RISK FACTORS OF ABNORMAL VISUAL CERVICAL INSPECTION IN HIV INFECTED WOMEN ON ANTIRETROVIRAL TREATMENT.

Dissertation in partial fulfillment for the Degree of Masters of Medicine (M.MED) in Obstetrics and Gynecology, University of Nairobi

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This is to certify that this dissertation is the original work of Dr. David Mwithiga Thuo, Master of Medicine student in Department of Obstetrics and Gynecology, registration number H58/68067/2011, University of Nairobi. The research was carried out in the department of Obstetrics and Gynecology, school of medicine, college of Health Sciences .It has not been presented in any other University for award of degree.

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DEDICATION

This thesis is dedicated to Michael Mwithiga.

LIST OF ABBREVIATIONS

HPV – Human Papilloma virus

HAART – Highly Active Antiretroviral agent.

VIA – Visual Inspection Using Acetic Acid

VILI - Visual Inspection Using Lugol's Iodine

UoN – University Of Nairobi

KNH – Kenyatta National Hospital

CD4 – Cluster of differentiation 4

MOH – Ministry of Health

ART – Anti Retro Viral therapy

BMI – Body Mass Index

WHO – World Health Organization

CCC – Comprehensive Care Center

DVI – Direct Visual Inspection

SCC – Squamous Cell Carcinoma

ddI – didanosine

ABC – Abacavir

LPV/r – Litonavir

D4T – Stavudine

3TC – Lamivudine

NVP – Nevirapine

EFV – Efavirenz

AZT – Zidovudine

PPV – Positive Predictive Value

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ABSTRACT

Background: HIV infected patients have a higher likelihood of multiple oncogenic HPV sub-types, get persistent HPV infections and progress faster to invasive cancer compared to their HIV uninfected counterparts. Unresolved HPV infection is the major cause of invasive cervical cancer. In Kenya, cervical cancer screening is opportunistic and not systematically done as in developed countries. Due to the challenges posed by the Pap smear protocol, the Kenyan Ministry of Health rolled out the visual cervical cancer screening with acetic acid and Lugol's iodine (VIA/VILI), yearly for HIV infected women. VIA/VILI is more sensitive but less specific than cytology in detection of cervical cancer and its pre–cancerous lesions. VIA/ VILI has acceptable test qualities and may in low resource settings be implemented as a large scale screening method.

Broad objective: To determine the factors associated with abnormal VIA/VILI among HIV infected women in KNH Comprehensive Care Clinic (CCC).

Study design: an unmatched case control study, with a ratio of 1:1 for cases to controls. Cases were HIV infected women with abnormal VIA/VILI screen while controls were patients who had no abnormalities on VIA/VILI screen. Cases and controls were compared for risk factors associated with abnormal VIA/VILI result.

Study setting: KNH CCC.

Study participants: HIV infected women who had undergone VIA/VILI at the CCC.

Analysis: Descriptive univariate statistics was calculated to summarize characteristics of HIV infected women (socio-demographic and clinical) according to findings of cervical visual inspection.

Results: The mean age of participants with abnormal VIA /VILI results was 40.9 years compared to 43.5 years in participants with normal findings.
Women aged 50-59 years were less likely to have abnormal VIA/VILI OR 0.29; p<0.001; 95%CI 0.15-0.57. Those who had a CD4 count less than 500 cells/mm^3 were more likely to have an abnormal VIA/VILI OR 1.65; p=0.017; 95CI 1.1-2.5. CD4 Counts less than 200 were also more likely to have an abnormal VIA/VILI result to a larger extent than those women with CD4 counts less than 500 cells/mm^3 OR 2.74; p=0.028; 95CI 1.12-6.73. Due to missing data the association between ARV regimen and abnormal VIA/VILI was inconclusive. The following were not associated with an abnormal VIA/VILI: marital status, WHO clinical staging, body mass index and AIDS defining illness.

Conclusion: Our study revealed a high prevalence of abnormal VIA/VILI among the study participants, and a high abnormal VIA/VILI risk for participants with low CD4 count less than 500 cells/mm^3.

INTRODUCTION AND LITERATURE REVIEW

Cervical cancer is the second most common malignancy globally and accounts for the greatest number of deaths from cancer in women worldwide [1]. Human immunodeficiency virus (HIV) infection also represents a tremendous health burden worldwide. Cervical cancer was made an acquired immunodeficiency syndrome (AIDS-) defining illness in 1993 [2].

In 2005, there were almost 260,000 deaths from cervical cancer and more than 500,000 new cases worldwide. Women in developing countries are at greater risk of death from cervical cancer, primarily because few have access to the screening and treatment services that have greatly reduced mortality in the industrialized world over the past four decades. About 75% of women in industrialized countries have been screened for cervical cancer in the previous five years, compared to less than 5% in developing countries [3]. According to World Health Organization (WHO) in 1986, it was estimated that only about 5% of women in developing countries have been screened for cervical dysplasia in the past 5 years, compared with 40% to 50% of women in developed countries [4].

