

PAP SMEAR CYTOLOGICAL FINDINGS IN WOMEN WITH ABNORMAL VISUAL  
INSPECTION TEST RESULTS REFERRED TO KENYATTA NATIONAL HOSPITAL

BY

PATRICK JOSEPH CHAGWA

A dissertation submitted in partial fulfillment for the award of the degree of Master of Science in  
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## DECLARATION

I .....declare that this dissertation is my original work under the guidance of the supervisors written below and has not been submitted to the University of Nairobi or any institute of higher learning.

Signature \_\_\_\_\_

Date \_\_\_\_\_

Principal Investigator

## SUPERVISORS' DECLARATION

This dissertation has been developed under our guidance and approval as University supervisors.

Dr. WAWERU W.

Lecturer, Anatomic Pathology Thematic Unit

Department of Human Pathology

University of Nairobi

Signature \_\_\_\_\_

Date\_\_\_\_\_

Prof. KIGONDU C.

Associate Professor, Clinical Chemistry Thematic Unit

Department of Human Pathology

University of Nairobi

Signature \_\_\_\_\_

Date\_\_\_\_\_

## **DEDICATION**

To my family for their support throughout my study.

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# TABLE OF CONTENTS

DECLARATION .....	ii
DEDICATION .....	iii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
LIST OF APPENDICES .....	xi
LIST OF ABBREVIATIONS.....	xii
ABSTRACT.....	xiv
1.0 INTRODUCTION .....	1
2.0 LITERATURE REVIEW .....	3
2.1 The Pap smear.....	3
2.1.1 Normal Cervical Cytology.....	4
2.1.1.1 Cells of the squamous epithelium .....	5
2.1.1.1.1 The basal cell layer .....	5
2.1.1.1.2 Parabasal cell layer.....	5
2.1.1.1.3 Intermediate cell layer.....	5
2.1.1.1.4 Superficial cell layer .....	6
2.1.1.2 Endocervical epithelium .....	6
2.1.2 Fundamental Concepts of the Pap smear .....	6
2.1.3 Abnormal Pap smear.....	7
2.1.3.1 General features of intraepithelial lesion .....	7
2.1.4 The Bethesda Classification.....	8
2.1.4.1 Negative for intraepithelial lesion or malignancy (NILM) .....	9

2.1.4.2 Low Grade Squamous Intraepithelial Lesion (LSIL).....	9
2.1.4.3 Atypical squamous cells (ASC) .....	10
2.1.4.3.1 Atypical squamous cells of undetermined significance (ASC-US) .....	10
2.1.4.3.2 Atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H). .....	10
2.1.4.4 High grade squamous intraepithelial lesion (HSIL).....	10
2.1.4.5 Atypical Glandular cells (AGC) .....	10
2.1.4.6 Adenocarcinoma in situ (AIS) .....	11
2.1.5 Sensitivity and specificity of Pap smear .....	11
2.1.6 False positive and false negative Pap smear results.....	11
2.2 Visual inspection methods .....	12
2.2.1 Visual inspection with acetic acid (VIA).....	13
2.2.1.1 Categories of VIA test .....	13
2.2.1.2 False positive result.....	13
2.2.2 Visual inspection with Lugol’s iodine (VILI).....	14
2.2.2.1 False positive results .....	14
2.2.2.1.1 Effect of increased false positivity rate.....	14
2.3 Colposcopy .....	15
2.3.1 Indications for colposcopy.....	15
2.3.2 Normal colposcopic findings .....	16
2.3.3 Abnormal colposcopic findings .....	16
2.4 Uterine cervical biopsy .....	16
2.4.1 Cervical intraepithelial neoplasia grade I (CIN I).....	17
2.4.2 Cervical intraepithelial neoplasia grade II & III (CIN II/III) .....	17
2.4.3 Invasive cervical carcinoma.....	17
2.4.4 Treatment for cervical intraepithelial lesions and cancer. ....	17

2.5 Justification .....	19
2.6 Research Question .....	19
2.7 Broad Objective .....	19
2.8 Specific Objectives .....	19
3.0 METHODS AND MATERIALS .....	20
3.1 Study Design .....	20
3.2 Study Area .....	20
3.3 Study population .....	20
3.3.1 Inclusion criteria .....	20
3.3.2 Exclusion criteria .....	20
3.4 Sample size determination .....	21
3.7 Selection of study participants .....	21
3.8 Specimen collection, processing, examination and reporting of the smears.....	22
3.9 Quality assurance .....	22
3.10 Ethical considerations .....	22
3.12 Data collection instruments.....	23
3.13 Data management and statistical analysis plans.....	23
4.0 RESULTS .....	24
4.1 Demographic characteristics .....	24
4.1.1 Age (years) distribution .....	25
4.1.2 Menstrual history .....	25
4.1.2.1 Pattern of menstrual cycles according to participant’s age group.....	25
4.1.3 Parity and sexual history .....	26
4.1.4. Contraceptive methods used .....	26
4.1.5 Reasons for seeking VIA/VILI screening service.....	27

4.1.6 Duration from VIA/VILI to Pap smear.....	27
4.1.7 Comparison of duration from visual inspection test to Pap smear test against Pap smear results. .....	28
4.1.8 Pap smear history .....	29
4.2 Comparing Pap smear with VIA/VILI results .....	29
4.2.1 Specific cytological findings on Pap smear .....	30
5.0 DISCUSSION .....	34
CONCLUSIONS.....	38
RECOMMENDATIONS .....	38
STUDY LIMITATIONS .....	38
REFERENCES .....	39
APPENDICES .....	43



## LIST OF TABLES

	Page No.
Table 1: Rates of spontaneous regression, persistence and progression of CIN.....	7
Table 2: Main reporting systems for cytological squamous intraepithelial abnormalities.....	9
Table 3: Benign conditions mimicking cervical intraepithelial neoplasia .....	12
Table 4: Treatment modalities of cervical intraepithelial neoplasia .....	18
Table 5: Baseline demographic characteristics .....	24
Table 6: Comparing Age with Menstrual history .....	26
Table 7: Comparing duration against Pap smear results.....	29

## LIST OF FIGURES

	Page No.
Figure 1. Age distribution histogram.....	25
Figure 2: Pattern of contraceptive methods used.....	27
Figure 3: Duration from VIA/VILI test to Pap smear test .....	28
Figure 4. Proportion of Pap smear positive test results.....	30
Figure 5: Distribution of intraepithelial lesions.....	30
Figure 6: A-J: Photomicrographs for intraepithelial lesions and infections.....	31

## LIST OF APPENDICES

	Page No.
Appendix I A: Client information and consent Form .....	43
Appendix I B: Fomu ya idhini .....	47
Appendix II: Questionnaire .....	50
Appendix III: Report formats .....	52
Appendix IV: Papanicolaou staining procedure .....	55
Appendix V: The Bethesda system of reporting cervical cytology (2001) .....	56
Appendix VI: Preparation of 5% Acetic acid .....	59
Appendix VII: Preparation of Lugol's iodine .....	60
Appendix VIII: Kenya Government guidelines for management of women after Pap smear test.....	61
Appendix IX: Kenya Government Guidelines for Management of Women after VIA/VILI test.....	62

## LIST OF ABBREVIATIONS

AGC	Atypical glandular cells
AIDS	Acquired immunodeficiency syndrome
AIS	Adenocarcinoma in situ
ASC	Atypical squamous cells
ASC-H	Atypical squamous cells cannot exclude high grade squamous intraepithelial lesion
ASC-US	Atypical squamous cells of undetermined significance
Ca	Carcinoma
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in-situ
DES	Diethylstilbestrol
ERC	Ethics and Research Committee
FP	Family planning
HCGs	Hyperchromatic crowded groups
HIV	Human immune deficiency virus
HPV	Human papilloma virus
HSIL	High grade squamous intraepithelial lesion
IQR	Interquartile range
IUD	Intra-uterine device
KNH	Kenyatta National Hospital

LEEP	Loop electrosurgical excision procedure
LSIL	Low grade squamous intraepithelial lesion
NILM	Negative for intraepithelial lesion or malignancy
NPV	Negative predictive value
OCP	Oral contraceptive pills
PPV	Positive predictive value
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
SD	Standard deviation
TZ	Transformation zone
UoN	University of Nairobi
USD	United States Dollars
VIA	Visual inspection with acetic acid
VILI	Visual inspection with Lugol's iodine
WHO	World Health Organisation

## **ABSTRACT**

### **BACKGROUND**

Cervical cancer is the leading cause of cancer mortality in women in the developing countries. The challenge of cost in establishing cytology and/or Human Papilloma Virus (HPV) mass screening for cervical cancer in these resource limited settings prompted adoption of visual inspection techniques as alternative screening methods for cervical cancer. Kenyatta National Hospital (KNH) receives women with abnormal visual inspection test results from peripheral health care centers for further management.

### **STUDY OBJECTIVES**

Broad objective: To describe the Pap smear cytological findings in women with abnormal visual inspection test results referred to Kenyatta National Hospital.

Specific Objectives: The study objectives were to describe the pattern of cervical epithelial cell abnormalities, to determine infective and other non-neoplastic findings in cervical smears in women with abnormal visual inspection with acetic acid/ visual inspection with Lugol's iodine (VIA/VILI) referred to KNH and to compare VIA/VILI with Pap smear results.

### **STUDY DESIGN**

This was a cross-sectional descriptive study.

### **METHODOLOGY**

Study area: the study was conducted at KNH in the Family planning clinic, cytology and histology laboratory facilities.

Study population: Consenting women 18 years and above with abnormal VIA/VILI referred to KNH for further management. A total of 232 women were recruited.

Specimen collection, processing and reporting: Pap smears were collected by qualified health care personnel, processed by the investigator using Papanicolaou staining procedure, reported by the investigator and signed out by a pathologist.

## **RESULTS**

All participants in this study (232) had positive result for visual inspection tests (VIA/VILI). Of these, 175(75.4%) had a report of negative for intraepithelial lesion or malignancy (NILM) while 57 (24.6%) had a report of atypical squamous cells of undetermined significance (ASCUS) or worse on Pap smear. The commonest lesion reported was high grade squamous intraepithelial lesion (HSIL), 20/57(35.1%) followed by squamous cell carcinoma, 18/57(31.2%). Of the 57(24.6%) abnormal cases 39 representing 16.8% of the total number of study participants were referred for colposcopy and biopsy while participants with reports of ASCUS and low grade squamous intraepithelial lesion (LSIL) were recommended for follow up after 6 and 12 months respectively. Out of the 232 participants, 11(4.7%) were reported as having infections. Of the 232 study participants, 33(14.2%) were more than 50 years of age. Kenya Government policy does not recommend cervical cancer screening using visual inspection tests in women who are more than 50 years of age.

## **CONCLUSIONS**

Majority of women in this study had normal Pap smear cytological findings and only few had significant lesions and were therefore triaged for definitive diagnosis and/or treatment.

## **RECOMMENDATIONS**

- Pap smear should continue being used to triage women with positive visual inspection test results.
- Increase awareness to service providers and the general public about the Government Policy on the use of visual inspection tests in women more than 50 years of age since 14.2% were inappropriately screened by the visual inspection test.

## 1.0 INTRODUCTION

Invasive carcinoma of the uterine cervix develops over a period of time from precursor lesions or abnormal surface epithelium (1). This natural history enables detection and treatment of this disease in its early stages thereby preventing its progression to advanced stages and cancer. Death caused by cervical cancer is therefore preventable through screening. Hence the goal of cervical cancer screening is the detection and treatment of pre-malignant lesions before cancer develops (2).

Prevention of cervical cancer death has been achieved in developed countries where screening using Papanicolaou smears (usually called Pap smears) and treatment of early lesions are well developed. In fact, as stated by Kumar et al (3) no form of cancer better documents remarkable effects of screening, early diagnosis and therapy than cancer of the cervix.

However, cancer of the cervix remains a common disease with a high mortality rate in developing countries (1) including Kenya where resources for conducting Pap smears and related services are limited. In view of this, alternative methods of screening for cervical intraepithelial neoplasia and cancer were developed and recommended for use in these resource limited countries even though they are not perfect. Although 'establishing diagnoses is an imperfect process resulting in a probability rather than a certainty of being right' (4)) that probability must be acceptable.

Visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) are examples of these tests which involve application of acetic acid or Lugol's iodine to the cervix, results reported as negative or positive according to the reaction upon visual inspection of the cervix. Nairobi Cancer registry recorded 475 cancer cases between 2003 and 2006 (5). Stages at diagnosis were also documented. Of all the lesions, only 3 (0.6%) were carcinoma in situ, the stage with the best prognosis. The rest were stage I and above with prognosis worsening as grade increases indicating late presentation which is preventable with screening. This is in agreement with the findings of Were and Buziba of Moi Teaching and Referral Hospital (6). This late presentation makes curative treatment difficult or almost impossible leaving palliative care as the only option.



The World Health Organization (2006) recommended use of cytology for large-scale cervical cancer screening programmes, where sufficient resources exist (7). At the same time VIA/VILI screening methods were recommended for use only in pilot studies or other closely monitored settings but not recommended for postmenopausal women (7).

