
AGC/Adenocarcinoma	1	1.4%	1	1.4%	0.999

***Significant Results**

Table 8 also shows comparison of LBC results with the gold standard-Colposcopic biopsy.

Out of 73 women referred with abnormal pap smears, 8.2% were normal on LBC while 11% were confirmed by histology as normal. The number of unsatisfactory smears was significant with LBC.

There were no inflammatory results with LBC compared with 15.1% on histology.

LBC detected 6.8% of the samples as ASCUS. Out the five ASCUS samples on LBC two were reported as inflammatory, two as LSIL and one as HSIL on histology.

Of the 21.9% of samples with LSIL on LBC, 28.8% were confirmed on histology while for HSIL, 39.7% of the samples were detected on LBC compared with 27.4% on histology.

LBC detected one sample as SCC with histology confirming 9.6% of the samples. One case of glandular lesion detected by LBC was confirmed on histology.

Sensitivity Analysis for Repeat CPAP and LBC

Table 9:

		Biopsy.GS							
		Normal		Abnormal		Total			
		n	%	n	%	n	X^2	Kappa	P value
Repeat CPAP	Normal	4	50.0%	28	43.1%	32	0.139	0.03	0.710
	Abnormal	4	50.0%	37	56.9%	41			
	Total	8	100.0%	65	100.0%	73			
Liquid Based	Normal	1	12.5%	5	7.7%	6	0.218	0.05	0.640
	Abnormal	7	87.5%	60	92.3%	67			
	Total	8	100.0%	65	100.0%	73			

Table 10:

Test	Sensitivity	Specificity	PPV	NPV
Repeat CPAP	57%	50%	0.90	0.13
Liquid Based	92%	13%	0.90	0.17

Table 9&10 shows sensitivity analysis of the two tests with Colposcopic biopsy as the gold standard.

Liquid based cytology had a higher sensitivity of 92% compared with conventional pap smears' 57%. However, it had low specificity of 13% compared with 50% for conventional Pap smear. The positive predictive values for the two tests were the same at 0.9. The negative predictive value for liquid based cytology was higher (0.17) compared to that of conventional Pap smear (0.13)

CHAPTER 4: DISCUSSION

4.1: DISCUSSION:

In this study a total of 73 patients referred to KNH with abnormal Pap smear who met the inclusion criteria were interviewed and samples taken. The mean age of the patients was 38 yrs (SD +-10). More than75% were below 44years with most with peak in the age range of 40-44 years. These findings are similar to those of Oguntayo O et al who in a retrospective study done in Nigeria found the mean age of CIN to be 37.6 years with a combined prevalence of 48/1000. [49] In another separate study, Okewole et al found the mean age of CIN to be 39.6+/- 9.6 SD[50].

The study found out that more than 80% of the patients had formal education with the majority (43.8%) having completed secondary education and 38.4% primary education. Only 6.8% had had no formal education. .

The majority of the patients (71%) had come for routine tests before referral with most coming for first test 36(49%) and 16(22%) for repeat test. About 10% each had been referred due to either dyspareunia or vaginal bleeding and 8% due to vaginal discharge. About 3% of the patients had been referred due to post coital bleeding and only 1% for preoperative requirement.

More than 80% of the patients had had their sexual debut before the age of 20 years of which 68% debuted between 15-20 years and 15% before 15years. More than 99% had less than four recent sexual partners with most, (86%) having less than two sexual partners. Only 1 patient had had more than five recent sexual partners. Berrington de González et al[52] found that the relative risk of CIN and therefore invasive cervical cancer was increased with increasing number of sexual partners, younger age at first intercourse, increasing parity, younger age at first full-term pregnancy and increasing duration of oral contraceptive use.