Human papillomavirus (HPV) is a DNA virus from the papilloma virus family that is capable of infecting humans. There are more than 30 to 40 sub-types of HPV. Typically transmission of HPV is through sexual contact and infects the ano-genital region. The High-risk HPV sub-types 16 and 18 are together responsible for over 65% of cervical cancer cases. Other high risk HPV subtypes include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 82: [5]

The public health importance of assessing the interaction between HIV and HPV infection with respect to cervical disease is suggested by increased rates of dysplasia persistence and recurrence among HIV-positive women and shorter survival for women with HIV infection and cervical cancer. HIV-positive women have a higher prevalence and incidence of cervical precancerous lesions than HIV negative women [6].

In Kenya, cervical cancer screening is opportunistic meaning that it is not systematically done. Pap smear screening has been shown to have challenges of implementation in Kenya because of a need of cytological support which is limited and a high patient lost-to-follow up because a Pap smear protocol requires patients to have multiple clinic visits. Due to the challenges posed by the Pap smear protocol, the Kenyan Ministry of Health (MOH) rolled out the visual screening method Visual inspection with Indole Acetic Acid and Lugols Iodine (VIA/VILI) to screen for cervical cancer. The advantages that guided the Kenyan MOH of roll out of the VIA/VILI approach were: patients get same sitting results, possibility of the see-and-treat with cryotherapy, does not need cytological support, relatively cheap, easy to implement and the fact that it can be done by lower carder staff. In addition, VIA/VILI results are easy to report either as negative, positive or suspicious for cancer [7].

In low resource settings, where cervical cancer screening by Pap smear is a challenge, visual methods like VIA/VILI, can be comfortably used because evidence has shown that they are more sensitive than the Pap smear. Even though they are less specific than cytology, but to a lesser extent, in detection of cervical cancer and its pre–cancerous lesions it is still an acceptable test in low resource settings; if large scale cervical cancer screening is to be implemented.

Sensitivity of VIA/VILI was 94.3 %versus 74.3 % for cytology. VIA/VILI specificity was 82.6 % versus 93.7 % for cytology. [8]`VIA/VILI can be performed at any time during the menstrual cycle, including during menses (provided flow is not too heavy), during pregnancy, at a postpartum examination, or during a post abortion check-up. It can also be done when a woman comes for care related to STIs, HIV screening, or follow-up care. The Kenya guidelines for screening in HIV positive women with history of sexual activity and aged 18-65 years old should be screened at diagnosis of HIV, 6 monthly in the first year and then yearly if normal. Health care providers need to provide a careful explanation of the procedure, why it is being done, what the possible findings might be, as well as what follow-up care might be necessary [9].

The VIA/VILI procedure begins with a reproductive health history and counselling for the procedure. The components of the history include menstrual history, bleeding pattern (irregular or post-coital bleeding), parity, age, current pregnancy status or contraceptive method, and family history of mother or sister with cervical cancer. The important pre-procedure preparation is bladder emptying. The VIA/VILI procedure is performed, when the woman is in a lithotomy position, draped and the transformation zone of the cervix exposed with a bivalve speculum. During the procedure, the transformation zone is checked carefully on and near the squamocolumnar junction. In the VIA procedure, lesions are any dense non-movable acetowhite, raised and thickened white plaques in the transformation zone. In VILI procedure, lesions are yellowish or safferon in color. VIA/VILI is reported as negative, positive, or suspicious for cancer. Abnormal VIA/VILI constitutes VIA/VILI positive and suspicious for cancer. The characteristics of lesions for VIA/VILI procedure are summarised in Box

1 [7].

Box 1 - Classification of results for VIA/VILI

DIAGNOSIS	APPEARANCE OF CERVIX ON VIA	APPEARANCE OF CERVIX ON VILI
Suspicious for cancer	Cervical ulcer or exophytic growth suspicious for cancer	Cervical ulcer or exophytic growth suspicious for cancer
Positive	Acetowhite lesion with well circumscribed border	Yellow lesion
Negative	No Acetowhite lesion visible	brownish

The risk factors for abnormal VIA/VILI result is similar to those of HPV infection, which is the aetiological cause. The risk factors include early coitache, multiple sexual partners, and sexually transmitted infections including HIV, high parity, oral contraceptive pills, low socio-economic status, cigarette smoking and older age group. Persistent infection with oncogenic HPV subtypes is a key factor in development of high-grade cervical lesions [10-17].

The government of Kenya guidelines on antiretroviral therapy (ART) [18] recommends ART for all HIV-positive adults and adolescents with WHO clinical stage 1 or 2 and a CD4 count ≤ 350 cells/mm3, WHO clinical stage 3 or 4 regardless of CD4 count, HIV and TB co-infection regardless of the CD4 count, patients with HIV/HBV co-infection with evidence of active liver disease (elevated ALT), cirrhosis or other evidence of chronic liver disease. HIV-positive women have a 2-fold to 4-fold greater rate of HPV infection than HIV-negative women. The prevalence of HPV among HIV-positive women is associated strongly with low CD4 counts and high HIV viral load (VL) [19, 20].Of the risk factors, HIV infection has been shown consistently

to increase the likelihood of an abnormal VIA/VILI. Highly active antiretroviral therapy (HAART) has been shown to decrease HIV viral load, increase CD4 cell counts, and decrease most opportunistic infections. Since the introduction of HAART there has been a decline in certain malignancies in HIV-infected individuals [21, 22]. However, studies on the impact of HAART on the natural history of cervical squamous intraepithelial lesions (SILs) have produced inconsistent and conflicting results with some studies showing a reduction of SILs and other studies showing no reduction of SILs [23, 24].There is an agreement by most studies that there is an increased risk of HPV infection and squamous intraepithelial lesions (SIL) the precursor of cervical cancer among HIV infected women [25, 26]. The ART Lopinavir has been shown to have anti–HPV properties which is a constituent of ARV regimes in Kenya, though not used as a common first line ART [27].

