

Classification of squamous intraepithelial lesions among HIV-positive women attending Mbagathi district hospital in Nairobi county Kenya.

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Abstract

Cervical cancer is one of the most frequent cancers among women in Kenya and the most frequent cause of cancer deaths in resource-poor countries. About 0.5 million new cases are diagnosed each year globally, and about 0.3 million women die from this disease. Cervical cancer, caused by persistent infection with high-risk human papillomavirus (HPV), is potentially preventable through primary prevention by HPV vaccines and by secondary prevention through screening by a variety of methods. Visual inspection with acetic acid (VIA) has been used alone as a "screen and treat" approach and may give false-positive results. **Objective:** The purpose of this study was to describe visual inspection with acetic acid and cytological findings in HIV-positive women. **Design:** This was a descriptive cross-sectional study. **Settings:** Comprehensive care clinic, Mbagathi District Hospital, Nairobi. **Study population:** HIV positive women attending the Mbagathi District Hospital. **Sample size:** Seventy-five HIV-infected women attending the Mbagathi District Hospital. **Methodology:** Permission for the study was obtained from KNH-UoN/ERC, Nairobi Metropolitan Services, and Mbagathi District Hospital. Seventy-five HIV-infected women were recruited into the study after meeting the inclusion criteria and a questionnaire was used to gather clinical and social demographic data. A qualified nurse collected seventy-five cervical smears from all the participants, followed by visual inspection with an acetic acid test. The cervical smears were

Keywords

squamous
intraepithelial
lesions,
HIV-positive
women.

stained with the papanicolau stain and examined microscopically. Analysis of the data was done using IBM SPSS statistics 26. **Results:** Out of the 75 women in the study 69(92%) had negative VIA results. Visual inspection with acetic acid was positive in 1(1.3%) and suspicious in 5 (6.7%). The prevalence of cervical intraepithelial lesions in women infected with HIV was 22.7%. High false-negative results by VIA was noted in this study. **Conclusion:** The prevalence of cervical intraepithelial lesions in women infected with HIV was 22.7%. High false negative results by VIA was noted in this study.

Introduction

Cervical cancer is the second most common cancer in women and a frequent cause of death in women(3). Cervical cancer has been complicated by the rise of HIV(16,17). The study on epidemiology of cervical squamous intraepithelial lesions in HIV infected women reported an increase of squamous intraepithelial lesion, the precursor for cervical cancer among HIV positive women(18). A study suggests that women whose immunity is low due to HIV infection are 2.7 times likely to develop cervical intraepithelial lesions(19–21). Precancerous stages or pre-invasive precursor lesions are interrupted before translating to cervical cancer. Therefore, the urgent need for Pap smear tests in health facilities. Although cervical cancer is a menace to the health of women worldwide, it is easily preventable through early identification of pre-cancerous lesions to prevent the transformation to invasive cervical cancer (1). There are effective methods put in place to prevent cervical cancer, including HPV vaccines, cervical smear screening, VIA, and HPV testing. However, implementation and affordability remain challenging in most developing countries(2). Although these methods are in place, cervical cancer has continued to increase, causing a lot of suffering in women. Among the many cancers affecting women in Kenya cervical cancer is the second cancer causing morbidity and mortality in women(3). The government has put measures to alleviate cervical cancer, including primary and secondary prevention of cervical cancer.

The HPV vaccine has been introduced and licensed as primary prevention in Kenya so that young women can be vaccinated before their

sexual debut(4). Women in Kenya have continued to suffer due to cervical cancer because the vaccine is expensive and not affordable. Cervical cancer has been complicated by the HIV epidemic in Kenya, becoming a threat to the health of HIV-positive women(5). The high incidence of cervical cancer, poor prognosis, and immune suppression due to HIV infection poses a significant challenge to women's health in Kenya(6). HIV-positive women have reduced CD4 T-cells, which is vital for resolving HPV infections(7). Studies suggest that 70% of cervical cancer cases are etiologically associated with HPV strains type 16 and 18(8–10). HIV-positive women are more vulnerable to the risk of high risk HPV, and prolonged HPV might result in squamous intraepithelial lesions and the transformation to cervical cancer.