There was overall high contraceptive use in all patients interviewed with the most common ever used methods of contraception being DMPA(36%) and IUCD(20%). For current use of contraception, the most widely used methods were OCPs and barrier method, 16% and 14% patients respectively. The 2008-09 Kenya Demographic and Health Survey (KDHS) [85] report showed that the most commonly ever used methods among all women and currently married women are injectables and pills, whereas sexually active unmarried women are most likely to have ever used the condom. Modern methods of contraception are more commonly used (39 percent) than traditional methods (6 percent). Of the modern methods, injectables are the most widely used, while the rhythm method is the most popular traditional method. Current use is higher among sexually active women and lowest among all women, a group that includes women who have not married, are not sexually active, or both. Appleby P et al [59] in a collaborative analysis of individual data for women with and without cervical cancer and hormonal contraceptives from 24 epidemiological studies found that the relative risk of cervical cancer is increased in current users of oral contraceptives and declines after use ceases. About 10 years' use of oral contraceptives from around age 20 to 30 years is estimated to increase the cumulative incidence of invasive cervical cancer by age 50 from 7·3 to 8·3 per 1000 in less developed countries and from 3·8 to 4·5 per 1000 in more developed countries.

More than 50% of the patients interviewed were aware of Pap smear. Less than 21% of these had done a pap smear before referral while 34% despite being aware Pap smear had not done any before. About 45% of the patients had no awareness about the Pap smear (Fig.4). Studies have shown that many women fail to comply with screening recommendations, and many patients with cervical cancer acknowledge that they have not been screened adequately.[9]

The reasons for this are not clear, but studies suggest that significant reasons for inadequate screening include patient's ignorance of guidelines, dislike of pelvic examination, lack of access to the medical care system, fear of cancer, fear of pain from diagnostic procedures, and mistrust of medical providers.[10] Compliance with screening guidelines has been shown to improve by education and physician-initiated reminders.[12, 13]

Of the 73 patients referred with abnormal CPAP, 32 (44%) were normal on repeat Pap smear for the same patients.

This study showed increased number of unsatisfactory smears in liquid based slides contrary to other studies done 19% against 6% for conventional pap smears. This result has been noted in other split-sample studies [33]; the first portion of the sample was used to make the conventional smear and may have contained most of the endocervical component. Another factor may have been the necessity of introducing the 'broom' sampling device for this study. The spatula-endocervical brush combination might have yielded a higher proportion of samples with endocervical component.

More than 90% of cervical intraepithelial lesions in the study population were squamous with HSIL/CIS accounting for 62% and LSIL 21%. Similar observations were made on repeat CPAP with HSIL/CIS reducing to 33% and LSIL to 11%. Histology further confirmed this finding. The reduction could be explained by the fact that about 57% of pap smears reported as LSIL regresses on repeat pap smear with 32% persisting while for HSIL i.e. CIN 2 & 3, 45% & 32% respectively regresses (Appendix 6). Eighty percent of cervical cancers are squamous, whereas 15 percent are adenocarcinomas and therefore majority of premalignant lesions would therefore be expected to be squamous as in this study. [68]

Liquid based cytology showed better performance as a screening test for cervical cancer than conventional Pap smear. Overall liquid based cytology had a higher sensitivity of 92% compared with conventional pap smears' 57%. However, it had low specificity of 13% compared with 50% for conventional Pap smear.

The positive predictive values CPAP and LBC were the same at 0.9. The negative predictive value for liquid based cytology was higher (0.17) compared to that of conventional Pap smear (0.13). These findings confer superiority to LBC as a screening test over CPAP for detecting those patients with actual disease and those without. These findings concurs with other studies done using split sample technique to compare the performance of CPAP and LBC.