Factors associated with HIV infection, including the prevalence of cervical lesions, their size and character and the concomitant presence of genital infections can influence the PPV of VIA or VIA/VILI and affect the overall impact and cost of a screening programme. [28,29] In fact, a wide range of PPVs has been reported for visual screening tests performed in populations of HIV-infected women, [30-32] making it difficult to estimate disease prevalence and plan for the costs and resources necessary for programme implementation.

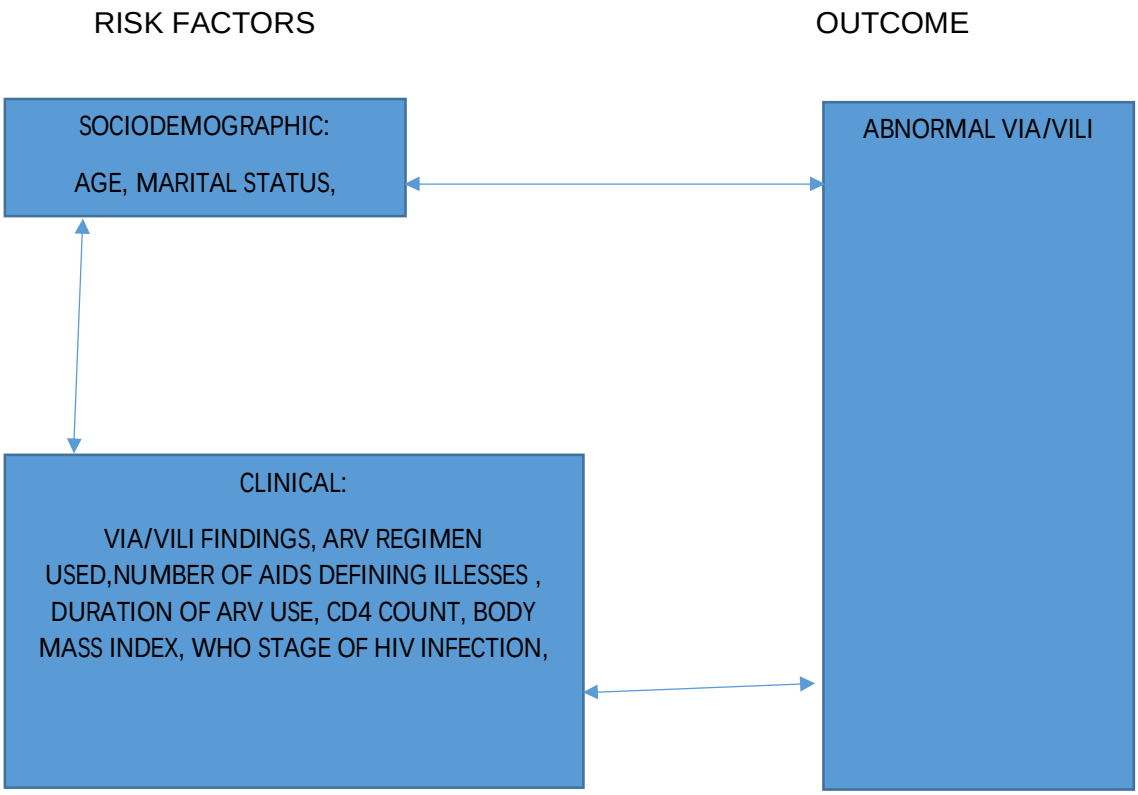
The risk of both HPV infection increases with increasing degrees of immunosuppression (as measured by lower CD4 counts and higher HIV RNA load) [33-35].For the most part, genetic, familial, dietary, and endogenous hormonal factors are not thought to play a role in development of CIN or cervical cancer [36, 37]. Although familial factors were implicated in some studies of the pathogenesis of

squamous cell cervical cancer, familial aggregation due to shared environmental exposures could not be excluded [38, 39]. HIV-infected women, who have an increased risk of developing the disease, carry a disproportionate amount of the burden. Therefore, appropriate screening strategy for this population is needed.