A research study on predictors of cervical cancer screening among Kenyan women had a limitation because of scarcity of archived information on the frequency of cervical cancer screening and the methods applied to screen the disease (11). The gap identified on predictors of cervical cancer screening led the PI to study and describe Pap smear findings and VIA findings in HIV-infected women attending Mbagathi District Hospital. Pap smear findings and VIA findings have been described and classified according to the Bethesda system of reporting pap smears (12). The study was conducted at Mbagathi District Hospital because of its strategic area. The facility neighbors Kibra slums, where women engage in activities including prostitution, female sex workers, homosexuality, multiple sexual partners, and casual sex, resulting in STDs, including HIV and HPV infections to earn a living(13).

Epidemiology of cytological abnormalities

Research studies reports that HIV-infected women have an increased risk of pre-cancerous lesions than HIV-negative women(14). There is variation in the prevalence of cytological abnormalities in different countries, including Southern Ethiopia 22.1 %, Nigeria 22.6%, South Africa 17.3%, and Cambodia 6.3%(48). A research study reported cervical epithelial lesions prevalence as 15 % among commercial sex workers in Nairobi County, Kenya (15).

The variation is due to the sample size used in different studies, social demographics, and exposure. The measures in place to prevent cervical lesions contribute to the difference in variation observed in other countries.

Parity

Multiparity may be a risk factor for cervical epithelial lesions, leading to cervical cancer (16). The study further suggests that women with more than four children are 10.9 times likely to develop epithelial cell abnormalities than women with fewer children.

Symptoms of abnormal cervical lesions

Unexplained cervical bleeding may be a sign of unusual cervical lesions (17). The foul smell emanating from the vaginal area, fatigue, nausea, and weight loss may be signs of abnormal squamous lesions.

Management of cervical lesions

Treatment of cervical lesions involves two modalities, including excisional and ablative therapy. Excision biopsy is sent to the laboratory to confirm the irregular lesions. In ablative procedure, biopsy is removed, denatured, and no further study. Cryotherapy and laser therapy are examples of ablative treatment. When cryotherapy method is an option as part of the cure, the abnormal cervical lesion is frozen, and

then it sloughs off. Treatment involving laser therapy subjects light to destroy cervical lesions. The types of excision biopsies include (LEEP) procedures and conization. LEEP's method comprises electrosurgical with a thin wire loop that removes the abnormal area of the cervix.

Abnormal cytology findings

An abnormal cytology is reflected when the nuclear of squamous cells show atypia and is larger than that of an intermediate cell. The nuclear of an intermediate cell is used as a reference to measure abnormal squamous cells. A normal intermediate cell nuclear measures 35microns. Bethesda system for reporting cervical cytology classifies squamous cell abnormalities into epithelial cells abnormalities. The epithelial cell abnormalities includes, atypical squamous cell of undetermined significance (ASCUS), atypical squamous cells – cannot exclude a high grade squamous intraepithelial lesion (ASC-H). Atypical squamous cell of undetermined significance measures two and half to three times that of an intermediate squamous cells and refers to nuclear changes that are suggestive of low grade squamous intraepithelial lesion. Atypical squamous cells - cannot rule out high grade squamous intraepithelial lesion refers to squamous cell changes related to high grade squamous intraepithelial lesions. Squamous cell abnormalities involve a spectrum of non-invasive epithelial abnormalities associated with human papilloma virus (HPV) (53).High grade means that the nuclear has irregularities, increased nuclear-cytoplasmic ratio, hyperchromatic nuclear, coarse chromatin granules or salt and pepper chromatin: Anisocytosis, syncytial patterns, tumor diathesis, and Indian ink files in high-grade squamous lesions. The atypical endocervical cell displays nuclear changes that exceeds reactive or reparative changes. Atypical endocervical cell criteria include nuclear changes, including nuclear overlap, sheets, and strip of cells. In endocervical adenocarcinoma, the requirements include single cells, twodimensional sheets, syncytial aggregates, and a three-dimensional cluster of abnormal cells(53).