Both Pap smears and these visual inspection tests are screening tests with inherent limitations. The gold standard for evaluation of the abnormal uterine cervical epithelium on screening is biopsy which is often colposcopically directed (1, 8, 9).

Kenya is one of the countries implementing VIA/VILI as cervical cancer screening along with Pap smear cytology. Human Papilloma virus (HPV) test is done in selected institutions including teaching and research institutions.

In its guidelines, Government of Kenya through the Ministry of Public Health and Sanitation and Ministry of Medical Services recommend that all women who test positive with any of these visual inspection screening tests should have cryotherapy or referred for colposcopy and/or biopsy depending on the set criteria (10).

Women with abnormal visual inspection test results who are referred to Kenyatta National Hospital are currently managed by doing Pap smears. This excludes selected cases where colposcopy and/or biopsy is/are done directly. This is because the 'see-and-treat' approach is not fully enrolled because of inadequate supplies including cryotherapy equipment.

## **2.0 LITERATURE REVIEW**

Currently, cervical cancer is the second most common cancer among women in developing countries and the largest cause of cancer mortality among women in these countries. “By 2030, cervical cancer is expected to lead to the death of over 474,000 women per year and over 95% of these deaths are expected to occur in low and middle-income countries” (11).

With the high prevalence of human immune deficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) the burden of cancer including cervical cancer is increasing. The risk of HIV positive women having a cervical lesion is more than five times than for those who are HIV negative(12).

Pap smear remains the most successful cancer screening test ever developed. Death from cervical cancer has been reduced by as much as 99% in countries where screening by Pap smear is well developed and with efficient treatment and follow-up. Reduction in mortality has been attributed to this screening program (4,13).

Since its discovery the Pap smear has and is still being extensively used as a primary screening test for cancer especially in developed countries. While Pap smear may detect endometrial disorders, like polyps, hyperplasias and endometrial cancers it does so in less than 50% of cases (14).

High cost to run the Pap smear screening programme including lack of adequate personnel to interpret the test makes it difficult to establish and/or maintain in resource limited settings. This prompted adoption of low technology, cost effective visual inspection tests, Visual inspection with acetic acid (VIA) and visual inspection with Lugol’s iodine (VILI). The government policy in the National Cervical Cancer Prevention Programme strategic plan 2012-2017 recommended first screening with VIA/VILLI and cytology using Pap smear and HPV testing where available (15).

### **2.1 The Pap smear**

Also known as Pap test, the Pap smear is a screening test used to detect pre-cancerous and cancerous uterine cervical lesions. Origins of Pap smear can be traced back to the middle of the 19<sup>th</sup> century when the microscopic appearance of cells from the vagina was illustrated by early observers including Donné and Beale (1). Dr George N. Papanicolaou (1883-1962), an American of Greek descent with the interest in endocrinology of the reproductive tract described cancer

cells he incidentally observed in vaginal smears from women with cervical cancer. Consequently the test we know today as the Pap smear named after Dr George N. Papanicolaou (1). The stain used on this smear was and still called the Pap stain.

The Pap smear test involves collection of material from the uterine cervix using special brushes or spatula and transfer them onto glass microscope slide. This preparation is then fixed using liquid or spray fixative and sent to the laboratory where they are processed, examined and reported. The first cervical cancer screening clinics were established in the 1940s (13).

The primary purpose of the Pap smear is to detect pre-invasive uterine cervical neoplasia and treat the lesion before it progresses to invasive cancer. However non-neoplastic findings including organisms can also be detected in Pap smears and their detection is useful for definitive treatment.

Cellular details are the most important parameters when evaluating the Pap smear. This demands that the cytologist be well trained in recognizing cell morphology in health and disease. The smears are usually screened by cytotechnologists who eliminate unsatisfactory smears, identify 'normal' smears, selects and marks abnormal cells. These 'abnormal' smears are reviewed and signed out by a pathologist while normal smears with exception of reactive or reparative cellular changes may be signed out by the cytotechnologist (13).

### **2.1.1 Normal Cervical Cytology**

The cervix, which is the terminal end of the uterus protruding into the vagina is made up of the ectocervix and endocervix (16). The ectocervix is covered by stratified squamous epithelium that normally lacks keratin. The endocervix is lined by mucus secreting simple columnar epithelium. The point where these two epithelia meet is called the squamocolumnar junction (SCJ). The position of the SCJ varies throughout reproductive life (17).

Squamous metaplasia of the columnar epithelium usually occurs within the SCJ. This is the reason why the SCJ is also known as the transformation zone (1). It is well known that the transformation zone is the most vulnerable area for development of most cancers and for this reason sampling of this area is critical for screening of squamous intraepithelial lesions and cancer (18).

### **2.1.1.1 Cells of the squamous epithelium**

The uterine cervical squamous epithelium is made up of four cell types making up four layers named after their position within the epithelium. Starting from the more immature to more mature layer this epithelium is made up of basal cell layer, parabasal cell layer, intermediate cell layer and superficial cell layer (1, 18).

#### **2.1.1.1.1 The basal cell layer**

This is the innermost layer made up of basal cells. Basal cells are small cells measuring about 10-15 $\mu$ m in diameter. When occurring singly they resemble small histiocytes. Nuclei of basal cells are round to oval, centrally located and vesicular with smooth margins and small amount of delicate cytoplasm. Basal cells are rare in Pap smear except in severe atrophy because Pap test does not scrape off the entire thickness of the epithelium but only the outer few layers. Basal cells anchor the epithelium to the basement membrane and are also responsible for continual renewal of the epithelium and may also participate in producing basement membrane. Together with parabasal cells, basal cells are a hall mark of atrophy when found in Pap smears (13, 19).

When they are found on Pap smear they appear as apparently syncytial aggregates (hyperchromatic crowded groups-HCGs) of small cells with bland nuclei and scant cytoplasm sometimes creating a diagnostic dilemma in distinguishing them from cells of high grade squamous intraepithelial lesion (HSIL). But bland nuclear features should betray their true nature (19).

#### **2.1.1.1.2 Parabasal cell layer**

Second upper outer layer is the parabasal cell layer made up of parabasal cells. Parabasal cells, measuring 15-30  $\mu$ m in diameter are the next larger cell type in uterine cervical epithelium. They have finely granular round to oval nuclei, smooth nuclear margins and rounded cell boundaries with moderately dense cytoplasm staining mainly blue-green or grey. The chromatin is evenly distributed with inconspicuous or absent nucleoli. Predominance of parabasal cells in Pap smear indicates atrophy which is common in childhood, postmenopause and postpartum (19).

#### **2.1.1.1.3 Intermediate cell layer.**

Forming the bulk of the epithelial thickness is the intermediate cell layer made up of intermediate cells. Measuring from 35-50 $\mu$ m the intermediate cell takes cytoplasmic features of parabasal cell (rounded borders) and superficial cell (polygonal) except for the size of nucleus which is about the size of the red blood cell. The nucleus is centrally placed, round to oval with

finely granular, evenly distributed chromatin with nucleoli normally inconspicuous. Intermediate cell nucleus is the reference for nuclear abnormalities in evaluating the Pap smear (19).

#### **2.1.1.1.4 Superficial cell layer**

Superficial cell layer is the top most layer in a normal cervix which is made up of superficial cells. Superficial cells are nonkeratinized squamous cells measuring about 45-50 $\mu$ m and have pyknotic, so called India ink dot non-functional nuclei measuring about 4 $\mu$ m. The cytoplasm is abundant with distinct cell borders and polygonal outlines (1, 19).

#### **2.1.1.2 Endocervical epithelium**

The endocervical epithelium is formed by a single layer of mucus-producing and ciliated tall columnar cells, the endocervical cells (18, 19). The nucleus is eccentrically placed and the chromatin is finely granular with inconspicuous nucleoli. The cytoplasm is abundant and vacuolated in mucin producing cells and dense in ciliated cells (13, 19). Often arranged in strips or sheets, endocervical cells appear as a picket fence and honeycomb respectively (13). Their presence in Pap smear signifies that the transformation zone has been sampled but their absence doesn't make the preparation unsatisfactory for evaluation (19). Due to lysis of the delicate cytoplasm, bare nuclei may be found in the smear (18).

Neoplastic processes of the endocervical epithelium are a minority as compared to their squamous counterpart. Therefore screening of these lesions is the secondary role of the Pap smear. There is reliable prediction of these lesions nowadays owing to their well-described morphologic characteristics but their diagnoses should be rendered when appropriate cytological features are present. Pap smear is generally unreliable in detecting endocervical glandular lesions in women over the age of 35 due to the change in position of the endocervical epithelium (19).

#### **2.1.2 Fundamental Concepts of the Pap smear**

When examining the Pap smear, the cytologist must determine the histogenesis of a cell and its nature in terms of neoplasia. The cytoplasm determines the histogenesis while the nucleus determines the health of the cell in terms of neoplastic changes (19). The cells exfoliated from the mucosal surface faithfully reflect the morphology of the hidden underlying epithelium and this is the basis of Pap smear diagnosis (19). Because of the primary purpose of the Pap smear, an abnormal smear is generally understood as one having intraepithelial lesion or dysplasia.

The report of the Pap smear is important for the management of the lesion. A false report leads to wrong or unnecessary treatment. This therefore demands that the Pap smear be sensitive enough to detect all true lesions where treatment is required and specific enough to detect absence of lesion where there is no need for treatment.

### 2.1.3 Abnormal Pap smear

The reporting system was unified and standardized by the Bethesda system (see below). This system proved to be consistent, easy to use and better reproducible. This also made the clinicians better understand the reports according to the natural history of cervical cancer enabling institution of appropriate management for the grade of lesion as some may regress, others persist and yet others progress to high grade intraepithelial neoplasia and even to cancer . Table 1 shows the behavior of different types of intraepithelial lesions if no intervention is made.

**Table 1: Rates of spontaneous regression, persistence and progression of CIN**

Disease course	CIN I	CIN II	CIN III
Regression to normal	60%	40%	30%
Persistence	30%	35%	48%
Progression to CIN III	10%	20%	N/A
Progression to cancer	□1%	5%	22%

**Source; Decherney et al (14)**

#### 2.1.3.1 General features of intraepithelial lesion

The following features are associated with neoplastic intraepithelial lesion although some may overlap with non-neoplastic lesions (19, 20).

- Increased staining characteristics known as hyperchromasia
  - Unevenly distributed chromatin pattern
  - Clumped chromatin
  - Presence of conspicuous nucleoli
  - Multinucleation

- Irregular nuclear membrane
- Atypical mitoses
- Loss of nuclear polarity (most appreciated in tissue)
- Increased nuclear to cytoplasmic ratio
- Pleomorphic nuclei

The above individual features are references only and are usually applied in combination rather than in isolation.

#### **2.1.4 The Bethesda Classification**

Initially the Pap smears were reported as classes which were formulated by Dr George Papanicolaou as Pap I through V. With time it was thought appropriate to make cytological and histological nomenclature similar and this was made possible from the meeting in Bethesda, Maryland first in 1988 (1), The Bethesda System for reporting cervical cytology subject to modification. Table 2 shows different reporting systems for reporting cervical intraepithelial lesions but only the Bethesda system will be discussed.

**Table 2: Main reporting systems for cervical cytological squamous intraepithelial abnormalities.**

Papanicolaou	WHO	CIN	Bethesda
Class I			NILM
Class II			ASC
	Mild dysplasia	CIN I	LSIL
Class III	Moderate dysplasia	CIN II	HSIL
	Severe dysplasia	CIN III	HSIL
Class IV	Carcinoma in situ	Carcinoma in situ	HSIL
Class V	Carcinoma	Carcinoma	Carcinoma

**Source: Bibbo and Wilbur (18)**

According to the Bethesda reporting system, the following are given as reports based on Pap smear findings:

#### **2.1.4.1 Negative for intraepithelial lesion or malignancy (NILM)**

This report indicates that there is no evidence of neoplasia in the cells examined on the Pap smear. About 91% of Pap tests are reported as NILM. Non-neoplastic lesions like non-infectious conditions, infections or cellular changes associated with infections are included under this category. These include parasites, bacterial, fungal and viral infections (13, 19).

#### **2.1.4.2 Low Grade Squamous Intraepithelial Lesion (LSIL)**

Previously known as mild dysplasia and histologically called cervical intraepithelial neoplasia grade I (1). Most LSILs carry little risk of oncogenesis unlike most HSILs. Encountered in about 2% of all Pap samples LSILs are caused by both low and high risk HPV types with types 6 and 11 as the most common culprits. Most LSILs regress spontaneously, others persist for longer time, small proportion progress to high grade and yet a very small proportion progress to cancer if left untreated (13). This lesion affects the superficial and intermediate cells with associated abnormalities including nuclear and cytoplasmic abnormalities.



### **2.1.4.3 Atypical squamous cells (ASC)**

Atypical squamous cells (ASC) is used for cellular changes suggestive of squamous intraepithelial lesion (SIL) but are either quantitatively or qualitatively insufficient for a definitive interpretation (18). ASC account for about 4% of all Pap samples but should generally be kept to less than 5% (13, 18).