Hutchinson et al[78] in a population based study using split sample technique to compare LBC and CPAP found increased sensitivity for LBC in detection of HSIL(92.9%) and 100% for carcinoma cases compared with CPAP-77.8% for HSIL and 90.9% for carcinomas. Baker JJ[75] showed that LBC was significantly better than CPAP in detecting biopsy-proven disease and in screening of benign abnormalities. A recent meta-analysis found sensitivity of conventional Pap smear to be 51 percent (CI 95 percent; 0.37 - 0.66) for detection of any grade of CIN by a single Pap test, but a higher sensitivity for high-grade lesions.[67]

Of the 73 patients with abnormal CPAP, 44% were normal on repeat CPAP while 6% were normal on LBC. Histology confirmed 11% of the samples as normal. The difference was significant for repeat CPAP ($P < 0.0001$). False-negative Pap tests have been shown in studies to be between 15-30% and may result from sampling error, in which abnormal cells are not present in the Pap test; from screening error, in which the cells are present but missed by the screener; or from interpretation error, in which abnormal cells are misclassified as benign.[8, 70]

LBC also showed better detection of LSIL than repeat CPAP. There was increased detection of low grade lesions with LBC 22% compared to conventional pap smears 11% of which 29% of the samples were true LSIL on histology which was a significant difference for CPAP (P 0.007). Both CPAP and LBC performed well in detection of HSIL/CIS with no significant difference when compared with histological diagnosis. However, in detection of more serious lesions, LBC performed better than repeat CPAP. For SCC, LBC detected 1(1%) of which 7(10%) were confirmed on histology (P 0.063) while repeat CPAP detected none which was significant (P 0.013). There was no significant difference in the detection of glandular lesions for CPAP and LBC.

These findings reaffirms those of studies done with split sample technique; Weintraub J et al[31] showed better performance of liquid based cytology in detection of both HSIL and LSIL/ASCUS; the study concluded high diagnostic sensitivity and sample adequacy for liquid based cytology. Sara J. Bernstein et al, [76], in a Meta analysis of prospective studies comparing cytologic diagnosis and sample adequacy, found that liquid based cytology improved sample adequacy and led to improved diagnosis of low-grade and high-grade squamous intraepithelial lesions. However, there was no difference in the rate of atypical cells of undetermined significance diagnosis between liquid based cytology and conventional smear groups.

The results of repeat CPAP and LBC were in agreement with a kappa of 0.84.

4.2: CONCLUSION:

Most of the cervical intraepithelial lesions in the study population were squamous with the majority being HSIL and LSIL.

Liquid based cytology showed better performance as a screening test compared to conventional Pap smear. However, there were more unsatisfactory smears with liquid based cytology 14(19%) compared to 4(6%) for conventional pap smears.

There were more false negative results on CPAP (P <0.0001) compared to LBC (P 0.574)

LBC performed better in detection of low grade cervical intraepithelial lesions compared to CPAP (P 0.007). However, both tests performed well in detection of HSIL/CIS with no significant difference when compared with histological diagnosis.

LBC performed better than repeat CPAP in detection of SCC, 1(1%) of which 7(10%) were confirmed on histology while repeat CPAP detected none (P 0.013). There was no significant difference in the detection of glandular lesions for both tests. In general, there was good agreement for cytological results of repeat CPAP and LBC with a kappa of 0.84.

Overall liquid based cytology had a higher sensitivity of 92% as compared with conventional pap smears' 57%. However, it had low specificity of 13% as compared with 50% for conventional Pap smear. The positive predictive values for the two tests were the same at 0.9.The negative predictive value for liquid based cytology was higher (0.17) compared to that of conventional Pap smear (0.13). Overall, this study concludes higher sensitivity of liquid based preparations than conventional pap smears.

More than 50% of the patients interviewed were aware about Pap smear. Less than 21% of these had done a pap smear before referral while 34% despite being aware about Pap smear had not done any before. About 45% of the patients were not aware about the Pap smear.

4.3: RECOMMENDATION:

1. In patients referred with abnormal pap smears requiring a repeat pap smear, liquid based cytology is recommended due to its higher sensitivity compared to conventional Pap smear. However, the cost of this new technology in cervical cancer screening is more than of conventional pap smears. In normal population, conventional Pap smear remains the screening test of choice.

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APPENDICES

APPENDIX 1: CONSENT

TITLE: A COMPARATIVE ANALYSIS OF LIQUID-BASED PREPARATIONS AND CONVENTIONAL PAP SMEARS WITH COLPOSCOPIC BIOPSY AT KENYATTA NATIONAL HOSPITAL.