A study by Megan J Huchko et al comparing VIA and VIA/VILI among HIV-infected in two HIV clinic sites in western Kenya between October 2007 and October 2010 [40], where 2338 women were screened with VIA and 1124 with VIA/VILI. In the VIA group, 26.4% of the women tested positive for CIN 2+; in the VIA/VILI group, 21.7% tested positive ($P < 0.01$). Histologically confirmed CIN 2+ was detected in 8.9% and 7.8% ($P = 0.27$) of women in the VIA and VIA/VILI groups, respectively. The Positive Predictive Value (PPV) of VIA for biopsy-confirmed CIN 2+ in a single round of screening was 35.2%, compared with 38.2% for VIA/VILI ($P = 0.41$) and exhibited that VIA/VILI had better diagnostic efficiency than VIA alone. [40]

Entities such as inflammation, cervical condyloma and leukoplakia can give false positive results of VIA test [41]. It also has a low positive predictive value resulting in over diagnosis and overtreatment. VIA has severe limitations with lesions above the endocervical canal which cannot be visualized; this represents a major problem especially for postmenopausal women where the endocervical junction recedes [42]. There is no permanent record of the test to be reviewed later. Between community centres high variability has been observed, and even in a study from Nigeria of 2013 VIA was not reproducible nor sensitive, this lead to discourage the method in that country [43].

Figure 1 - Conceptual framework algorithm



Conceptual Framework narrative

The cases and controls were analysed for selected clinical factors(VIA/VILI findings, ARV regimen used, number of AIDS defining illnesses , duration of ARV use, CD4 count, body mass index and WHO stage of HIV infection and socio demographic factors (age, marital status).

A comparative analysis was then done to identify any significant associations between the factors and abnormal VIA/VILI result.

JUSTIFICATION

HIV-positive women have a higher prevalence and incidence of cervical precancerous lesions than HIV negative women. The prevalence of HPV among HIV-positive women is associated strongly with CD4 counts and HIV viral load. Highly active antiretroviral therapy (HAART) has been shown to decrease HIV viral loads, increase CD4 cell counts, and decrease most opportunistic infections. Since the introduction of HAART there has been a decline in certain malignancies in HIV-infected individuals. However, studies on the impact of HAART on the natural history of cervical squamous intraepithelial lesions (SILs) have produced inconsistent results.

In Kenya public facilities, screening for cervical cancer is opportunistic which creates a gap in knowledge on the burden and risk factors of precancerous lesions among the susceptible HIV infected women. A study within routine care and using visual inspection in HIV patients has not been done before at the Comprehensive Care Centre (CCC) of Kenyatta National Hospital (KNH), the findings of this study will help further understand factors influencing cervical dysplasia among HIV-infected women.

RESEARCH QUESTION

What are the factors influencing abnormal VIA/VILI findings among HIV infected women in KNH CCC?

NULL HYPOTHESIS

There are no factors influencing abnormal VIA/VILI findings among HIV infected women in KNH CCC?

BROAD OBJECTIVE

To determine the factors associated with abnormal VIA/VILI among HIV infected women in KNH CCC

SPECIFIC OBJECTIVES

Among HIV infected women in KNH screened with VIA/VILI from January 2012 to July 2014:

1) To determine the proportion of abnormal VIA/VILI results
2) To determine the socio demographic factors associated with abnormal VIA/VILI result between those who had abnormal VIA/VILI result and those who had a normal VIA/VILI result
3) To determine clinical factors associated with abnormal VIA/VILI result between those who had abnormal VIA/VILI result and those who had no abnormalities on VIA/VILI screen.

STUDY METHODOLOGY

Study design

This was an unmatched case control study with a ratio of 1:1 for cases to controls. Cases were HIV infected women with abnormal VIA/VILI screen while controls were patients who had no abnormalities on VIA/VILI screen. Cases and controls were compared for risk factors associated with abnormal VIA/VILI result.