#### **2.1.4.3.1 Atypical squamous cells of undetermined significance (ASC-US)**

This is reported in smears with squamous cell abnormalities that are greater than those in reactive changes but fall short of clear features of intraepithelial lesion either qualitatively or quantitatively (18). In laboratories that serve low risk populations the rate of ASC-US should not exceed 5% while in laboratories that serve high risk populations the rate could be higher but should not exceed 2-3 times the rate of SIL (18).

#### **2.1.4.3.2 Atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H).**

When cellular features are suggestive of but do not satisfy definite criteria of HSIL the report is given as ASC-H which represents 5-10 % of all ASC cases (13,18). The prevalence of CIN 2/3 is substantially higher in women with ASC-H (37–40%) as compared to ASC-US cases (11.6%) (18) and this explains why these cases are managed as HSIL cases. The most common pattern for ASC-H is that of immature squamous cells with mild to moderate nuclear atypia commonly called atypical squamous metaplasia (13).

#### **2.1.4.4 High grade squamous intraepithelial lesion (HSIL)**

HSIL previously known as moderate and severe dysplasia encompasses the histological grade of cervical intraepithelial neoplasia II/III.<sup>1</sup> It is encountered in about 0.5% -3% of all Pap samples depending on populations (1, 13). Almost all women in this grade category test positive for high risk HPV (13). Usually a lesion of parabasal sized cells, HSIL can affect any cell type with nuclear features more severe than its LSIL counterpart.<sup>13</sup> HSIL is followed by colposcopy and biopsy if indicated by colposcopic findings.

#### **2.1.4.5 Atypical Glandular cells (AGC)**

This report is rendered when cell morphology do not show completely normal endocervical cells but fall short of definitive features of AIS or invasive adenocarcinoma. It is clinically a more significant lesion than ASCUS and therefore requires a more rigorous follow-up because about 30% of patients with AGC have a significant lesion .Endometrial cells and other cells outside of

uterine cervix are included in this category (13,18). Women with a report of AGC are followed up by performing a procedure called endocervical curettage followed by histological evaluation of the curettings for neoplasia or cancer.

#### **2.1.4.6 Adenocarcinoma in situ (AIS)**

Characterized as the precursor lesion to invasive endocervical adenocarcinoma this glandular abnormality is a challenge to diagnose on Pap smear mainly because of its low incidence (13). Representing 0.2% to 0.3% of all Pap samples this interpretation is used when cellular changes fall between those of a definite benign reactive process and those of unequivocal

adenocarcinoma in situ or adenocarcinoma. Definite benign reactive processes should be included in the NILM category (18).

#### **2.1.5 Sensitivity and specificity of Pap smear**

Studies have shown that Pap smear is less sensitive in detection of cervical intraepithelial lesion. In five studies conducted in South Africa, Zimbabwe and India between 1999 and 2004 with a total of 32,839 participants the sensitivity of conventional Pap smear ranged from 44% to 78% and the specificity ranged from 91% to 96% for high grade squamous intraepithelial lesion (HSIL) threshold (22). In a prospective study conducted on 400 women by Goel *et al* (23) the sensitivity of Pap smear was 50% with specificity of 97%. Cibas and Ducman reports a mean sensitivity of 47% and mean specificity of 95% (13).

#### **2.1.6 False positive and false negative Pap smear results**

A false positive result means that the test has detected the intraepithelial lesion when it is actually absent while a false negative result means that the test has not detected intraepithelial lesion when it is actually present. A number of benign cervical lesions and normal physiological cervical changes are culprits in this phenomenon and most are confused with particular grades of dysplasia (see table 3). The confusion lies in the fact that the epithelium reacts to the insults or when other non-epithelial cells are confused with neoplastic cells. “The differential diagnosis between inflammatory change and dysplasia is a common, everyday problem in Pap smear diagnosis” (19). False positive Pap smear diagnoses of cervical cancer occur in 10% to 15% of cases with atrophic smear with benign squamous atypical, pseudonecrotic background and reparative changes as the chief culprits (13). Failure to recognize these abnormal cellular changes in otherwise benign conditions therefore leads to over diagnosis of cervical intraepithelial lesion or malignancy. The rate of false negative Pap test results range from 10% to 20% with sampling

errors as the main cause (4). Failure of tumors to exfoliate abnormal cells, poor specimen collection technique, laboratory processing and reading error are among the causes of false negatives (24).

**Table 3. Benign conditions mimicking intraepithelial lesions.**

Mimics LSIL <sup>a</sup>	Mimics HSIL <sup>b</sup>	Mimics cancer
Candida, Trichomonas	Herpes effect	Repair
Other inflammation	IUD <sup>c</sup> effect	Reactive endocervical cells
Squamous metaplasia	Squamous metaplasia	Arias-Stella reaction
Vitamin deficiency	Atrophy	
Radiation	Follicular cervicitis	
Histiocytes	Histiocytes	
Decidual cells	Decidual cells	
	Reserves cell hyperplasia	
	Arias-Stella reaction	
	Endometrial cells	

*a. low grade squamous intraepithelial lesion; b. high grade squamous intraepithelial lesion. c.intrauterine device*

**Source: Modified from DeMay (19)**

## **2.2 Visual inspection methods**

In developing countries, cervical cancer screening using cervical cytology and human papillomavirus testing is difficult. This prompted introduction of low technology, low cost tests such as visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) in as alternative cancer screening techniques (25). This involve application of acetic acid or Lugol's iodine respectively to the cervix and observe color change of the cervical epithelium after specified time. The principle behind these techniques lies in the composition of the cervical

epithelium. However, one of the weaknesses of these visual inspection techniques is the high rate of false positive findings which may lead to unnecessary greater number of colposcopies (26).

### **2.2.1 Visual inspection with acetic acid (VIA)**

Application of 3-5% Acetic acid is believed to reversibly coagulate or precipitate cellular proteins and also helps in clearing mucus secretions on the cervix (25). Normal squamous epithelium appears pinkish in colour. An increase in cellular proteins characteristic in cervical intraepithelial lesion results in increased coagulation and therefore obliterating the colour of the stroma, resulting in acetowhitening (25).

#### **2.2.1.1 Categories of VIA test**

Results for VIA are reported as negative, positive or suspicious for cancer (27).

- A negative report is given when there is no acetowhitening or when there are faint acetowhite lesions, polyp, cervicitis, inflammation, Nabothian cysts.
- A positive report is given when there are sharp, distinct, well-defined, dense acetowhite areas with or without raised margins touching the squamocolumnar junction (SCJ); leukoplakia and warts.
- A result suspicious for cancer is given when there is a clinically visible ulcerative, cauliflower-like growth or ulcer; oozing and/or bleeding on touch.

#### **2.2.1.2 False positive result**

Acetowhitening is not unique to CIN as other conditions also cause accumulation of cellular proteins. These include (25);

- Immature squamous metaplasia,
- Healing and repair as in inflammatory processes.
- Leukoplakia (hyperkeratosis)
- Condyloma (this is a neoplastic lesion – indication of HPV infection and therefore classified as LSIL/CIN I)

In a study done by Davis-Dao et al (28) in which they assessed the effect of cervicitis on Visual

Inspection with Acetic Acid it was found that women with cervicitis were twice as likely to have a positive VIA result as women without cervicitis (odds ratio = 2.0, 95% CI: 1.0-3.7). Sensitivity of visual inspection tests in detection of cervical intraepithelial lesion can be as high as 97% with specificity as low as 36% (23).

### **2.2.2 Visual inspection with Lugol's iodine (VILI)**

Normal mature uterine cervical epithelium contains glycogen which is the basis of this test (14).

Iodine is glycophilic and hence when applied to normal uterine cervical epithelium, is taken up by the cells and changes colour to deep mahogany- brown or dark (14, 25) and areas with little or no glycogen do not take up the stain and hence no colour changes to mahogany brown or dark.

Therefore areas taking up the stain are considered normal and therefore reported as negative for intraepithelial lesion while areas that do not take up the stain are considered abnormal and are reported as positive for intraepithelial lesion.

#### **2.2.2.1 False positive results**

Failure by the epithelium to take up iodine stain doesn't always mean the presence of intraepithelial lesion as factors other than CIN may contribute to this. These include (14, 25);

- Immature squamous metaplastic and atrophic epithelia since cells in these epithelia do not produce glycogen
- In leukoplakia (hyperkeratosis)
- Endocervical epithelium since endocervical cells does not produce glycogen.
- Cyst formation

##### **2.2.2.1.1 Effect of increased false positivity rate.**

Perkins et al (29) did a study in which they assessed the impact of patient adherence and test performance on the cost effectiveness of cervical cancer screening in developing countries. The results revealed that population wide screening with VIA was more costly than screening with Pap smears because more women received treatment. This overtreatment is attributed to the high rate of false positivity.

In a comparative study of visual inspection with acetic acid and Pap smear conducted by Ibrahim *et al* (30) done in Sudan it was found that the combination of VIA and Pap had better sensitivity and specificity than each independent test (82.6% and 92.2%). This suggests that after an abnormal visual inspection test result, Pap smear may reduce the false positivity given its higher specificity than visual inspection tests.

### **2.3 Colposcopy**

Colposcopy is the procedure used to visualize the vagina or uterine cervical epithelial surfaces using a binocular operating microscope called the colposcope (21). The colposcope was discovered in 1925 by a German gynecologist, Hinselmann (1). Using the colposcope whose magnification is between 5 and 20 times, the cervical epithelial surface can be examined in detail to identify cervical intraepithelial neoplasia and preclinical invasive cancer (17). Magnification of up to 60x is available with some equipped with a camera for taking photographs of cervixes as necessary. The diagnosis is based on color tone, opacity, surface configuration, vascular pattern and intercapillary distance with blood vessels highlighted as black lines (17, 31). Chemical agents like acetic acid and Lugol's iodine are usually used to improve visualization. This increases the sensitivity of biopsy since it is directly obtained from suspicious areas and not blindly taken (13). Visual inspection techniques are discussed in 2.2.

#### **2.3.1 Indications for colposcopy**

The following prompts the clinician to perform or refer patient for colposcopy (10,14):

- Abnormal cervical cytology
- Clinically abnormal or suspicious looking cervix
  - Unexplained intermenstrual or postcoital bleeding.
  - Vulvar or vaginal neoplasia
  - History of in-utero diethylstilbestrol (DES) exposure
  - Persistently unsatisfactory quality on cytology
  - HPV positive test in women above 30 years of age
- VIA or VILI positivity

- To map abnormalities before cryotherapy or loop electrosurgical excision procedure (LEEP)

In colposcopic determination of precancerous or cancerous lesions of the uterine cervix, the client lies on appropriate table in a lithotomy position. Using appropriate speculum the cervix is visualised using a colposcope. First, normal saline is used to remove the mucus in order to visualize any obvious signs of cancer such as erosions surface contour abnormality leukoplakia or exophytic lesions. The prominent abnormal cervical vasculature is more visible using green filter even before application of acetic acid or Lugol's iodine in HSIL and cancer (32).

Acetic acid (3-5%) is then gently applied to the cervix followed by assessment of margins of the lesion, color and vascular pattern. Grading is done according to observed features and biopsies obtained from areas that are judged as most severe. If results after application of acetic acid are unsatisfactory, Lugol's iodine is applied to help determine the most abnormal areas (32).

### **2.3.2 Normal colposcopic findings**

The original squamous epithelium appears smooth and pink due to the reflection of light from the underlying stroma, which is rich in blood vessels while the squamocollumnar junction is identified by a clinically visible line on ectocervix or within distal canal (25).

### **2.3.3 Abnormal colposcopic findings**

The following are considered abnormal on colposcopic evaluation (14):

- Leukoplakia or hyperkeratosis
- Mosaism or punctuation of vessels
- Atypical vessels with bizarre capillaries
- Acetowhite epithelium

### **2.4 Uterine cervical biopsy**

The gold standard for evaluation of the abnormal uterine cervical epithelium on screening is biopsy which is often colposcopically directed (1).

Histological report of cervical intraepithelial neoplasia of uterine cervical biopsy was suggested by Richart (1). On biopsy, diagnosis of intraepithelial lesion is based on identification of nuclear

atypia while the grading into CIN I (LSIL) through III (HSIL) is based on expansion of immature cell layer from its normal basal location (4).

#### **2.4.1 Cervical intraepithelial neoplasia grade I (CIN I)**

This histological grade of cervical intraepithelial lesion is equivalent to low grade squamous intraepithelial lesion (LSIL) in the Bethesda system of reporting cervical cytology. This histological grade is given when dysplastic cells are confined to the lower third of the epithelium in which the fundamental structure of the epithelium of the squamous epithelium is reasonably well preserved (1, 4). The epithelium may or may not show koilocytic atypia.