HOSPITAL NUMBER: …….

DATE OF BIRTH: …….. AGE: …….. SEX: ……

ADDRESS: …….. TELEPHONE: ……..

CLIENT CONSENT EXPLANATION:

Purpose of the study

Conventional Pap smear is a test done to detect the possibility of a woman getting cervical cancer and it is the one used here in Kenya and other countries in the world. Liquid based Pap smear is a modification of the above test to improve detection of cervical cancer. Several studies done have shown improved sensitivity, specificity and less inadequacy of the specimens collected thus reduced need for repeat of the Pap smear. Furthermore the earlier pap smear taken and shown abnormality could actually be normal so the need for colposcopy and biopsy. I will be comparing the two tests.

The study is being carried out by Dr. Macharia H. Chege, Postgraduate student OBS/GYN, U.O.N. It has been approved by the KNH-ERC.

Procedure to be followed

First, I will obtain information about yourself and ask several questions about your social life. You will be asked to prepare yourself before the procedure after which you will lie on the examination couch and placed in lithotomy position. This will enable using an instrument we call a speculum to expose your cervix from where I will collect the samples. Pap smear will then be collected and split into two for the two tests. While still in the same position, colposcopy will be done.

For this, a solution we call Lugol's iodine will be applied on the cervix to identify abnormal areas then using a colposcope to magnify the areas, biopsy will be taken from these areas. You will be given instructions to follow after the procedure and when results will be available.

Confidentiality

All information obtained in this study is for research only and will be treated with utmost confidentiality; so, feel free to answer all the questions. No identity of any specific patient in this study will be discussed in any public reports or publications.

Risks and benefits:

Collection of the samples may cause discomfort and pain but is safe. The tests results also might not be the same i.e. one normal and the other abnormal. If any problem arises you will receive prompt and appropriate medical attention. Treatment for the abnormal results confirmed by biopsy will be as per the set guidelines.

Financial considerations

You will not be required to pay more money than you have already paid.

Obtaining additional information

You are encouraged to ask any questions that occur to you at this time or ask questions at any time during your participation in the study.

Basis of participation

You are free to withdraw your consent to participate in this study at any time. If you choose to do so, your rights to present or future services at KNH will not be affected.

CONSENT FORM:

I have read and explained to the above information and had an opportunity to ask questions and all my questions have been answered. I accept to take part in this study on my own free will as explained to me by the Investigator/Research assistant.

Signature of the patient: ……. Date: …….

Signature of the Investigator: ……. Date: …….

CONTACTS:

1. Principal Supervisor Dr. E. Cheserem 0722722440
2. Principal investigator Dr. Macharia H. Chege 0723802016
3. Secretary KNH/U.O.N.-ERC Tel 726300-9 EXT. 44102

POST COLPOSCOPY INSTRUCTIONS:

There will be slight per vaginal bleeding and change to dark brown discharge for 2-4 days. If bleeding persists and become heavy or becomes foul smelling, come back to hospital immediately. You might have some cramps (pain) also for a day. Avoid sexual intercourse for 1 month to enable healing of biopsy areas.

APPENDIX 2: QUESTIONNAIRE

TITLE: A COMPARATIVE ANALYSIS OF LIQUID-BASED PREPARATIONS AND CONVENTIONAL PAP SMEARS WITH COLPOSCOPIC BIOPSY AT KENYATTA NATIONAL HOSPITAL.

SERIAL NUMBER: …….

HOSPITAL NUMBER: …….

Date of data collection: ……..

Initials of person collecting the data: …….