Problem statement

Mbagathi District Hospital has a high population serving people from the surrounding slums. Most of the HIV positive women attending CCC in Mbagathi District Hospital are poor and unable to pay for the HPV vaccines, and therefore are at risk of infection with oncogenic HPV 16 and 18, found in 70% of all cervical cancers. The harsh environment in the slums could lead the immunocompromised HIV positive women to have sexual intercourse early before the HPV vaccine, and therefore likely to have acquired STDs including high risk HPV. The HIV positive women attending the facility could be having undetected cervical epithelial lesions. HIV infected women with Precancerous lesions are referred to Kenyatta National Hospital for better management and end up absconding because of the high cost of services. Kenyatta National Hospital handles referrals, outpatients, and inpatients; therefore, access to the facility becomes a challenge to the HIV infected women. Although VIA has been recommended as a screening method, HIV infected women who are 40 years and above, are likely not to benefit from the method because transformation zone recedes to the endocervical canal, and as a result cervical intraepithelial lesions are likely to be missed and progress into cervical cancer. The PI desired to pick all the squamous intraepithelial lesions by usage of pap smear and VIA.

Study design

Descriptive cross-sectional study

Study site

The study was carried out at Mbagathi District Hospital's comprehensive care clinic. The hospital is an urban health facility located within the Nairobi County, Ngumo Estate, off Mbagathi road. The facility is a public health hospital bordering Kibra slums where underprivileged people lived in slums near Kenyatta market. The hospital treats both inpatients and outpatients and has a capacity of 250 beds. Mbagathi District Hospital is in the category of level 5 county

hospital, although its name has not changed. The hospital serves a population of over 3 million people(19). All HIV-positive women of childbearing age are routinely screened for cervical cancer using VIA / VILI tests. HIV-infected women testing VIA /VILI positive are referred to Kenyatta National Hospital for Pap smear and further management.

Study population

HIV-positive women whose age group ranged from 18 years – 50 years consenting to the study.

Inclusion criteria

Women infected with HIV, aged 18 years - 50 years with formal consent.

Exclusion criteria

Those already diagnosed to have cervical cancer
Women who have undergone a hysterectomy

Sample size estimation

The number of samples for the study population was calculated using a prevalence of 26.7%, as was observed in a study assessing the prevalence and identified associated risk factors for precancerous cervical lesions among HIV-positive women in resource-limited settings in Kenya. The prevalence of CIN1, CIN11, CIN111, and ICC was 26.7 % (20).

$$N = \frac{Z^2 p (1-p)}{D^2}$$

N = Sample size

Z = confidence level on a standard distribution
1.96 is the critical value and corresponds to 95 % CI

P = Expected prevalence of proportions expected at to a particular characteristics=**26.7%**

D = degree of precision set at 10% n=
 $1.96^2 \times 0.267 (1-0.267) = 75 + 10$, the sample size was 75 HIV infected women.

(0.1)2

Sampling method

Convenience sampling method was employed to recruit the study participants

Recruitment and consenting

HIV-positive women referred for visual inspection with the acetic acid test, attending Mbagathi District Hospital, and meeting recruitment criteria were incorporated in the study. All the patients signed the consent form. The nurse and P.I. informed the study participants of the rationale and benefits expected after completing the study; no inducement to participate in the research was given. The nurse recorded the social demographic data and the clinical summary of the patient. In every case, a record of a complete history, clinical details, along with clinical diagnosis was made.

Administration of the questionnaire and consenting

The nurse/research assistant and P.I. administered the questionnaire to the patient. The questionnaire form captured the client's name, phone number, and social-demographic data.

Sample collection and Laboratory analysis

A trained and experienced nurse in Mbagathi District Hospital collected the pap smear and performed the VIA test. Daily the principal investigator educated the participants on cervical cancer, pre-cancerous lesions, cervical smear test, and VIA tests. The teachings were conducted frequently for the participants who arrived in CCC at different times. The study nurse coordinated the activities in MCH and assisted the

principal investigator in issuing the consent forms and in cervical smear collection. A complete pap smear kit with a cyto-fixative was used, and therefore, the trained nurse had no challenge fixing the Pap smear. The already fixed cervical smears were appropriately packed on a slide box carrier, transported to KNH laboratory Cytology unit for processing. The patient sample was labeled with a unique code that matched the request form identification. The Papanicolaou method of staining was applied in the processing of the cervical smears. Preparation of the stain followed the standard operating procedures in the laboratory. The smears were evaluated and interpreted using the current 2014 Bethesda System for Reporting Cervical smears.