#### **2.4.2 Cervical intraepithelial neoplasia grade II & III (CIN II/III)**

This histological grade of cervical intraepithelial lesion is equivalent to high grade squamous intraepithelial lesion (HSIL) in the Bethesda system of reporting cervical cytology. This report is given when highly abnormal smaller cells expand to the middle third (CINII) or cover the entire thickness of squamous epithelium (CINIII) (4). CIN III carries a higher risk of progression to cancer than CIN I or CIN II (33).

#### **2.4.3 Invasive cervical carcinoma**

This report is given when nests and/or tongues of keratinizing or non-keratinizing squamous epithelium invade the underlying cervical stroma. This category also include adenocarcinoma in where malignant endocervical cells proliferate and invade the stroma of the endocervical epithelium (4).

#### **2.4.4 Treatment for cervical intraepithelial lesions and cancer.**

Precancerous lesions are usually treated using ablative or excisional methods which can be done on an out-patient basis. The methods involve destroying abnormal tissue by heating or freezing and by surgical removal of abnormal tissue respectively. Determinants for the choice of treatment modality include provider experience, nature of the lesion in relation to its location and extension and the cost of the method. Advantages and disadvantages are also another factor when deciding treat modality for particular lesion (10). Treatment for invasive cancer depends on the stage of the tumour and include surgery, radiotherapy, chemotherapy or in combination (10, 34). Treatment modalities for cervical intraepithelial lesions including their advantages and disadvantages are outlined in Table 4 below.



**Table 4: Treatment modalities for cervical intraepithelial neoplasia**

Method	Method summary	Pros	Cons
Loop excision of the TZ	High frequency current wire loop	Easy outpatient procedure	Cervical incompetency* and stenosis
Radical electrodiathermy	Monopolar high frequency current-cervical cautery	Easy outpatient procedure	No tissue available for pathology.  Depth of tissue destruction not known
Cryotherapy	Freezing the cervix with nitrogen probe	Easy outpatient procedure	No tissue available for pathology. Depth of tissue destruction not known
Laser vaporisation	Destroying lesion with CO <sub>2</sub> laser	Easy outpatient procedure	No tissue available for pathology
'Cold' coagulation	Heating to appr. 100°C	Easy outpatient procedure	No tissue available for pathology.  Depth of tissue destruction not known
Cone biopsy	Surgical excision	Large specimen obtained  Tissue available for pathology	Often needs general anaesthesia

\*=*small association*; TZ= *transformation zone*

**Source: Modified from Magowan et al (21)**

## **2.5 Justification**

Uterine cervical cancer screening using visual inspection techniques is being used in developing countries including Kenya. Government of Kenya recommended use of visual inspection tests as the primary screening tests for cervical cancer while Pap smear or Human papillomavirus tests may also be used as primary tests where they are available (10). According to eligibility criteria, women with abnormal results on visual inspection test are to be treated with cryotherapy (10), the so called 'see-and-treat' approach. While useful as screening tests for uterine cervical cancer in low resource settings, these visual inspection tests have a limitation of high false positivity rate making the value of 'see-and-treat' approach or direct management by colposcopy and biopsy questionable. Given the high negative predictive value of these tests, most true negatives can be eliminated with high degree of certainty thereby relieving the facility for only those women at higher risk of harboring neoplasia for follow up. The see-and treat approach may over or under treat the lesions while referring all positive cases would overwhelm the referral system and make it very expensive to manage thereby defeating the purpose of simplicity and effectiveness of the use of these visual screening methods. Using a pap smear, that is highly specific to triage patients for colposcopy can reduce the rate of referrals to tertiary facilities. This would ensure that only women at higher risk receive definitive diagnostic tests and enable proper utilisation of these limited resources. This would also minimise unnecessary complications associated with diagnostic and treatment procedures on the lesions in women who are otherwise free of disease.

## **2.6 Research Question**

What were the Pap smear cytological findings in women with abnormal visual inspection test results referred to Kenyatta National Hospital?

## **2.7 Broad Objective**

To determine Pap smear cytological findings in women with abnormal VIA/VILI test results referred to Kenyatta National Hospital.

## **2.8 Specific Objectives**

- To describe the cervical cytological findings in women with abnormal VIA/VILI referred to KNH.

- To describe infective and other non-neoplastic findings in cervical smears in women with abnormal VIA/VILI referred to KNH.
- To compare VIA/VILI findings with Pap smear findings.

### **3.0 METHODS AND MATERIALS**

#### **3.1 Study Design**

This was a cross-sectional descriptive study

#### **3.2 Study Area**

Recruitment of study participants and specimen collection were done at Kenyatta National Hospital (KNH) Family Planning Clinic while specimen processing including staining was done in KNH cytology laboratory. Apart from running family planning and other services, the Clinic also runs cervical cancer screening programme where it receives women who come for routine screening for cervical cancer. The other group of women comes after being referred for among others, having a positive test for VIA/VILI and their number may reach close to 20 per week. The cytology laboratory is located in the anatomic pathology unit comprised of histopathology and cytology. This laboratory processes both gynecological and non-gynaecological specimens where about 3000 gynaecological specimens are processed annually.

#### **3.3 Study population**

Women with abnormal VIA and/ or VILI results referred to Kenyatta National Hospital for further management.

##### **3.3.1 Inclusion criteria**

- Women 18 years and above referred to KNH with abnormal VIA/VILI
- Women referred to KNH with abnormal VIA/VILI who give consent to participate

##### **3.3.2 Exclusion criteria**

- Women with unsatisfactory specimens for evaluation

### 3.4 Sample size determination

The sample size was calculated using the disagreement rate of 18% between VILI and Pap smear as found in the study conducted by Shastri et al (9). In this study, maximum disagreement rates were 10% for VIA vs Pap smear and 18% for VILI vs Pap smear hence 18% has been used.

The Fisher's formula was used for calculating the sample size using the disagreement rate of 18% as indicated above.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where;

n is the minimum sample size for proposed study

Z is the normal standard deviation corresponding to 95% confidence interval

P is the known prevalence

d is the margin of error of precision set at  $\pm 5\%$

$$n = \frac{1.96^2 \times 0.18 \times 0.82}{0.05^2}$$

$$= 226.8081$$

$$= 227$$

### 3.7 Selection of study participants

Potential participants were identified by a nurse as having visual inspection test results as they visited the Family Planning Clinic which was verified by a referral letter. The nurse (research assistant) introduced themselves and explained the purpose of meeting with them. After that the consent was sought from them using ethical guidelines as indicated in the client consent information form (Appendix I). Consecutive sampling method was used until the required sample size was achieved.

### **3.8 Specimen collection, processing, examination and reporting of the smears**

The cervical specimens were collected by experienced health care providers under speculum examination. The specimen was applied on a frosted end glass slide, fixed immediately with 95% alcohol both of which are contained in the Pap collection kit and labelled properly. The specimens were processed by the principal investigator by staining with Papanicolaou stain. Procedure for staining was done following KNH cytology laboratory standard operating procedure for staining gynaecological specimens. (See Appendix III for the Papanicolaou staining procedure).

The principal investigator performed the primary microscopic evaluation of the preparations followed by signing them out together with a pathologist (supervisor). The Bethesda System 2001 for reporting cervical cytology was used when examining and reporting the smears (See Appendix IV)

### **3.9 Quality assurance**

Specimen collection was done by experienced nurses who do the procedures on day to day basis. The specimens were processed at Kenyatta National Hospital cytology laboratory using standard operating procedures put in place and approved by the cytology laboratory-in-charge. The reagents and stains were prepared using relevant standard operating procedures (SOPs) and filtered before each use to ensure good quality staining. Deteriorated stains were discarded and replaced where necessary. On microscopic examination, the investigator evaluated the smears first followed by review together with a supervisor. All 'abnormal' and 10% of 'normal' smears were reviewed by second pathologist and there was a high degree of agreement ( $\kappa=0.8$ ). Any discrepant result was referred to a third pathologist as the tie-breaker.

### **3.10 Ethical considerations**

Permission to conduct this study was sought from KNH/UoN-ERC and clearance was sought from heads in respective departments. Informed consent was sought in writing from the prospective study participants using ethical guidelines. Participant's information was kept confidentially. Results found during the study were sent to the service provider for further management within the same period as routine Pap smears results. After the study, hard copies of data collected will be in the custody of the chairman, Department of Human Pathology of the University of Nairobi for a minimum period of five years before being completely destroyed by

designated personnel. Electronic version of the documents will also be irretrievably destroyed from the computer after a period of five years.

### **3.12 Data collection instruments**

Data was collected using predesigned questionnaire and report forms.

### **3.13 Data management and statistical analysis plans**

Data was coded, entered and managed in a pre-designed Microsoft Access database. Data entry was done continuously in the course of data collection. Data cleaning was performed at the end of data entry and analysis conducted using SPSS version 17.0 software.

The characteristics of the participants were summarized into means/medians and proportions for continuous and categorical variables respectively. Categorical variables were compared using chi-square test. The findings were presented using tables and graphs. All statistical tests were performed at 5% level of significance (95% confidence levels).

## 4.0 RESULTS

### 4.1 Demographic characteristics

Out of 240 participants recruited, 8 (3.3%) were excluded from the study, (1= inadequate squamous cell component; 7= heavy inflammatory cells obscuring cellular details). One participant with inadequate squamous cellular component re-entered the study upon repeat specimen collection and had a high grade lesion.

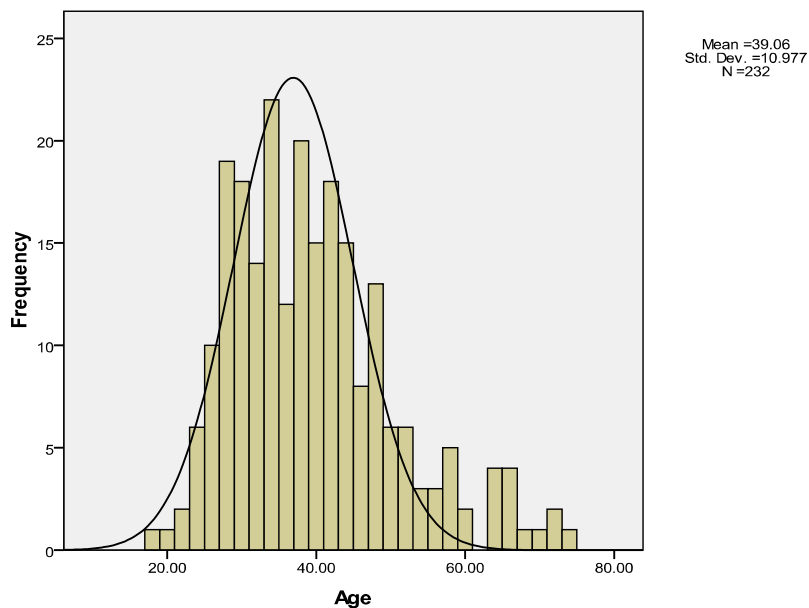
**Table 5: Demographic characteristics**

<b>Variable</b>	<b>Frequency (%)</b>
Mean age (1SD)(Years)	39.1 (11.0) years
Range	18-74 years
Menstrual history (n=232)	Frequency (%)
Regular periods	160 (69.0)
Irregular period	15 (6.5)
Amenorrhea	12 (5.2)
Menopause	42 (18.1)
Missing information	3 (1.3)
Parity	
Median (IQR)	3 (2 – 4)
Range	0 – 12
Age at first sexual intercourse	
Mean (SD)	19 years
Range	9-32 years

### 4.1.1 Age (years) distribution

The mean age of the study participants was 39.1 years (1SD=11.0 years) with the range of 18-74 years. Figure 1 is a Gaussian distribution of age in years with a few over the age of 60 years. There were 33 women representing 14.2% of the total number of the study population who were aged 50 years and above (Table 6) and only 3 (7.1%) had abnormal results on Pap smear.

Fig.1: Age (years) distribution



### 4.1.2 Menstrual history

Regular periods were reported by 160 (69.0%) while 15 (6.5%) had irregular periods, 12 (5.2%) had amenorrhea and 42 (18.1%) were postmenopausal. Information on menstrual history was missing in 3 (1.3%) of the participants (Table 5)

#### 4.1.2.1 Pattern of menstrual cycles according to participant's age group.

When age was compared with menstrual history, it was found that 42 (26.2%) were postmenopausal women (Table 6).