1. Age of patient in years/D.O.B [ ]
2. Parity [ ] LMP [ ]
3. Age at menarche [ ]
4. Age at first sexual intercourse:

   0 = <15 yrs. [ ]

   1 = 15 – 20 yrs. [ ]

   2 = 20 – 25 yrs. [ ]

   3 = 25 – 30 yrs. [ ]

   4 = > 30 yrs. [ ]
5. Marital Status:

   0 = Single never married [ ]

   1 = Married

   01 Monogamous [ ]

   02 Polygamous [ ]

   2 = Separated [ ]

   4 = Divorced [ ]

   3 = Widowed [ ]

6. Number of recent sexual partners:

   0 =< 2 [ ]

   1 = 2 – 4 [ ]

   2 = >5 [ ]

7. Family Planning method used: Duration

	Ever Used (Dur.)	Current Use (Dur.)	
0=None	[ ]	[ ]	0= < 2yrs. 1= 2-5 yrs.
1=Natural Method	[ ]	[ ]	2= 5-10yrs. 3= >10yrs
2=Barrier Method	[ ]	[ ]	
3=OCPs	[ ]	[ ]	
4=IUCD	[ ]	[ ]	
5=Injectable (DMPA)	[ ]	[ ]	
6=Implants	[ ]	[ ]	
7=BTL	[ ]	[ ]	

8. Level of Education:

   0 = No formal Education [ ]

   1 = Primary Education [ ]

   2 = Secondary Education [ ]

   3 = tertiary [ ]

9. Reason for pap smear(index)

   1. Routine

      01 First Test [ ]

      02 Repeat Test [ ]

   2. P.V. Discharge [ ]
   3. P.V. Bleeding [ ]
   4. Post-coital bleeding [ ]
   5. Dyspareunia [ ]
   6. As pre-operative requirement [ ]

10. Pap smear awareness(index)

0= Never heard before [ ]

1= Heard but didn't know importance [ ]

2= Knew but had never done [ ]

3= Knew had done one [ ]

12. Pap Smear/Colposcopy and Biopsy Results:

OUTCOME	INITIAL CPAP	RPT CPAP	LBC	BIOPSY
Normal				
Inflammatory smear				
ASCUS				
LSIL				
HSIL				
SCC				
Unsatisfactory				
AGC/Adenocarcinoma				

APPENDIX 3: BUDGET

ACTIVITY	APPOX. COST (Kshs)
Statistical consultations	20,000
Reagents for thin films	50,000
Cytological reading of both tests	40,000
Research assistants	10,000
Stationery, photocopying and printing, staplers	5,000
Binding	2,000
TOTAL	**127,000**

APPENDIX 4: STAINING

After collection the samples are stained using Papanicolaou stain (also Pap stain). It is a multichromatic staining histological technique developed by George Papanicolaou, the father of cytopathology.

Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be gynecological smears (Pap smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells.

Pap staining is a very reliable technique. As such, it is used for cervical cancer screening in gynecology. The entire procedure is known as Pap smear.

The classic form of Pap stain involves five dyes in three solutions:

- A nuclear stain, haematoxylin, is used to stain cell nuclei. The unmordanted haematein may be responsible for the yellow color imparted to glycogen.
- First OG-6 counterstain (-6 denotes the used concentration of phosphotungstic acid; other variants are OG-5 and OG-8). Orange G is used. It stains keratin. Its original role was to stain the small cells of keratinizing squamous cell carcinoma present in sputum.
- Second EA (Eosin Azure) counterstain, comprising three dyes; the number denotes the proportion of the dyes, e.g. EA-36, EA-50, EA-65.
    - Eosin Y stains the superficial epithelial squamous cells, nucleoli, cilia, and red blood cells.
    - Light Green SF yellowish stains the cytoplasm of all other cells. This dye is now quite expensive and difficult to obtain, therefore some manufacturers are switching to Fast Green FCF, however it produces visually different results and is not considered satisfactory by some.

Procedure:

Filter the Harris Haematoxylin immediately before use.