Quality assurance in the laboratory

The P.I ensured that the questionnaire was accompanied by the request form, identified with a unique number that matched the patients' sample. Internal quality control slides were stained first and compared with pre-stained slides to ensure the new batch's quality of the stains used. The P.I screened the slides, later lecturer anatomic pathology unit re-examined the slides again. For quality control in cytology, 10 % randomly picked negative slides, and all the positive slides were reconfirmed by an independent senior lecturer anatomic pathology unit who was the tiebreaker.

Quality assurance in VIA

This was a clinical procedure and the nurse followed the SOPs provided by the ministry of health.

Results

4. 5 Table 3: Comparison of Visual inspection with acetic acid and Pap smear results

VIA	positivity	Pap smear	Number
SCC	1	SCC	1
HSIL	1	HSIL	4
LSIL	2	LSIL	6
ASCUS	0	ASCUS	6

VIA reaction due other causes	Number
Atrophy	1
Inflammatory smears	1

Statistical tests for Significance:

Statistical tests for significance was not done because some of the lesions confirmed by Pap smear, including ASCUS are not classified as precancerous lesions. Visual inspection with

acetic acid had a reaction in atrophic smear and inflammatory smears and could not be classified as precancerous lesions.

4.6 Photomicrographs

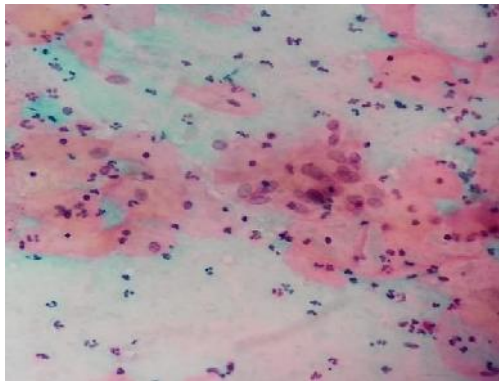


Image 1: Atypical squamous cell of undetermined significance, X 40

Atypical intermediate squamous cell with an increased N: C ratio 2 to 3 times the area of a normal squamous intermediate cell, poorly

defined nuclear halo, hyperchromatic, and irregular margins.