**Table 6: Pattern of menstrual cycles according to participant's age group. (n=227)**

Age(Yrs)	Menstrual History									
	Regular		Irregular		Amenorrhea		Postmenopausal		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
<20	1	0.6(100)	0	0 (0)	0	0 (0)	0	0(0)	1	(100)
20-29	37	23.9(78.7)	6	35.3(12.8)	4	30.8 (8.5)	0	0(0)	47	(100)
30-39	68	43.9 (85)	8	47.1 (10)	4	30.8 (5)	0	0(0)	80	(100)
40-49	47	30.3(71.2)	3	17.6 (4.5)	5	38.4 (7.6)	11	26.2(16.7)	66	(100)
50-59	2	1.3 (10)	0	0 (0)	0	0 (0)	18	42.9(90)	20	(100)
60-69	0	0 (0)	0	0 (0)	0	0 (0)	10	23.8(100)	10	(100)
70-79	0	0(0)	0	0 (0)	0	0(0)	3	7.1(100)	3	(100)
Total	155	100	17	100	13	100	42	100	227	

**Note: (row %)**

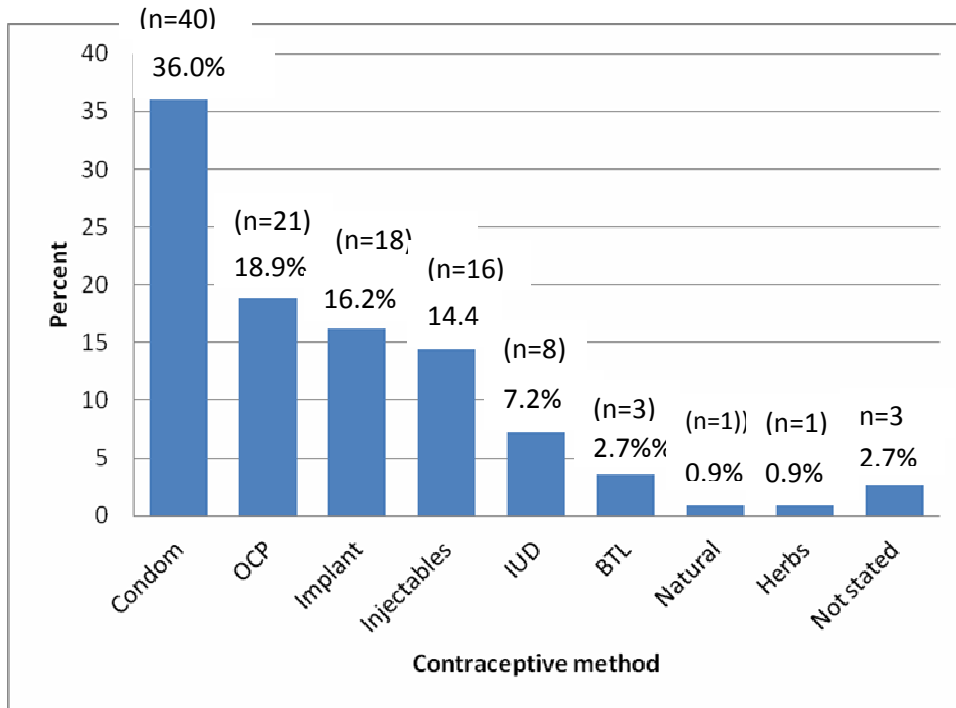
#### 4.1.3 Parity and sexual history

Parity ranged from 0-12 with a median of 3 (IQR=2-4). Median age at first sexual intercourse was 19 years. (Table 5)

#### 4.1.4. Contraceptive methods used

Out of 232 participants, 111 (47.8%) reported being on some contraceptive method and the rest 121 (52.2%) reported not being on any contraceptive method. The most commonly used contraceptive method was condoms, 40 (36.0%; n=111) followed by oral contraceptive pills (OCP) 21 (18.9%); implant 18 (16.2%); injectables 16 (14.4%). (Fig.2)

**Fig. 2: Contraceptive methods used (n=111)**



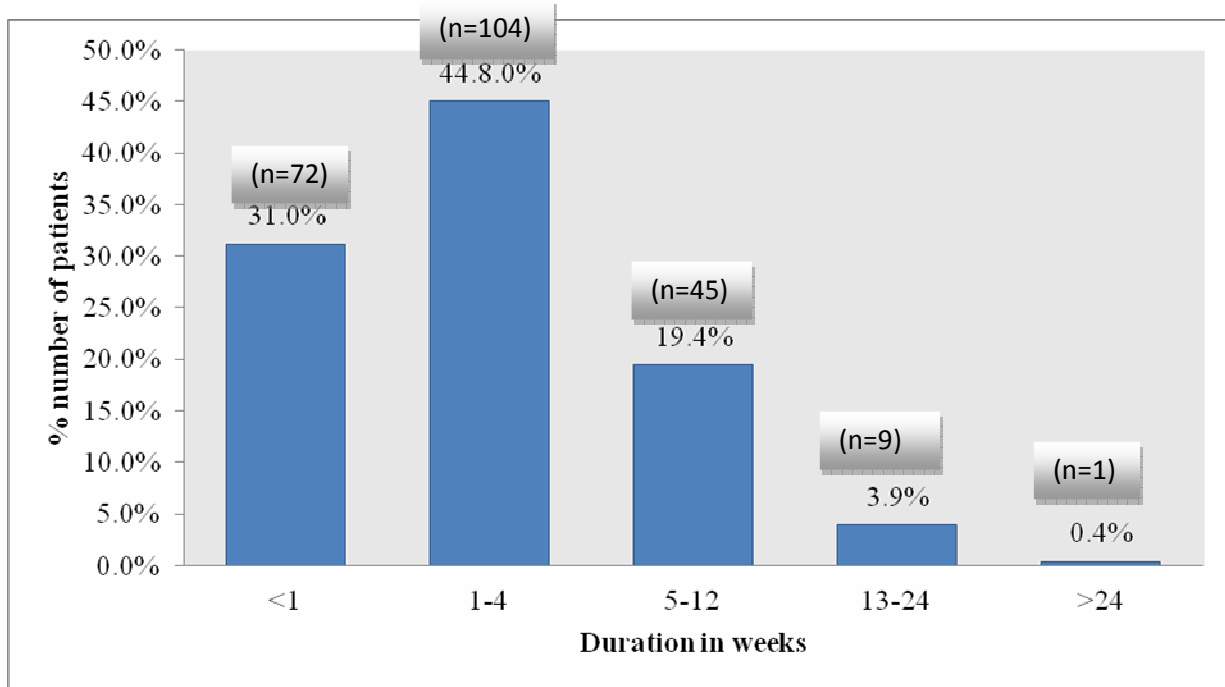
#### **4.1.5 Reasons for seeking VIA/VILI screening service**

There were three recorded reasons that prompted the women seek cervical cancer screening by visual inspection. All three reasons had almost equal number of women as follows; 74 (31.9%) came to have routine check, 69(29.7%) were referred by medical practitioners following symptoms and 71 (30.6%) were self referrals. Data was missing in 18(7.6%).

#### **4.1.6 Duration from VIA/VILI to Pap smear**

Time taken (in weeks) from the date VIA/VILI was done to the date Pap smear was done was recorded. Majority of patients (76.2%) came for Pap smear test within 4 weeks after VIA/VILI test was done. (Fig.3).

**Fig 3. Duration from VIA/VILI test to Pap smear test (n=231)**



**4.1.7 Comparison of duration from visual inspection test to Pap smear test against Pap smear results.**

Using ASCUS as cut off for abnormal Pap result, duration between the date VIA/VILI was done to the date Pap smear collection was done was compared with the Pap smear results to determine whether this could influence Pap smear results. Majority of women came for Pap smear within 4 weeks after VIA/VILI was done (Fig. 3). However, Pap smear results were not associated with duration of seeking Pap test after VIA/VILI, ( $p = 0.175$ ). (Table7).

**Table 7: Comparison of duration from visual inspection test to Pap smear test against Pap smear results (n=231)**

	Positive	Negative	P value
Duration in weeks			
<1	16 (28.6%)	56 (32.0%)	0.175
1-4	22 (39.3%)	82 (46.9%)	
5-12	13 (23.2%)	32 (18.3%)	
13-24	5 (8.9%)	4 (2.3%)	
>24	0 (0.0%)	1 (0.6%)	

#### **4.1.8 Pap smear history**

In the questionnaire study participants were asked if they have had a Pap smear test before. Out of 232 participants, 50, representing 21.6% reported having had Pap smear test before while the rest 182, representing 78.4% had not had any Pap smear test before.

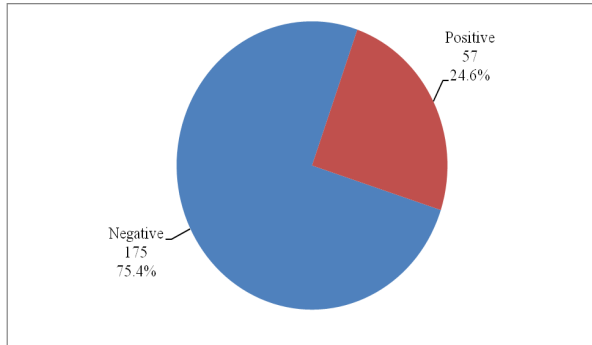
Of the 50 participants who had Pap smear test before, 43 (86%) had normal cytology report (NILM) while only 4 (8%) had abnormal cytology report. Out of these abnormal results 2 were LSILs and the other 2 could not remember the grade of the lesions in the previous Pap test report. Information on previous Pap smear test results was missing in 3 (6%) participants.

#### **4.2 Comparing Pap smear with VIA/VILI results**

All participants in this study (232) had positive result for visual inspection tests (VIA/VILI). Of these, 57 (24.6%) had a report of ASCUS or worse while the rest 175 (75.4%) had the report of negative for intraepithelial lesion or malignancy (NILM) (Fig. 4)

Out of the 232 participants, 11(4.7%) were reported as having infection as follows; 5(45.4%) were Bacterial vaginosis, 4 (33.4%) were Candida, 1(9.1%) was Herpes simplex and 1(9.1%) was reported as having Trichomonas vaginalis.

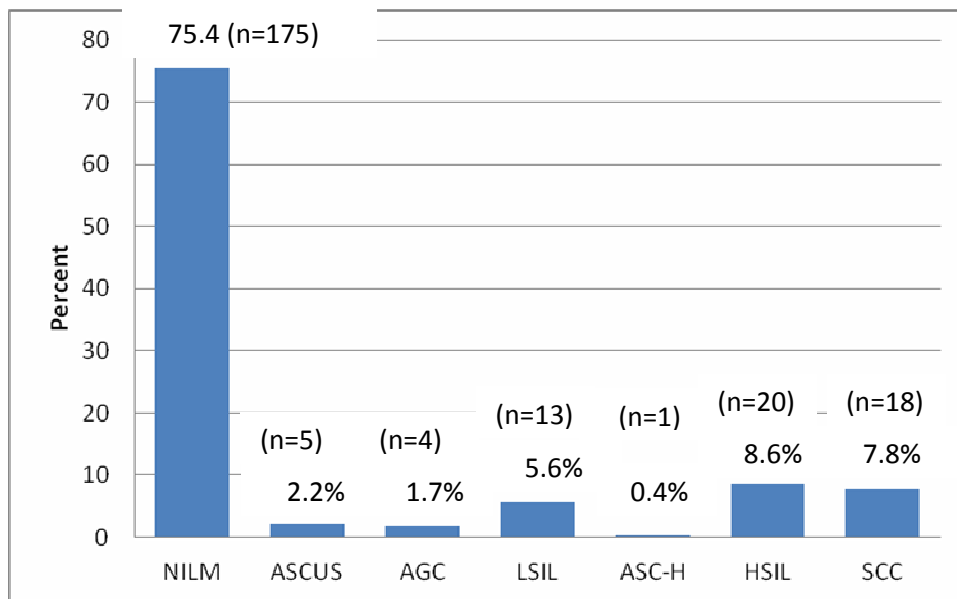
**FIG 4: Proportion of Pap smear positive test results (n=232)**



#### 4.2.1 Specific cytological findings on Pap smear

Out of the 57 participants with abnormal lesions 5 (2.2%) were reported as ASCUS, 13 (5.6%) were LSILs, 4 (1.7%) were AGCs, 1 (0.4%) was ASC-H, 20 (8.6%) were HSILs and 18 (7.8%) were reported as invasive carcinoma (Fig.5). This means that only 39/232 (16.8%) were referred for definitive management while ASCUS and LSIL were recommended for follow up after 6 and 12 months respectively.