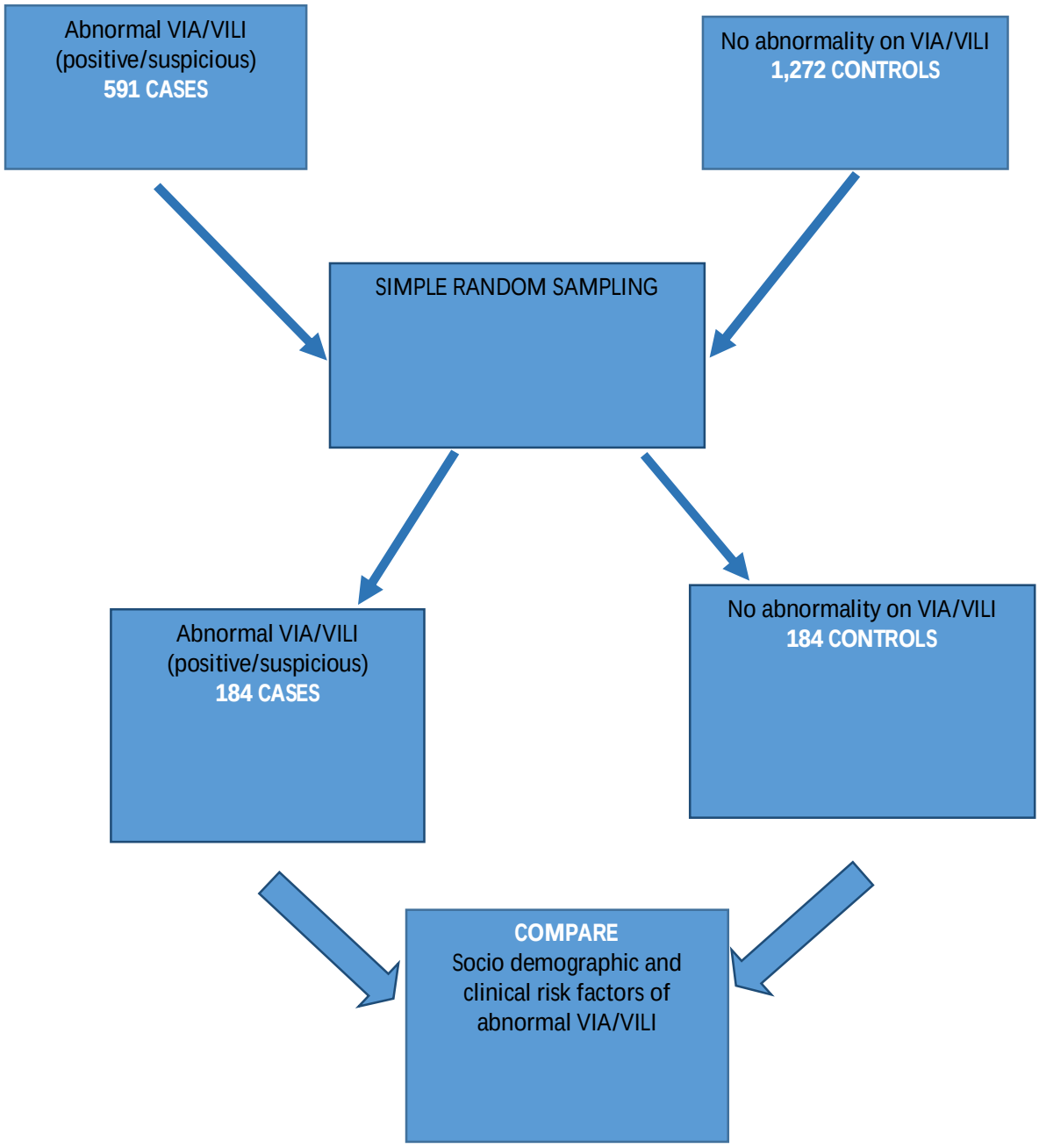
Study site and setting

This study was conducted at the cervical cancer screening clinic of the Kenyatta National Hospital (KNH) Comprehensive Care Clinic (CCC), which was established in 2012, and offers comprehensive care and treatment with various antiretroviral regimens based on a national guideline by the Ministry of Health to approximately 2,000 HIV- infected patients through offering voluntary visual screening with VIA/VILI on the weekdays from 9am to 4pm.

The clinic attends an average of 72 patients in a month and is run by a nurse competent in performing VIA/VILI which are done systematically with VIA followed by VILI and recorded as positive if both results were positive, negative if both results were negative and suspicious for cancer based on visible cervical lesions the mode of entry in the clinic did not specify the findings of VIA or VILI separately but instead the examination findings were logged as a collective for VIA/VILI. The KNH has a protocol for cervical visual inspection using VIA/VILI (appendix 3) where patients with a negative VIA/VILI results are asked to repeat the screening in 1 year, however if the patient has 2 negative VIA/VILI then they are scheduled to repeat a screening in 5 years. If the patient gets a positive result, they are sent for cryotherapy but if the

patient is not eligible for cryotherapy or where it is not available then they are sent for colposcopy followed by appropriate management based on the colposcopy histology result.

Figure 2- Study design algorithm



Study population

Inclusion criteria

1) Cases were women who had an abnormal VIA/VILI result
2) Controls were women who had no abnormalities on VIA/VILI screen

Exclusion criteria

1) Patients who had missing VIA/VILI results

2) Patient who had inconclusive VIA/VILI results

3) Patients who were excluded through sampling to fit sample size

Sampling size

Sample size calculation was done using the following formula for estimating sample size for comparison of two proportions (Kirkwood & Sterne 2001):

$$n = \left(\frac{r+1}{r}\right) + \frac{\bar{p}(1-\bar{p})(Z_{\alpha} + Z_{\beta})^2}{(p_1 - p_2)^2}$$

n = desired sample size per group

Z_{α} = statistic for 95% confidence = 1.96

Z_{β} = power of the study set at 80% = 0.84

r = ratio of cases to controls

p_1= proportion of women **with precancerous** lesions and on ARVS (8.9% i.e. 17 out of 191 in Memiah *et al* 2012) [44]

p_2 = proportion of women **without precancerous** lesions and on ARVS (OR of 2.5, represents a p_2 of 19.6%)

$\bar{p}$ = average of p_1 and p_2

$$n = \left(\frac{1+1}{1}\right) \times \frac{0.1425(1-0.1425)(1.96+0.84)^2}{(0.089-0.196)^2}$$

n = 184 women per group for objective 2; that is 184 participants with VIA/VILI abnormal and 184 participants with no abnormalities on VIA/VILI screen.