1. Dip slide(s) gently 5-10 times in 95% ethanol.
2. Dip slide(s) gently 5-10 times in 70% ethanol.
3. Dip slide(s) gently 5-10 times in distilled water.
4. Stain 5 minutes in Harris Haematoxylin.
5. Place smears in distilled water. Rinse in successive changes of distilled water until the water remains colourless.
6. Dip slide(s) gently 5-10 times in 70% ethanol.
7. Dip slide(s) in a 1% solution of HCl in 70% ethanol until the smear shows a salmon colour.
8. Rinse slide(s) well in 2 changes of 70% ethanol.
9. Dip slide(s) gently in a 3% solution of ammonium hydroxide in 70% ethanol until the smear takes on a blue colour.
10. Rinse the slide(s) in two changes of 70% ethanol.
11. Dip slide(s) 5-10 times in 95% ethanol.
12. Stain slide(s) in OG-6 for 2 minutes.
13. Rinse slide(s) in two changes of 95% ethanol.
14. Stain slide(s) in EA-50 or EA-65 for 3-6 minutes.
15. Rinse slide(s) well in two changes of 100% methanol.
16. Rinse slide(s) in one part absolute methanol one part xylene.
17. Clean smear in xylene.

Mounting Procedure:

1. After the smear has been completely cleaned in xylene it is mounted with a microscope slide cover glass preferably 22x40mm, #1 thinness.
2. A permanent clean mounting medium should be used.
3. The excess xylene should be drained, in order to avoid the appearance of air spaces when xylene evaporates.
4. Place the required amount of mounting medium along an edge of one of the longer borders of the cover slip.

5. Place the slide at right angles to the edge of the cover slip so that the side containing the cells is facing the mounting medium.

6. Slowly lower the slide and permit the mounting medium to spread between the slide and cover slip.

On a well prepared specimen, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink at once.

APPENDIX 5: Classification Systems for Cervical Cytology

Pap smears are most often classified according to The Bethesda System (TBS). It was developed in 1988 to replace numerical designations in the Papanicolaou System developed in the 1930s. Two dysplasia classification systems were developed by Richart and Reagan in 1953 and 1973 respectively. The National Cancer Institute (NCI) introduced TBS to standardize and expand designations and facilitate management decisions for further testing and treatment of cervical abnormality.

Bethesda System
Benign within normal limits
Infection: atypical squamous cells of unknown significance (ASCUS) Reactive and reparative changes: Atypical glandular cells of unknown significance (AGUS)
Low-grade squamous intraepithelial lesions (LGSIL), human papilloma virus (HPV), mild dysplasia
Cervical intraepithelial neoplasia (CIN1*)
High grade squamous intraepithelial lesion (HGSIL) including moderate dysplasia, severe dysplasia, carcinoma in situ, and CIN2*
HGSIL including severe dysplasia, carcinoma Insitu (CIS**), CIN3*.
Invasive carcinoma

Source: Shingleton HM, Patrick RL, Johnston WW, Smith RA.: The current status of the Papanicolaou Smear. CA-Cancer Journal for Clinicians. 1995; 45(5):309.

*Terminology developed by Richart, RM: Cervical intraepithelial neoplasia. Pathology Annual 1973; 8; 301-328.

**Terminology developed by Reagan JW, et al: The cellular morphology of carcinoma in situ and dysplasia or atypical hyperplasia of the uterine cervix. Cancer 1953; 6:224-235.

APPENDIX 6: Natural History of Cervical Intraepithelial Neoplasia Lesions				
	Regression (%)	**Persistence (%)**	**Progression to CIS (%)**	**Progression to Invasion (%)**
CIN 1	57	32	11	1
CIN 2	43	35	22	5
CIN 3	32	<56	–	>12

CIN = cervical intraepithelial neoplasia; CIS = carcinoma in situ.

From Ostor, 1993.