**Fig 5: Distribution of intraepithelial lesions on Pap smear (n=232)**



**Note on fig. 5:** 2 women had combined lesions of LSIL/AGC and other 2 women had combined lesions of HSIL/AGC.

**Figure 6 A-J: Photomicrographs of intraepithelial lesions and infections**

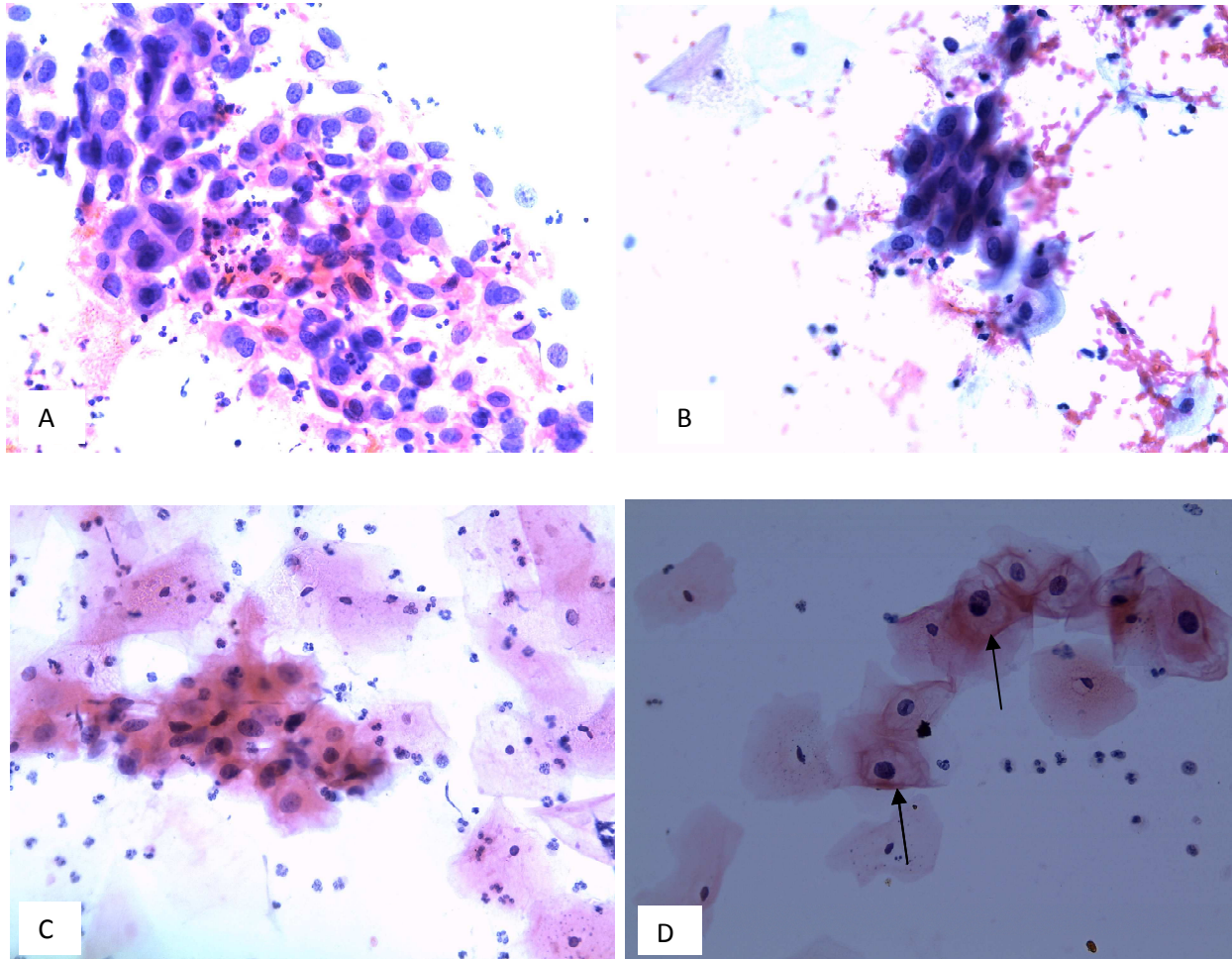


Fig. 6 A and B show atypical squamous cells of undetermined significance (ASCUS)(40x). C and D show Low grade squamous intraepithelial lesion (LSIL). See koilocytes in D (arrows). 40x

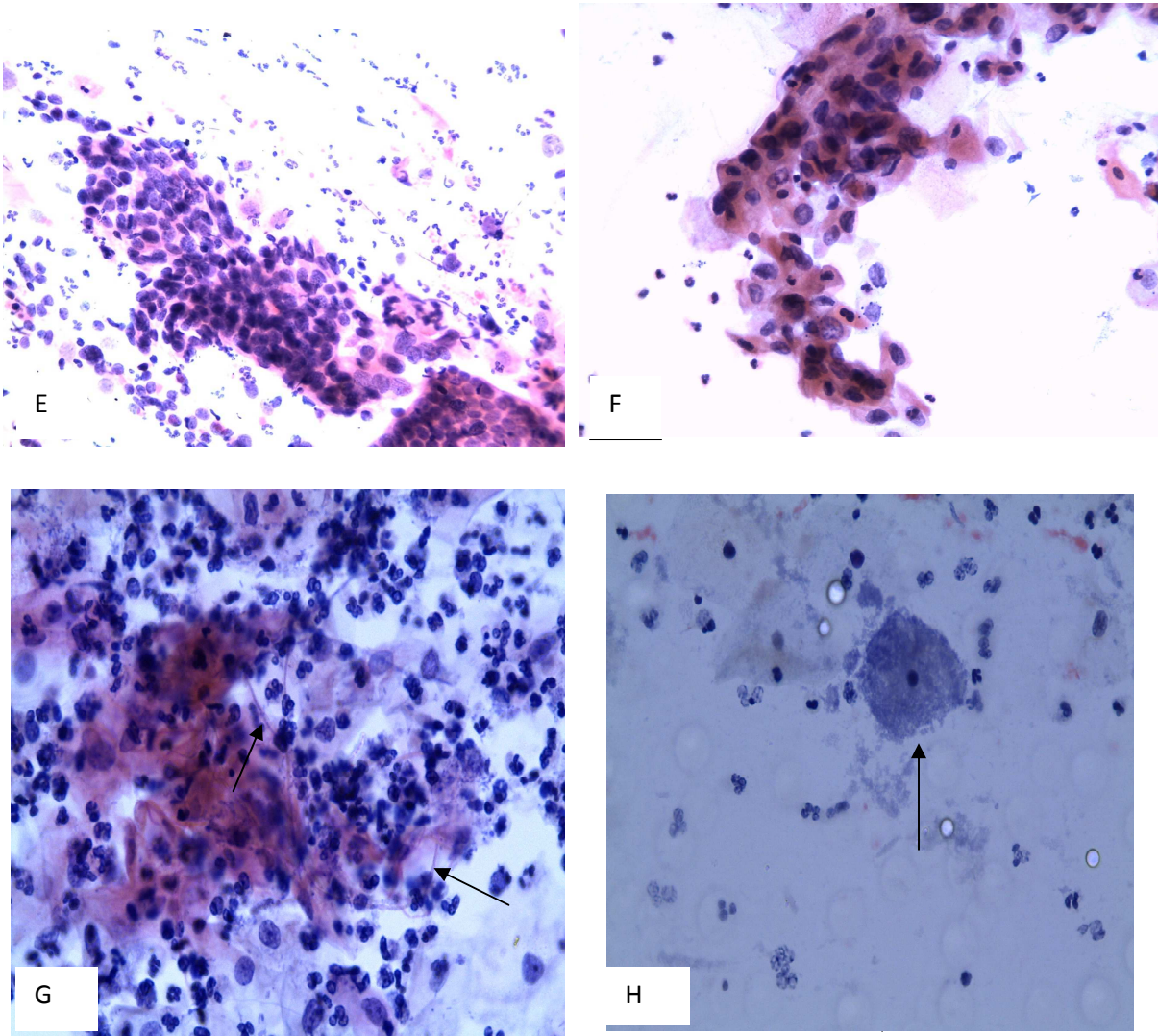


Fig 6 E shows high grade squamous intraepithelial lesion (HSIL)(40x). Note nuclear overcrowding, hyperchromasia chromatin coarseness and a raised N/C ratio. F shows squamous cell carcinoma (SCC) (40x). G shows Candida hyphae ; (arrows) 63x. H shows a clue cell (arrow), shift in flora suggestive of Bacterial vaginosis (63x)

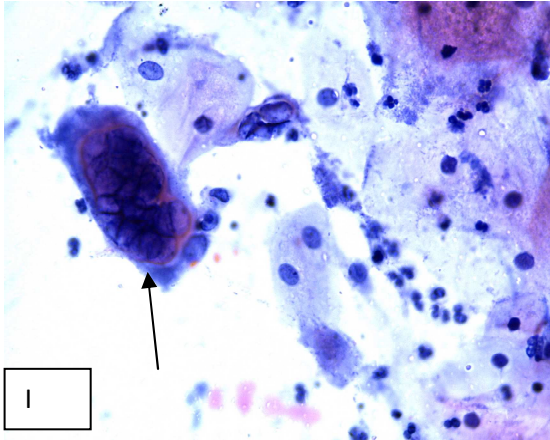


Fig. 6. I shows Herpes simplex virus cytopathic effects (arrow). Note the multinucleation, nuclear molding and chromatin margination.(63x)



## 5.0 DISCUSSION

Cervical cancer screening with cervical cytology remains a challenge in developing countries. Low cost and low technology techniques, visual inspection with acetic acid and visual inspection with Lugol's iodine were recommended in these countries. Government of Kenya adopted this recommendation such that women who test positive should either undergo cryotherapy or be referred for colposcopy and/or biopsy according to their eligibility. Visual inspection tests have a reputation of producing increased false positive results and may result in overtreatment or increase in referrals for definitive diagnosis (23). This study aimed at examining cervical smears from women with abnormal results on visual inspection referred to Kenyatta National Hospital.

The mean age of the study participants was 39.1 years (1SD=11.0 years) with the range of 18-74 years. Median age of onset of sexual activity was 19 years. These results were comparable to those found by Jeronimo et al (2).

There was particular interest on the number of women age 50 years and above who were recruited in this study whereby 14.2 % (n=232) of the study population were of this age group. In its recommendations for the use of visual inspection tests in developing countries, the WHO does not recommend screening postmenopausal women using visual inspection tests (7). This was adopted by Kenyan government where it does not recommend screening women over 50 years of age with visual inspection tests (10). The inclusion of this age group in this study may be due to lack of awareness to the service providers or the women themselves. Recommendation for this group is screening by cervical cytology or HPV test (10). These women should be referred for cytology because the visual inspection tests have low sensitivity to detect lesions because of the shift in position of the transformation zone deep inside the endocervical canal making its visualization difficult (7). Again the false positivity rate is likely to be high due to the nature of the epithelium which is prone to inflammation by both infective and non-infective agents (13) which could explain the results found in this study.

Of the 232 participants who met the inclusion criteria, only 57 (24.6%) had a result of ASCUS and above. Of these, 18(7.8%) were ASCUS or LSIL. The rest were AGC, ASC-H/HSIL or invasive carcinoma. Because of the natural history of cervical cancer, cases of ASCUS and LSIL are safely followed up after 6 and 12 months respectively when higher proportion regress while

only a small proportion (<1%) will progress to cancer (14). The rest of the lesions need to be investigated with colposcopy where treatment and biopsy may be indicated.

The proportion of women with high grade lesions and cancer detected by cytology in this study was higher compared to the general population. For example HSIL was (8.6%) in this study population as compared to general population (0.5%-3%) (1,13) as reported by Koss and Edmund. This could be due the population selected in this study whereby the participants were screen positive by VIA/VILI. In their study, Lewis et al (34) compared triage methods on Kenyan women who screen positive following visual inspection of the cervix with acetic acid. With similar cutoff point of ASCUS as abnormal test, the rate of cervical abnormality detection was 54% which is higher than findings in this study (24.6%). Lewis et al study included women in the 30-39 age groups while this study included any age starting from 18 years. However this could not explain the difference because similar age group in the present study had lower rate (31.2%) of abnormal results as compared to their counterparts in Lewis et al study. The difference could be attributed to the fact that this study underwent a rigorous quality control with four levels of sequential specimen evaluation while the study by Lewis et al evidently had two levels and with poor agreement ( $\kappa=0.08$ ) versus 0.8 in this study.

In a study by Shastri et al (9) where they compared VIA/VILI, Pap smear and HPV test, the detection rates of LSIL, HSIL and cancer on cytology were 0.2%, 0.8% and 0.5% respectively. This is lower compared to that found in this study i.e. LSIL, HSII and cancer were 5.6%, 8.6% and 7.8% respectively. This difference could be explained by the fact that Shastri et al selected asymptomatic, apparently health and previously unscreened women while this study included women who were screen positive for both or either of VIA/VILI . The detection rate of cervical intraepithelial lesions with ASCUS as a threshold was 44% in a study by Mabeya et al (40) who compared Pap smear with VIA in HIV-infected women. This is higher than that obtained in this study (24.6%) and the difference could be attributed to the fact that HIV-infected women are at higher risk of harboring cervical intraepithelial as found by Muchiri (12). At LSIL threshold, detection rate of cervical intraepithelial lesions was 2.2% in Sarian et al (26) study in which they evaluated VIA/VILI,cervical cytology and HPV testing. With similar threshold of LSIL, detection rate in this study was 19% which is higher than Sarian et al study. This could be due to study population difference where Sarian et al included apparently healthy women with no previous abnormal Pap smear result.

In this study the number of women referred for definitive diagnosis and/or therapy was further reduced to 39(16.8%) by cytology. This means that Pap smear has selected women who most likely do not require any treatment for neoplasia thereby saving them from potential complications associated with particular treatment.

Duration between the date VIA/VILI was done to the date Pap smear was done was recorded. Majority (76.2%) of women came for Pap smear within 4 weeks. Although reasons for this turnout within this short period of time in this study have not been explained, anxiety may be suggested as one of them as put down by Marcus (35) that *'the psychological..... impact of false positives, and the results of cancer screening can wind up being the opposite of what patients seek: Rather than peace of mind, they can come away with more questions, distress.....than they bargained for'*. Pap smear results were not significantly different among women coming at different durations ( $p=0.176$ ). Advantages of triaging with Pap smear after screen positive test may be two fold. First the anxiety that the woman has about having cancer reduces or disappears sooner or later if triage test result is negative. Secondly, only women at high risk of having cancer i.e. with HSIL and above may receive more invasive management in the form of definitive diagnosis with or without therapy.