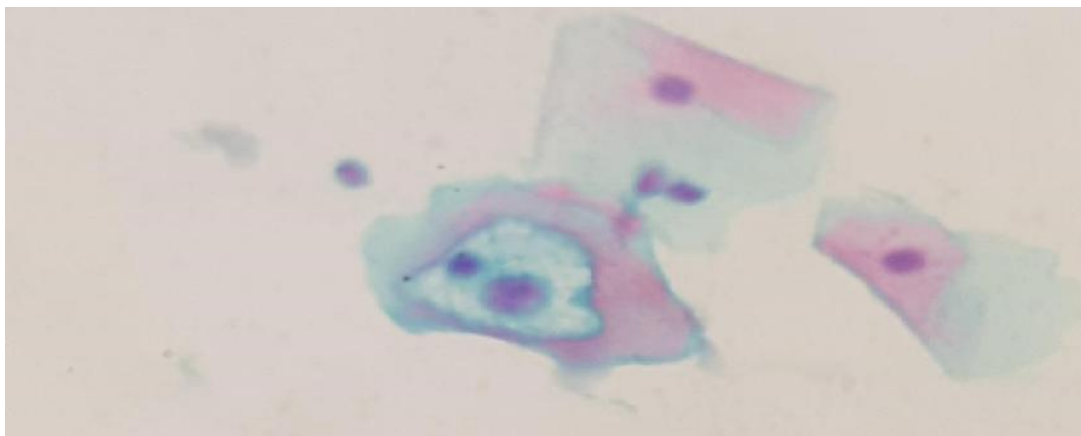


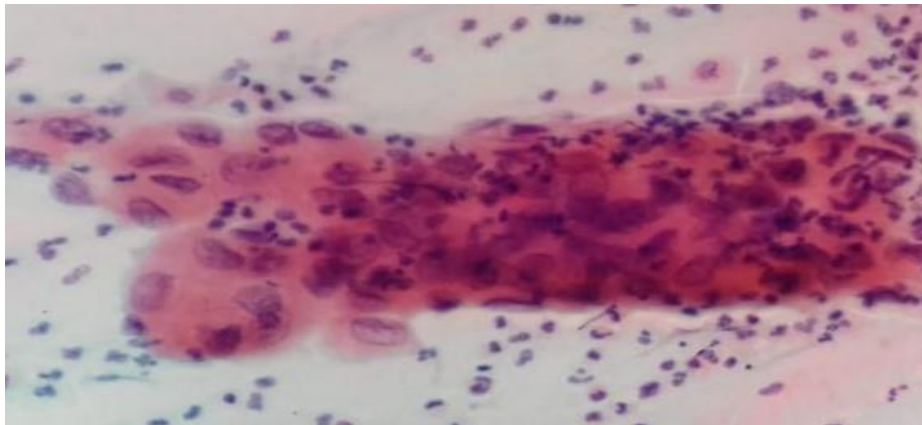
Image 2: Koilocytes, X 40

Abnormal squamous cell with an increased N: C ratio almost 3 times the area of a normal squamous cell, hyperchromatic nuclei, perinuclear

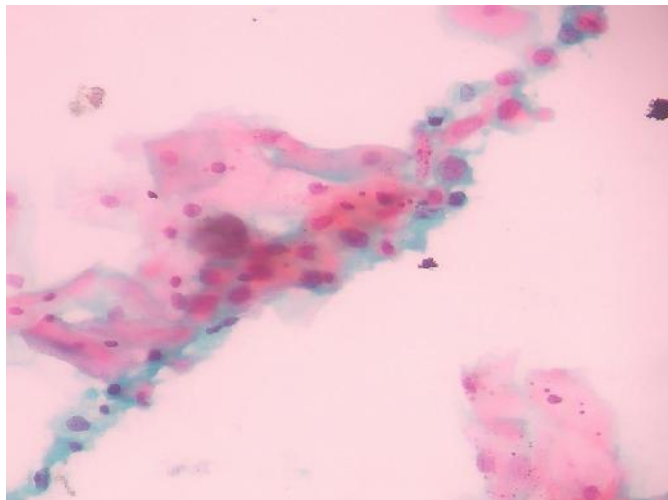
halo, and thick irregular cytoplasmic margins as shown in in images 3 B and C.