APPENDIX 7: Cervical Cytology Screening Guidelines			
	ACS	**ACOG**	**USPSTF**
Initiation of screening	Approximately 3 years after onset of vaginal intercourse; no later than age 21	See ACS	See ACS
Screening intervals for women at average risk	Age 30: annual if conventional smear; every 2 years if LBC test	Age 30: annual	At least every 3 years
	Age >30: every 2 to 3 years after 3 consecutive negative tests	Age >30: see ACS	
Screening intervals for women at higher risk	HIV + or other immunocompromised state: 2 tests during first year after immune disease diagnosis, then annually (per CDC)	HIV +: see ACS Other immunocompromised states, DES: may require more frequent screening	No specific recommendations
		History of CIN 2 or 3 or cervical cancer: annual	
Discontinuation of screening	Age 70: consider if 3 documented negative (and no abnormal) tests in prior 10 years	Age 70 in low-risk women	Age 65 if not otherwise at high risk for cervical cancer
	Continue if screening history uncertain, history of cervical cancer, DES, recent HPV +, HIV + status, other immunocompromised state	Continue if high risk, sexually active, history of multiple sexual partners, or history of abnormal cytology	
Screening after hysterectomy	Not indicated if removal confirmed for benign indication Subtotal hysterectomy: continue screening per guidelines	Not indicated if removal confirmed with benign pathology and past negative cytologies	Not recommended if total hysterectomy for benign disease
	Continue screening if history of DES or cervical cancer	See ACS Positive or uncertain history of CIN 2 or 3: annual screening until 3 negative tests obtained, then may discontinue	See ACS

ACOG = American College of Obstetricians and Gynecologists; ACS = American Cancer Society; CDC = Centers for Disease Control and Prevention; CIN = cervical intraepithelial neoplasia; DES = in utero diethylstilbestrol exposure; HIV+ = human immunodeficiency virus infection positivity; HPV+ = human papillomavirus DNA positivity; LBC = liquid-based cytology; USPSTF = U.S. Preventive Services Task Force. Data from American College of Obstetricians and Gynecologists, 2003, Saslow, 2002, and U.S. Preventive Services Task Force, 2003.

APPENDIX 8: Cervical Cytology: Initial Management of Epithelial Cell Abnormalities		
Epithelial Cell Abnormality	**General Recommendation**	**Special Circumstances**
ASC-US	Repeat cytology at 6 and 12 months Reflex HPV DNA testing Colposcopy	Refer to colposcopy for recurrent abnormal cytology, or initial positive HPV DNA test; adolescents[b] managed with repeat annual cytology
LSIL	Colposcopy for non-adolescent women	Adolescents managed with repeat annual cytology; HPV DNA test at 12 months or repeat cytology at 6 and 12 months are also acceptable for postmenopausal women
ASC-H, HSIL, squamous cell carcinoma	Colposcopy	
AGC, AIS, Adenocarcinoma	Colposcopy, endocervical curettage[a]; HPV DNA testing for AGC	Endometrial sampling[a] indicated if age >35 years, abnormal bleeding, chronic anovulation, or atypical endometrial cells specified

[a]Endocervical curettage and endometrial sampling are contraindicated in pregnancy.

[b]adolescents = age 13 to 20 years.

AGC = atypical glandular cells; AIS = Adenocarcinoma in situ; ASC-H = atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US = atypical squamous cells of undetermined significance; HPV = human papillomavirus; HSIL = high-grade squamous intraepithelial lesion; LSIL = low-grade squamous intraepithelial lesion.

Adapted from Wright, 2007.

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ A/178

14th July 2011

Dr. Macharia H. Chege
Dept. of Obs/Gynae
School of Medicine
University of Nairobi

Dear Dr. Chege

Research Proposal: "A comparative analysis of liquid-based preparation and conventional pap smears with colposcopic biopsy at Kenyatta National Hospital" (P88/03/2011)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal . The approval periods are 14th July 2011 13th July 2012.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept.of Obs/Gynae, UON
The HOD, Records, KNH
Supervisors: Dr. Eunice Cheserem, Dept.of Obs/Gynae, UON
Prof. Elizabeth Bukusi, Dept.of Obs/Gynae, UON
Dr. Lucy Muchiri, Dept.of Human Pathology, UON