Inflammation has been implicated as a cause of positive visual inspection test results among other causes with infections as one cause of such inflammation (25,28). The proportion of infections detected in this study was low (4.7%). This compares well with similar study by Lewis et al whose infection detection rate was 6.5%. Detection rate for *Candida albicans* in Pap smear was 7.6% in study by Avwioro et al (36) in which they determined the sensitivity of a Papanicolaou smear in the diagnosis of *Candida albicans* infection of the cervix which translated to sensitivity of 25.25%. Although sensitivity for infection detection was not calculated in the present study, Avwioro et al study mentioned above and several other studies have confirmed the low sensitivity of Pap smear in infection detection in general which could explain the results in this study. In addition there are other non-infective causes of inflammation that have not been evaluated in this study.

Sensitivity of visual inspection tests in detection of cervical intraepithelial lesion can be as high as 97% with specificity as low as 36% (23). Sensitivity of Pap smear in detection of cervical intraepithelial lesion in the general population can be as low as 47% with specificity as high as

95% (10, 13, 22, 23). The low specificity for the visual inspection tests could therefore explain the small number of abnormal results on Pap smear found in this study.

## **CONCLUSIONS**

Majority of women in this study had normal Pap smear cytological findings and only few had significant lesions and were therefore triaged for definitive diagnosis and/or treatment.

## **RECOMMENDATIONS**

- Pap smear should be used to triage women with positive visual inspection test results.
- Increase awareness to service providers and the general public about the Government Policy on the use of visual inspection tests in women more than 50 years of age because 14.2% of study participants were inappropriately screened using visual inspection tests.

## **STUDY LIMITATIONS**

- All women with normal cytological reports on Pap smear were recommended for routine follow-up as this is the standard practice at the study facility. This might have affected the results as Pap smear might have missed some abnormal cases due to its low sensitivity.

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## **APPENDICES**

### **APPENDIX I A: CLIENT CONSENT INFORMATION FORM**

**TITLE:** PAP SMEAR CYTOLOGICAL FINDINGS IN WOMEN WITH ABNORMAL VISUAL INSPECTION TEST RESULTS REFERRED TO KENYATTA NATIONAL HOSPITAL

My name is Patrick Joseph Chagwa, student at the University of Nairobi, School of Medicine in the Human Pathology department. I would like to conduct a study that I will introduce to you below. There is English and Kiswahili version for the same information. From these languages, feel free to choose the language you would understand better. A nurse will read to you the following information so that you understand about this study I would like you to participate if you agree.

#### **INTRODUCTION**

Cervical cancer is preventable in its early stage through screening programmes like the one/s you have undergone. They are easy to implement because they are less expensive to establish and are considered suitable for mass screening. Pap smear can be done where available although more expensive compare to the visual inspection methods. When visual inspection tests produce abnormal results as in your case, there is need to conduct further test/s before treatment. Pap smear may successfully select cases that may not need treatment or further tests. In addition, Pap smear may reveal conditions other than cancer e.g. infections which may improve care if reported and treated.

This study therefore aims at determining how Pap smear can improve the cervical cancer screening after an abnormal visual inspection test result.

#### **PROCEDURE**

This procedure is similar to the one you have already undergone. This time, the nurse will use something like a broom designed for collection of this specimen. The broom will be touched to the cervix to collect material for examination. The collected material will be spread on a special

glass ready for further processing. The investigator will collect and process the specimen after which the results will be compared with visual inspection test results which you have.

## **BENEFITS**

Your Pap smear result will be compared with visual inspection results which you already have. In addition, abnormalities (other than developing cancers) that are not picked by visual inspection tests may be revealed by Pap smear. This would lead to better decisions and hence better management would be given to you. There is no financial benefit arising from participating into the study.

## **RISKS AND INCONVENIENCES**

Pap smear collection procedure is not associated with any complications. However, slight discomfort may be felt and small amount of bleeding may be manifested.

All information gathered from you or generated from the processing of your specimen will be treated confidentially. The questionnaire data will be delinked and your name will not appear in the data base. No added cost will be asked from you as a result of participating in the study.

## **ABNORMAL PAP SMEAR FINDINGS**

Should the results from Pap smear turn out significantly abnormal, you shall be referred for confirmation tests. Results from these tests shall be followed up and used in this study for comparison with visual inspection test results and Pap smear results.

## **VOLUNTARISM**

Participation in the study is totally voluntary. Declining to participate will by no means affect the services you are seeking for. You are therefore, upon reading the above information, free to make up your decision to participate or refuse to participate in the study without any consequence.

Do you have any questions?                      YES                         NO  

If yes I will clarify them to you.

Do you agree to participate in the study?   YES                         NO  

**CONSENT**

I ..... have read and understood the purpose of the study, procedure to be done on me, the benefits, risks and inconveniences associated with participating in the study and have agreed to participate without force or coercion of any kind.

Thumb print/signature: .....                      Date: .....

Witness:      Name;                      .....

                    Signature:                      .....

Feel free to contact any of the mentioned persons below should you have any question anytime.

**CONTACTS**

Researcher

Patrick Joseph Chagwa

University of Nairobi, College of Health Sciences

P.O. Box 19676-00202, Nairobi.

Mobile number: +254 717402558

University of Nairobi, Department of Human Pathology

Dr. Waweru W. (Supervisor)

P.O. Box 19676-00202, Nairobi.

Tel: +254-02-7263000 Ext 43769 ; +254-02-2725102

KNH/UON Ethical Research Committee chairperson

P. O. Box 20732, Nairobi Kenya.

Tel: +254-02-726300 Ext 44102

## **APPENDIX I B: FOMU YA IDHINI**

KICHWA CHA UTAFITI: MATOKEO YA UCHUNGUZI WA KISAITOLOJIA (PAP SMEAR) KATI YA WANAWAKE WALIOGUNDULIWA KUWA NA MATOKEO YASIO YA KAWAIDA YA UKAGUZI KWA KUONA (VIA/VILI) WALIOELEKEZWA KATIKA HOSPITALI KUU YA KENYATTA

Jina langu ni Patrick Joseph Chagwa mwanafunzi katika chuo kikuu cha Nairobi idala ya Human Pathology. Ningependa kufanya utafiti ambao nitawaelezea, tafadhali soma ujumbe ufuatao kwa makini. Ujumbe huu emeelezwa kwa lugha ya kingleza na Kiswahili. Una uhuru wakuchagua lugha ambayo utaelewa vyema.

### **MAELEZO KWA UFUPI NA NIYA YA UTAFITI HUU**

Saratani ya njia ya kizazi yaweza kuzuiwa iwapo dalili zake zitatambuliwa mapema. Iyi ni kwakupitiya njia uchunguzi ambayo ushaipitiya (VIA/VILI). Njia hii ya uchunguzi ni rahisi kuitumia, ni ya bei nafuu na imetumika katika uchunguzi wa uma. Uchunguzi wa saratani hii kwa njia ya Pap smear yaweza kafanywa ingawa ni wa bei ya juu kulinganisha na njia ya VIA/VILI. Iwapo majibu ya VIA/VILI yataonyesha dalili ya saratani ya njia ya kizazi, basi kuna umuhimu wakufanya uchunguzi zaidi kabla ya matibabu. Njia ya Pap smear yaweza kubaini kati ya wanawake ambao hawatahitaji uchunguzi zaidi au matibabu. Matokeo ya uchunguzi kwa njia ya Pap smear yaweza kuonyesha magonjwa mengine mbali na saratani ambayo yataimarisha matibabu iwapo yatatambuliwa.

### **UTARATIBU WA KUSHIRIKI**

Utaratibu wakuchukuwa kipimo ni kama uliopitia. Muuguzi atatumia kifaa maalum kuchukuwa kipimo. Kipimo kitaperekwa katika mahabara ili kubaini dalili za saratani. Matokeo yatalinganishwa na yale ya VIA/VILI.

## FAIDA YA UCHUNGUZI HUU

Majibu ya Pap smear yatalinganishwa na yale ya VIA/VILI. Pap smear ina uwezo wa kubaini magonjwa mengine mbali na dalili za saratani ambayo hayawezi kubainiwa kupitia njia ya VIA/VILI. Hivyo basi, utafaidika kutokana na uamuzi bora wamatibabu utakayo pewa. Hakuna faida yoyote yaki fedha utakayo pata kutokana na utafiti huu.

## MADHARA YA UTAFITI

Ingawa utaratibu wakupata kipimo kwa njia ya Pap smear hauna madhara yoyote, muhudumiwa anaweza kuhisi usumbufu pia kiwangu kidogo cha damu chaweza andamana na kinapochukuliwa kwa kipimo.

Stakabadhi za matokeo yako zitashughulikiwa kwa njia ya siri; hakuna yeyote asiyeruhusiwa atakayezisoma. Hakuna malipo ya ziada utakayo hitajika kulipa kwakuhusika katika kwa uchunguzi huu.

## UTARATIBU UTAKAOFUATWA BAADA YA PAP SMEAR KUONYESHA DALILI ZA SARATANI YA KIZAZI

Iwapo matokeo ya Pap smear itaonesha dalili za saratani utaelekezwa kufanyiwa uchunguzi wa kina. Majibu ya uchunguzi huu wa kina yatafuatiliwa ili kuyalinganishwa na yale ya VIA/VILI na ya Pap smear.

## IDHINI YA MSHIRIKI

Watakao shiriki katika uchunguzi huu itakuwa kwa njia ya hiari bila kushurutishwa. Kutoshiriki hakutapoteza kwa njia yoyote haki yako yakuhudumiwa unavyostahili.

Una uhuru wa kuamua kushiriki au kutoshiriki katika uchunguzi huu bila madhara yoyote.

Una swali lolote? Ndio  La

Kama una swali nitakujibu kwa upana.

Utashiriki kwenye utafiti huu? Ndio  La

Kama utashiriki, tafadhali tia sahihi yako kwenye pengo lililoachwa hapa chini.

Mimi .....nimesoma na nimeelewa  
nia ya uchunguzi huu, utaratibu utaotumika kuchukuwa kipimo, faida na madhara yanayohusika  
na uchunguzi huu. Nimekubali kushiriki kwa hiari bila kushurutishwa.

Sahihi ya mshirika..... Tarehe.....

Sahihi ya Shahidi..... Tarehe.....

Unaweza wasiliana nasi wakati wowote kupitia nambali zifuatazo iwapo unaswali lolote.

ANWANI

Mchunguzi,

Patrick Joseph Chagwa

Chuo kikuu cha Nairobi

S.L.P. 19676-00202, Nairobi.

Nambari ya simu: +254 717402558

Chuo kikuu cha Nairobi, Idara ya Human Pathology

Dr. Waweru W. (Msimamizi)

S.L.P. 19676-00202, Nairobi.

Nambari ya Simu: +254-2-7263000 Ext 43769 ; +254-2-2725102

Maadili ya utafiti ya KNH/UON ERC

S.L.P. 20732, Nairobi Kenya.

Nambari ya simu: 02-726300 Ext 44102



**APPENDIX II: QUESTIONNAIRE**

PROJECT TITLE: PAP SMEAR CYTOLOGICAL FINDINGS IN WOMEN WITH  
ABNORMAL VISUAL INSPECTION TEST RESULTS REFERRED TO KENYATTA  
NATIONAL HOSPITAL

Date ..... Study No 

--	--	--

Referred from (Health facility name).....

Lab No: 

--	--	--	--	--	--	--	--

Date VIA/VILI was done (dd/mm/yy) 

--	--	--	--	--	--

Date on referral (dd/mm/yy) 

--	--	--	--	--	--

1. Age 

--	--

 years

2. A. Last menstrual period with regular periods (dd/mm/yy) 

--	--	--	--	--	--

B. Irregular periods (Tick) 

--

C. No periods for the last six months (Tick) 

--

D. Menopause (Tick) 

--

3. Are you currently using any Contraceptive? (Tick as appropriate)

No 

--

 Yes\* 

--

\*If yes specify (tick as appropriate)

Natural	<input type="checkbox"/>	Injectables	<input type="checkbox"/>
IUD	<input type="checkbox"/>		
OCP	<input type="checkbox"/>		
Condom	<input type="checkbox"/>	Others*	<input type="checkbox"/>

\*Specify.....