A

Image 3. LSIL – Low grade squamous intraepithelial lesion X40



B

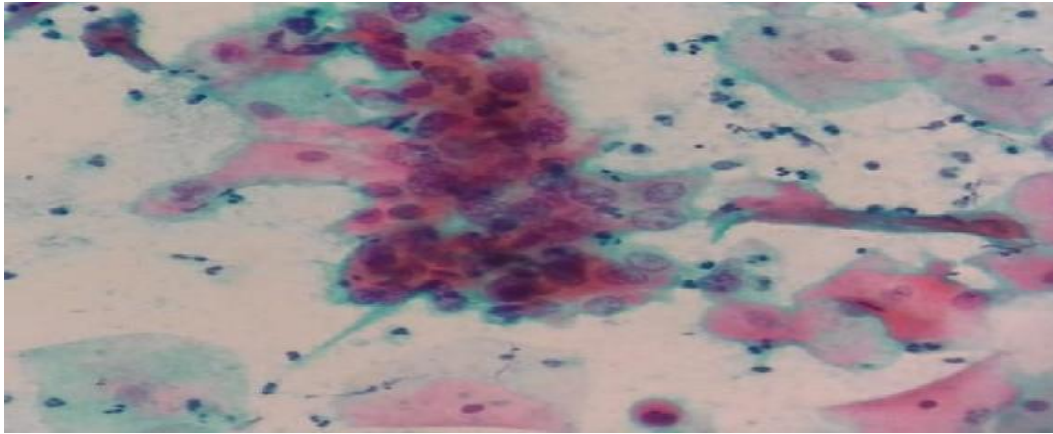


C

Image 3: A, B, C, above show Low grade squamous intraepithelial lesion, X40

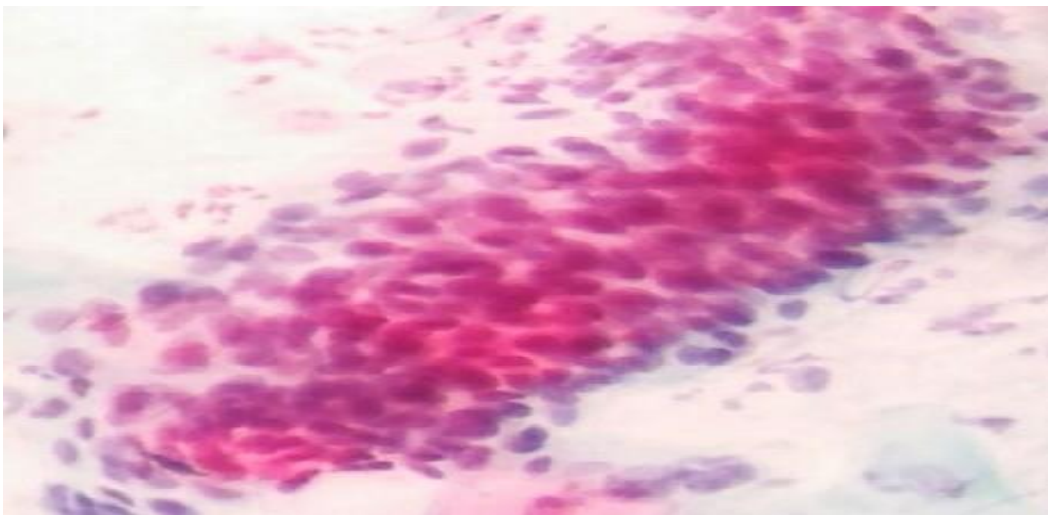
A group of squamous cells with an enlarged nucleus compared to a normal intermediate cell, hyperchromatic nuclei, well-defined perinuclear

halos, irregular nuclear margins, and some with an inflammatory cells in the background.



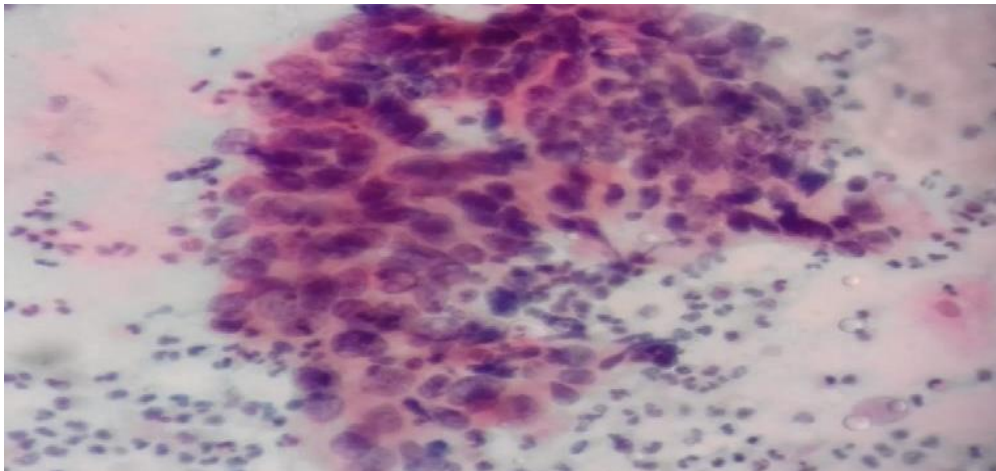
A

Image 4: High grade squamous intraepithelial lesion X40



B

HSIL - High grade squamous intraepithelial lesion X40



C

HSIL- High grade squamous intraepithelial lesion X40

Image 4A , a cluster of tightly cohesive squamous epithelial cells exhibiting a raised nuclear cytoplasmic ratio, pleomorphic nuclei, hyperchromatic group of cells, salt and pepper chromatin, irregular margins, flattening of cells at the edges of the cluster, a feature suggestive of HSIL. Images 4 B & C shows a tightly cohesive

cluster of epithelial cells exhibiting high N/C ratio, pleomorphic nuclear, open chromatin granules, and others salt and pepper chromatin, hyperchromatic, and others are spindle-shaped and flattening at the edges in the background with marked inflammation.