Sampling procedure

Participants were drawn from records at the CCC dating back to the time of inception of the cervical cancer screening clinic. The eligible patients with positive/suspicious VIA/VILI results and those with negative VIA/VILI results during the study period from January 2012 to July 2014 was done and found to be 591 and 1272 respectively with a total cohort of 1863.

These figures were divided by the sample size of 184. Recruitment into the study for abnormal VIA/VILI results (cases) was by simple random sampling of the first entry and every 3rd patient and recruitment for no abnormalities on VIA/VILI screen (controls) was simple sampling of the first and every 6th patient.

Data variables

Outcome measure: VIA/VILI result (abnormal or no abnormalities seen on screening) VIA/VILI.

Exposure measures: age, marital status, WHO stage of HIV infection, Body mass index, CD4 count, ARV regimen, number of AIDS defining illnesses and duration of ARV use.

Data collection procedure

Data was collected retrospectively from patient's records. All eligible participants were assigned a unique number, their VIA VILI results, age, WHO staging for HIV infection, body mass index, CD4 count, ARV regimen, number of AIDS defining illnesses, duration of ARV use and marital status data was extracted from the health management software at the CCC (IQ care) in September 2014 and fed directly into a data abstraction form. (Appendix 1).

Data analysis

Data analysis was done using SPSS software (version 17.0). Descriptive univariate statistics was calculated to summarize characteristics of HIV infected women (socio-demographic, clinical and reproductive) according to findings of cervical visual inspection. Continuous variables including age and clinical factors e.g. CD4 count, and duration of ARV use, were summarized using mean and standard deviation, unless non-normality was detected in which case the median, range and interquartile range was calculated. Categorical factors e.g. marital status, were summarized using univariate frequency distribution tables showing frequencies and percentage of mothers in each category.

Bivariate analysis was then conducted using logistic regression to identify risk factors showing association with abnormal cervical visual inspection. Each categorical risk factor including oral ARV regimen was cross tabulated according to abnormal findings of cervical visual inspection and comparisons was done using the chi-square or Fisher's exact test, as appropriate. Students T-test was used to compare means of continuous variables in the group with and without abnormal cervical visual inspection findings.

Logistic regression was used to conduct multivariable analysis with abnormal cervical findings as the outcome (dependent variable) and risk factors showing significant associations with abnormal cervical findings as the independent variables. Adjustment was made for important underlying socio-demographic and clinical factors. Statistical significance was based on an alpha level of 0.05. Associations from the logistic regression models was reported as Odds ratios (OR) and 95% confidence intervals (95% CI).

Ethical consideration

Ethics approval for this study was granted by Kenyatta National Hospital/ University of Nairobi Ethical Review committee. Permission to carry out the study was granted by the Comprehensive Care Center for approval before commencing the study. Patients' identity was protected by replacing their names with numbers and confidentiality on patient information was upheld. No additional data was collected beyond what was available in patient' records. Patients who had abnormal VIA/VILI were referred for further follow up which included pap smear, colposcopy and for gynecology oncology review.

Study limitations

There was no separate documentation of the VIA findings and VILI findings even as the screening is done in sequence and therefore an opportunity to assess the differences in the two methods among the HIV infected population was missed.

Limitations encountered included comparing VIA and VILI both of which have varying sensitivities and specificities and this has been overcome by ensuring that the sample size calculation was large enough so there was power to detect significant differences among them and also among the different ARV regimen, socio demographic and clinical characteristics if indeed they exist.

Another limitation encountered was missing data due to the retrospective nature of the study for various socio demographic factors including viral load, parity, number of sexual partners, age of sexual debut, condom use, hormonal contraceptive use, smoking history, here all missing data other than VIA/VILI results excluded from the study.

RESULTS

A total of 1,863 patients were eligible for this study. Out of these 184 cases were compared for risk factors with 184 controls. The mean age (SD) of participants with abnormal VIA /VILI results was 40.9 years (9.8) compared to 43.5 years (9.9) in participants with normal findings. Results are presented according to objectives.

1. Proportion of abnormal VIA/VILI results among HIV infected women in KNH

Figure 3: Enrolment algorithm for HIV infected women screened with VIA/VILI in KNH CCC from January 2012 to July 2014