4. How many pregnancies have you had?

5. Have you had Pap smear done before? (tick as appropriate)

NO  YES\*

5.1 \*If yes, Normal  Abnormal<sup>†</sup>

<sup>†</sup> If abnormal what were the results? (Tick as appropriate)

5.1.1 ASCUS  LSIL  HSIL  AGC

5.1.2 Cannot remember

6. What prompted you to seek cervical cancer screening service? (Tick as appropriate)

1. Recommended routine check

2. Medical advice following symptoms \*  \*specify.....

3. Self referral

4. Others

7. Age at first sexual intercourse .....

**APPENDIX III; REPORT FORMATS**

1. Pap smear report (Tick as appropriate)

NILM	<input type="checkbox"/>	ASCUS	<input type="checkbox"/>
LSIL	<input type="checkbox"/>	ASC-H	<input type="checkbox"/>
HSIL	<input type="checkbox"/>	AGC	<input type="checkbox"/>
AIS	<input type="checkbox"/>	SCC	<input type="checkbox"/>
ADENO CA	<input type="checkbox"/>	INFECTIONS	<input type="checkbox"/>

Comments if any.....

2. Colposcopic findings (Tick as appropriate)

NEGATIVE

CIN I

CIN II

CIN III

INVASIVE CANCER

Comments if any.....

3. Biopsy findings

NEGATIVE

CIN I

CIN II

CIN III

CIS

AIS

INVASIVE CA

INFECTIONS\*

\*Specify.....

Comments if any.....

## **APPENDIX IV: PAPANICOLAOU STAINING PROCEDURE**

### **Principle of the stain**

Hematoxylin, being basic, stains the nuclei blue by dye lake formation. The eosin azure solution being acidic stains the cytoplasm which is basic so that the eosin has affinity for the mature cells while light green has affinity for the young cells. Orange G is also an acidic dye and consequently has an affinity for the cytoplasm and stains and keratin.

### **Staining technique**

1. Slides with smears will be fixed in 95% ethanol for 15 minutes.
2. Hydrated in descending grades of alcohol, 80%, 70%, 50%, 10 dips in each.
3. Rinsed in tap water, 10 dips.
4. Stained with Harris Hematoxylin stain for 4 minutes.
5. Rinsed in tap water until excess stain is drained off.
6. Differentiated in three changes of 0.05% acid alcohol, 10 dips in each.
7. Rinsed in running tap water.
8. Blued in Scott's tap water for 1 minute, and rinsed in tap water, 10 dips.
9. Dehydrated in ascending grades of alcohol, 70%, 90%, 10 dips in each.
10. Stained with Orange G-6 for 2 minutes.
11. Dehydrated in three changes of 95% alcohol, 10 dips in each.
12. Stained in Eosin Azure EA-36 for 3 minutes.
13. Dehydrated in three changes of 95% alcohol, 10 dips in each.
14. Cleared in three changes of xylene, 10 dips in each.
15. Mounted with DPX (Diestrene Plasticizer Xylene), and observed under microscope.

## **APPENDIX V: THE BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY (2001)**

### **1. SPECIMEN NAME**

- Indicate conventional (Pap smear) vs. Liquid based

### **2. SPECIMEN ADEQUACY**

- Satisfactory for evaluation (indicate presence or absence of endocervical cells and /or transformation zone component, indicate other quality indicators e.g. Partially obscuring blood, inflammation, technical aspects etc.
- Unsatisfactory for evaluation (specify reason)

### **3. INTERPRETATION/RESULT**

#### **A. NON-NEOPLASTIC FINDINGS**

Organisms

- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* spp
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp
- Cellular changes consistent with Herpes simplex virus
- Other non-neoplastic findings (Optional to report; list not inclusive)

Reactive cellular changes associated with:

- Inflammation (includes typical repair)
- Radiation
- Intrauterine contraceptive device (IUD)

- Glandular cells status post hysterectomy  
Endometrial cells (in a woman  $\geq 40$  years of age)

## **B. EPITHELIAL CELL ABNORMALITIES**

### Squamous cell abnormalities

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

### Glandular cell abnormalities

1. Atypical:
  - Endocervical cells, NOS or specify in comments
  - Endometrial cells, NOS or specify in comments
  - Glandular cells, NOS or specify in comments
2. Atypical
  - Endocervical cells, favor neoplastic
  - Glandular cells, favor neoplastic



3. Endocervical adenocarcinoma *in situ*

4. Adenocarcinoma

- Endocervical
- Endometrial
- Extrauterine
- Not otherwise specified (NOS)

#### **C. OTHER MALIGNANT NEOPLASMS**

- Carcinomas
- Sarcomas
- Other tumors

## **APPENDIX VI: PREPARATION OF 5% ACETIC ACID (38, 39)**

### Ingredients

Glacial acetic acid – 5 ml

Distilled water – 95 ml

### Preparation

Carefully add 5 ml of Glacial acetic acid into 95 ml of acetic acid and mix well

### Storage

Discard any unused acetic acid at the end of each preparation day

### Label

5% dilute acetic acid

Note: it is important to remember to dilute the glacial acetic acid since the undiluted strength causes severe chemical burn if applied to the epithelium.

## **APPENDIX VII: PREPARATION OF LUGOL'S IODINE**

### Ingredients

Potassium iodide – 10 g

Distilled water – 100 ml

Iodine crystals – 5 g

### Preparation

Dissolve 10 g of potassium iodide in 100 ml distilled water.

Add 5 g of iodine after the potassium iodide is fully dissolved

Stir well until all iodine flakes have fully dissolved

### Storage

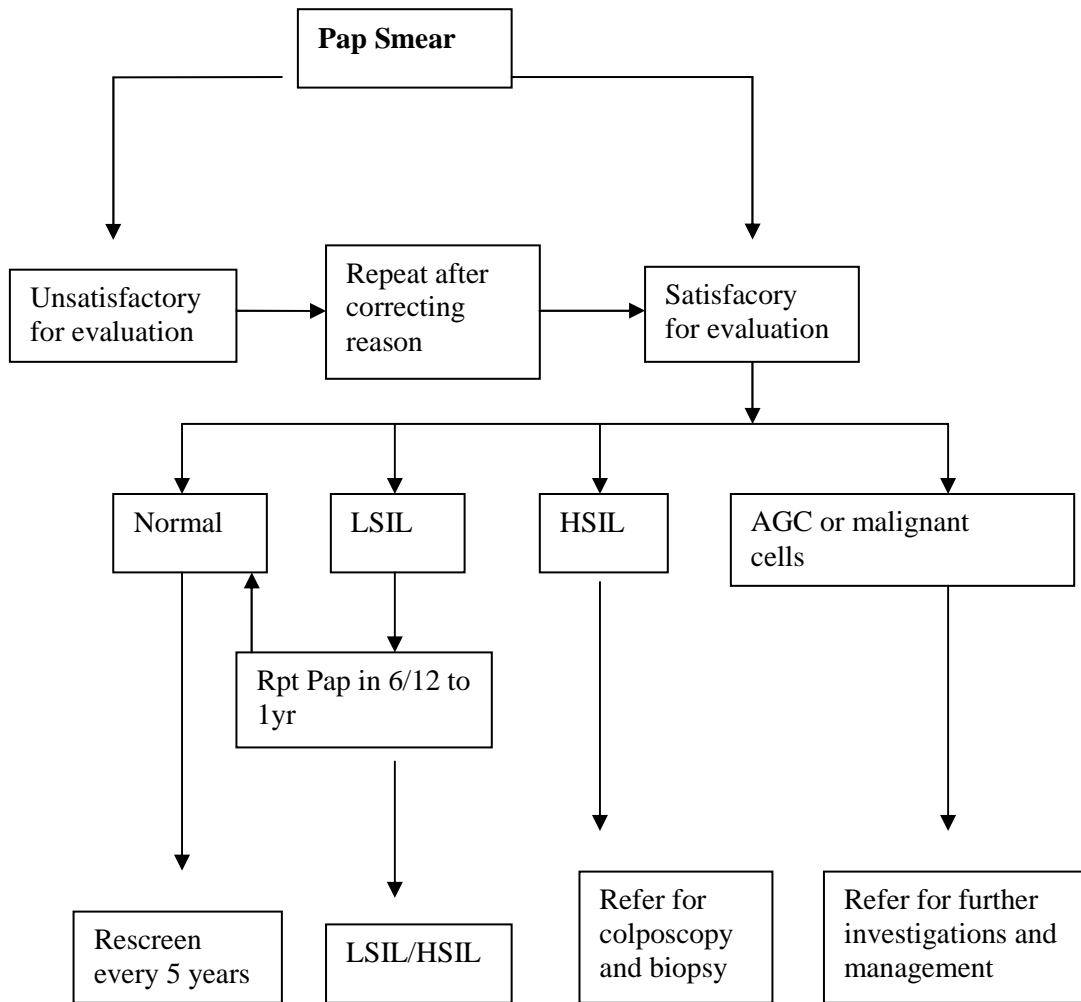
Store in sealed container to prevent evaporation of iodine and loss of staining activity.

Do not store for more than one month.

### Label

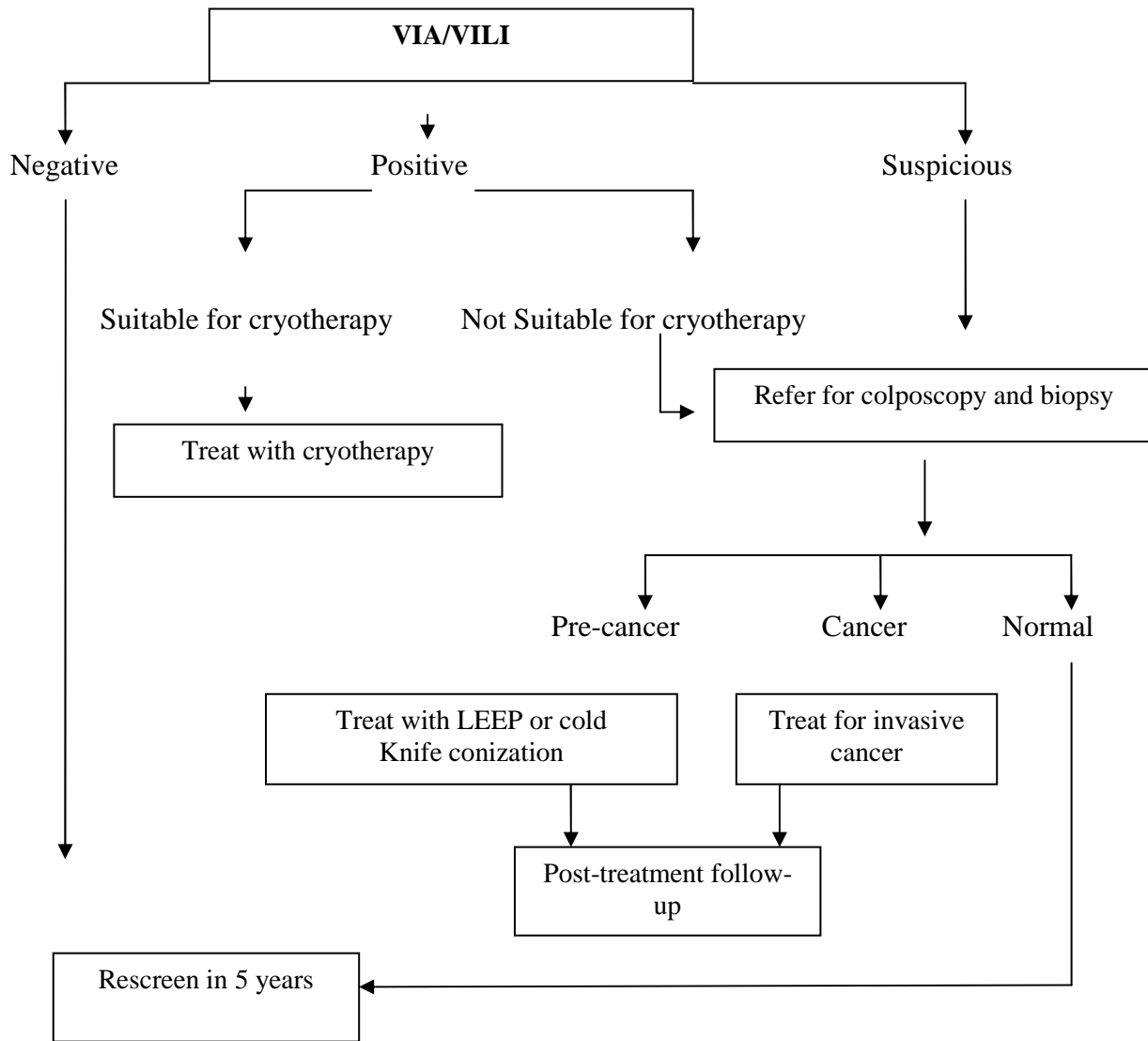
Lugol's iodine

**APPENDIX VIII: KENYA GOVERNMENT GUIDELINES FOR MANAGEMENT OF WOMEN AFTER PAP SMEAR TEST**



Source: Government of Kenya. Ministry of Medical Services and Ministry of Sanitation. National Guidelines for Prevention and Management of Cervical, Breast and Prostate Cancers. Jan 2012

**APPENDIX IX: KENYA GOVERNMENT GUIDELINES FOR MANAGEMENT OF WOMEN AFTER VIA/VILI TEST**



Source: Government of Kenya. Ministry of Medical Services and Ministry of Sanitation. National Guidelines for Prevention and Management of Cervical, Breast and Prostate Cancers. Nairobi. 2012.