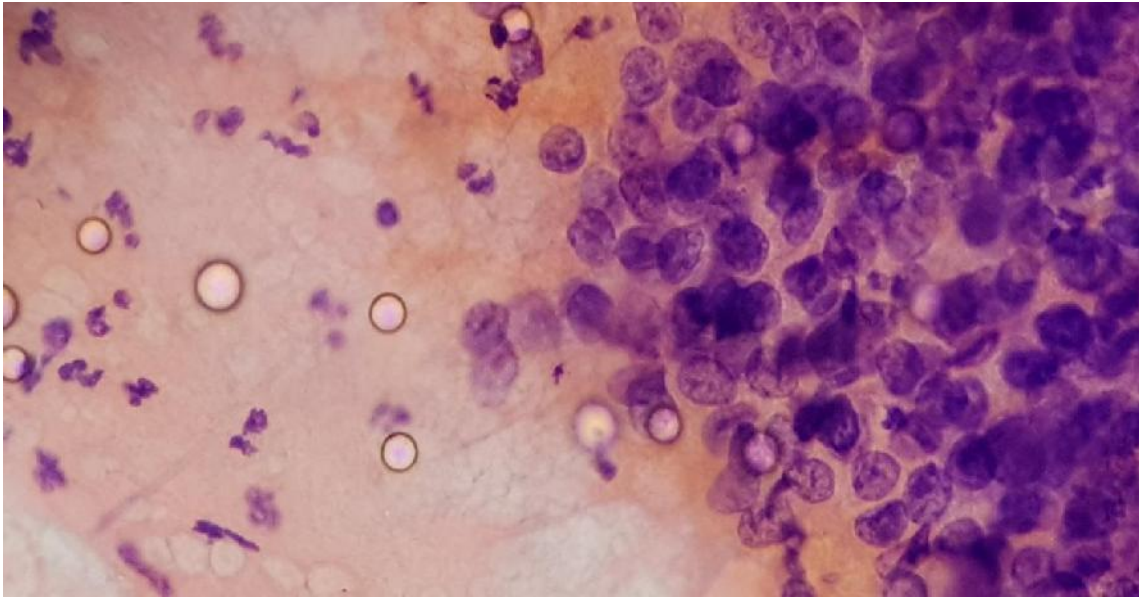


Image 5. Squamous cell carcinoma X 40

A tightly cohesive cluster of squamous epithelial cells with a high N: C ratio, pleomorphic, hyperchromatic, irregular margins, coarse

chromatin granules, open chromatin, Macro nucleoli, and tumor diathesis.