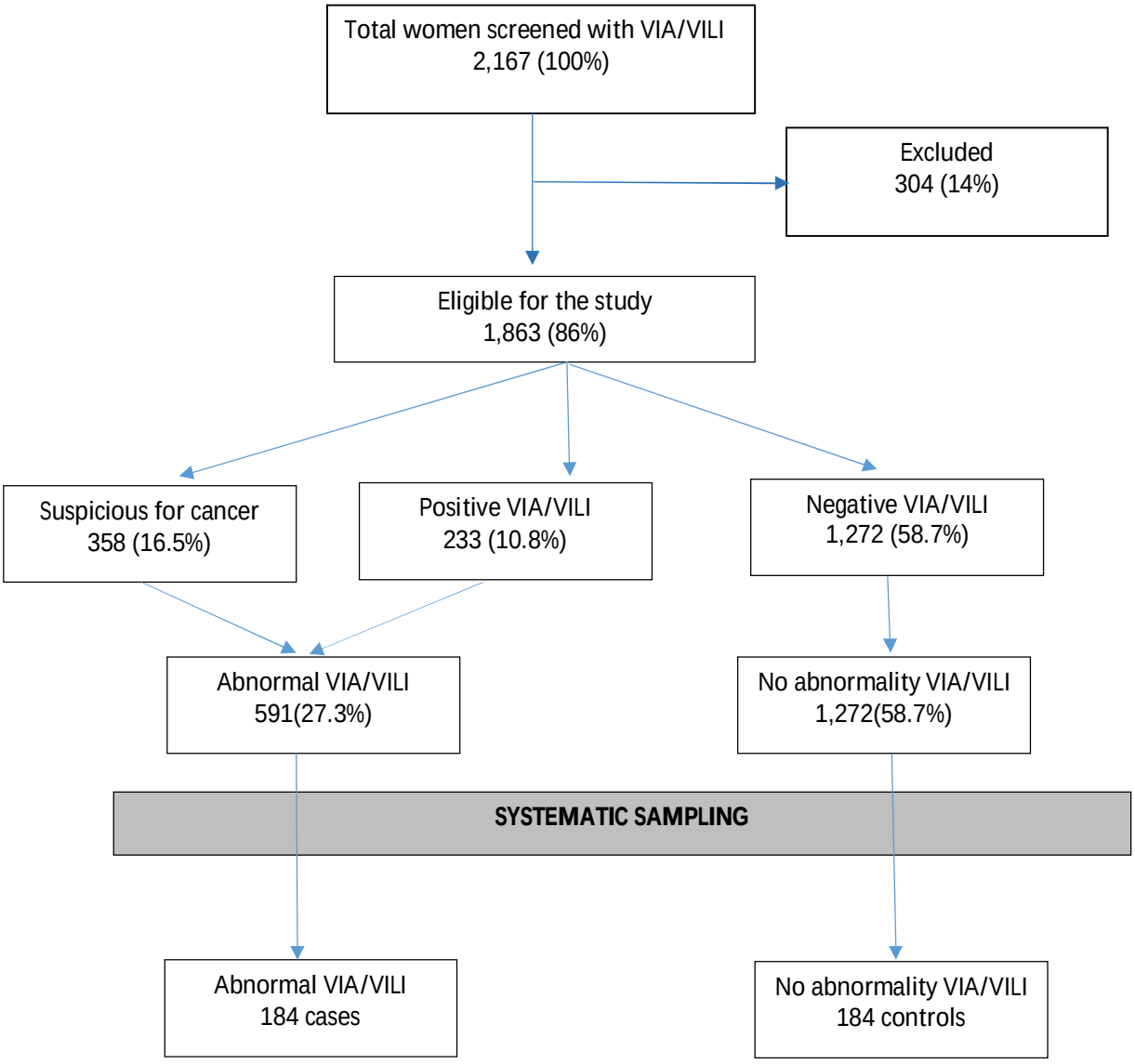


Table 1. Univariate analysis of socio demographic and clinical risk factors associated with abnormal VIA/VILI result

	Abnormal VIA/VILI	
	Yes(n=184)	**No(n=184)**
Age group (in years)	**n(%)**	**n (%)**
20-29	21(63.6)	12(36.4)
30-39	67(56.3)	52(43.7)
40-49	68(50.7)	66(49.3)
50-59	16(27.1)	43(72.9)
60-79	12(52.2)	11(47.8)
Marital status		
Widowed	27(14.7)	23(12.5)
Married	87(47.3)	88(47.8)
Separated	20(10.9)	16(8.7)
Single	42(22.8)	47(25.5)
Unknown	8(4.3)	10(5.4)
CD4 count		
<200 cells/mm3	18(9.8)	7(3.8)
>200 cells/mm3	166(90.2)	177(96.2)
WHO staging		
Not staged	3(50.0)	3(50.0)
Stage 1	87(50.6)	85(49.4)
Stage 2	32(53.3)	28(46.7)
Stage 3	54(50.0)	54(50.0)
Stage 4	8(36.4)	14(63.6)
BMI category		
Underweight (<18.5)	4(80.0)	1(20.0)
Normal weight (18.5-24.9)	60(50.0)	60(50.0)
Overweight (25-29.9)	78(52.3)	71(47.7)
Obese (>30)	42(45.2)	51(54.8)
AIDS defining illness		
No	154(83.7)	153(83.2)
Yes	30(16.3)	28(15.2)
Missing response	0(0.0)	3(1.6)
ARV regimen		
AZT+3TC+NVP	10(31.3)	22(68.7)
AZT+3TC+EFV	10(50)	10(50)
TDF+3TC+NVP	30(46.9)	34(53.1)
TDF+3TC+EFV	53(43.4)	69(56.6)
TDF+3TC+LPVr	9(52.9)	8(47.1)
Undocumented	72(63.7)	41(36.3)