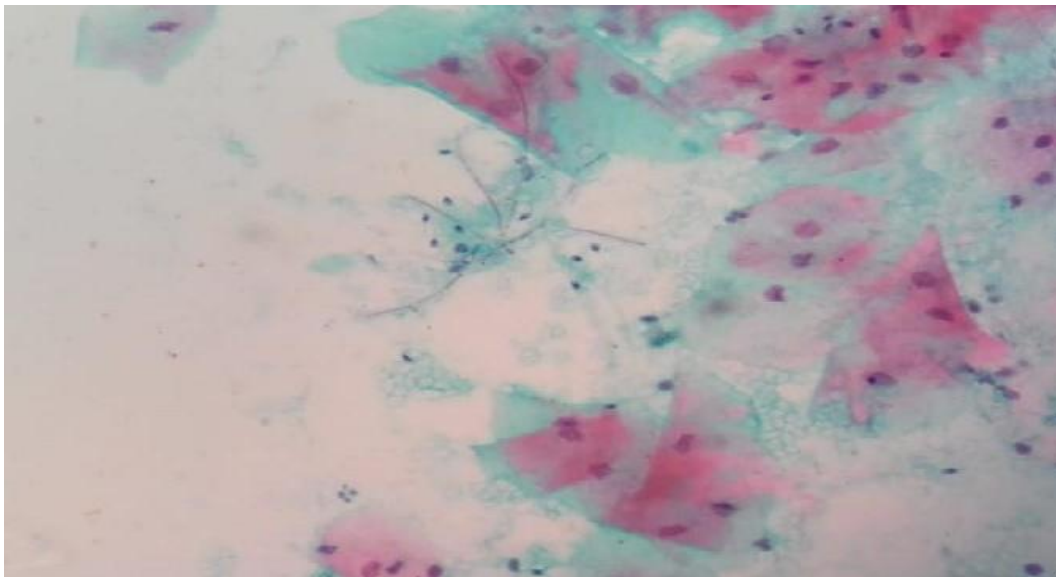


Image 6: Fungi organism consistent with candida species X40

Discussion

The study intended to pick several cervical intraepithelial lesions by the use of VIA and pap smear in HIV positive women attending Mbagathi District Hospital. Pap smear has a high specificity, but low sensitivity when compared to VIA. The two screening methods picked only a few cervical intraepithelial lesions. In this study, VIA had a positivity of 6(8%), with 1(1.3%) positive reaction and 5(6.7%) suspicious reactions for the cervical intraepithelial lesions. This study is comparable to that of Mustafa et al.2010 where VIA positivity was reported as 9.9%(21). In their study, 6.2% out of the 9.9% were diagnosed with precancerous lesions. In this study, 4(5.3%) out of 6(8%) were confirmed by pap smear to be cervical epithelial lesions. The interpretation of VIA results depends on the expertise of the personnel performing the test. A study done in Ethiopia by Kebede et al. 2017 among healthy women VIA positivity was reported as 6.7% (22). This is similar to the findings in this study at 6 (8%). Research study by Anderson et al.(2015) reported VIA positivity as 10%(60). The difference found in this study is that the study participants were HIV-positive women attending CCC, while Anderson et al.2015 study involved three countries with different infrastructure and screening programs. Chung et al.2013 study reported VIA positivity as 40% and pap smear 61.4%(60). The difference observed could be due to the duration of HIV management in this population. VIA had a negative reaction to 13(17.3%) cervical lesions confirmed by pap smear. Interpretation of VIA test, and the judgment of the personnel performing the test could have played a significant role in the outcome results. In the current study VIA missed a significant number of lesions confirmed by pap smear.

The current study reported abnormal findings as 22.7%, ASCUS and above. A similar study in Makueni reported abnormal cervical squamous lesions as 25% , ASCUS and squamous cell carcinoma (23). The difference in our study is that the study population was annually screened for cervical epithelial lesion using VIA screening

method. The PI expected pap smear positivity to rise, although the percentage of cervical epithelial lesions was low. Women in urban areas are more exposed to cervical cancer screening, are knowledgeable about pap smear test and the many satellite clinics in Nairobi county, could have forced many women to seek health services explaining the slight difference encountered in our study (24).

The study found VIA positivity to be 6(8%). Visual inspection with acetic acid positivity rate was expected to be high because the study population were on routine visit to CCC and some could be visiting CCC for the first time while others could be on ARVs for the first time. Cytology has a high specificity compared to VIA. The high sensitivity in VIA and high specificity in cytology could explain the difference experienced in the two screening tests. Although VIA has been confirmed as a “screen and treat” test nationally this study has proved that using VIA alone could be risky to the HIV positive women. This study has shown that VIA test missed significant number of lesions which were confirmed by pap smear test, subjecting HIV infected women at risk of developing high grade cervical intraepithelial lesions and SCC. Although the health personnel conducting VIA was experienced ,confounding factors including the batch of acetic acid used, and old age above 41 years could have caused VIA to miss the squamous epithelial lesions(62). A study has reported decrease of VIA sensitivity at ages 41 years and above, reflecting VIA inability to detect changes in the transformation zone. However in the same study pap smear positivity remained unchanged when compared to HPV and VIA methods (60). In the current study, recruited participants with ages 41 years to 49 years had the highest percentage (45.3%) and frequency of 34 years. In future studies, confounding factors related to age should be ruled out. In our study pap smear combined with VIA would offer excellent results for the management of HIV positive women. Pap smear had a positivity of 22.7%, becoming an alternative test for the management of HIV positive women. The results of pap smear in this study was confirmed

by two experienced pathologists in the Department of Human Pathology University of Nairobi.

Conclusion

The prevalence of cervical intraepithelial lesions by pap smear was 22.7%.

The prevalence of cervical lesions by VIA was 6(8%).

Recommendations

The presence of precancerous lesions among the recruited study participants calls for pap smear triage with VIA for proper management of HIV infected women.

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	Website: www.ijarm.com
	Subject: Medical Sciences
Quick Response Code	
DOI: 10.22192/ijamr.2022.09.12.029	

How to cite this article:

Charles M.M, Waweru. W, Joseph R. N, Josephine N.R. (2022). Classification of squamous intraepithelial lesions among HIV-positive women attending Mbagathi district hospital in Nairobi county Kenya. Int. J. Adv. Multidiscip. Res. 9(12): 349-361.

DOI: <http://dx.doi.org/10.22192/ijamr.2022.09.12.029>