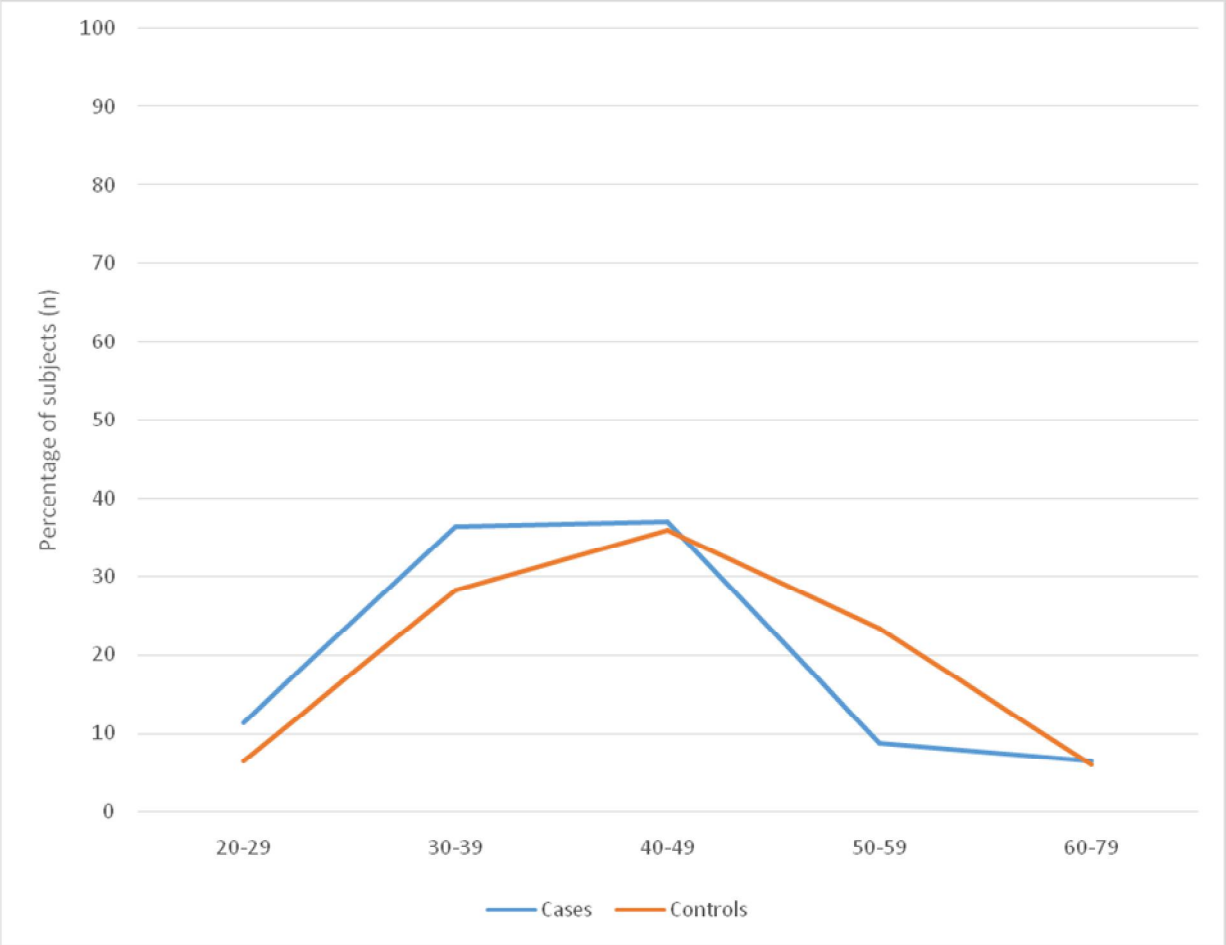
On univariate analysis both the cases (abnormal VIA/VILI) and controls (no abnormality on VIA/VILI), participants were aged 40- 49 years, married, had CD4 counts of >200 cells/mm3, in WHO stage 1, and had no AIDS defining illnesses.
For controls (no abnormality on VIA/VILI) most participants were on TDF+3TC+EFV while most cases (abnormal VIA/VILI) were on an undocumented ARV regimen

2. Determination of socio demographic risk factors associated with abnormal VIA/VILI result.

Table 2: Age group as a factor for abnormal VIA/VILI at KNH CCC.

	Abnormal VIA/VILI					
	Yes(n=184)	**No(n=184)**	**OR**	**95% CI**		**P**
Age group (in years)						
20-29	21(63.6)	12(36.4)	1.00			
30-39	67(56.3)	52(43.7)	1.36	0.57	3.32	0.450
40-49	68(50.7)	66(49.3)	1.70	0.73	4.10	0.184
50-59	16(27.1)	43(72.9)	4.70	1.72	13.0	<0.001
60-79	12(52.2)	11(47.8)	1.60	0.47	5.41	0.391

Figure 4: Relationship between age of participants and the outcome of VIA/VILI



Women aged 50-59 years had significantly less prevalence of abnormal VIA/VILI and were less likely than the youngest population aged 20-29 to have it, OR 4.70 p=<0.001; 95% CI 1.72-13.0. (Table 2)

2. Determination of clinical risk factors associated with abnormal VIA/VILI result between those who had abnormal VIA/VILI result and those who had no abnormalities on VIA/VILI screen

Table 3: Marital status as a factor for abnormal VIA/VILI at KNH CCC

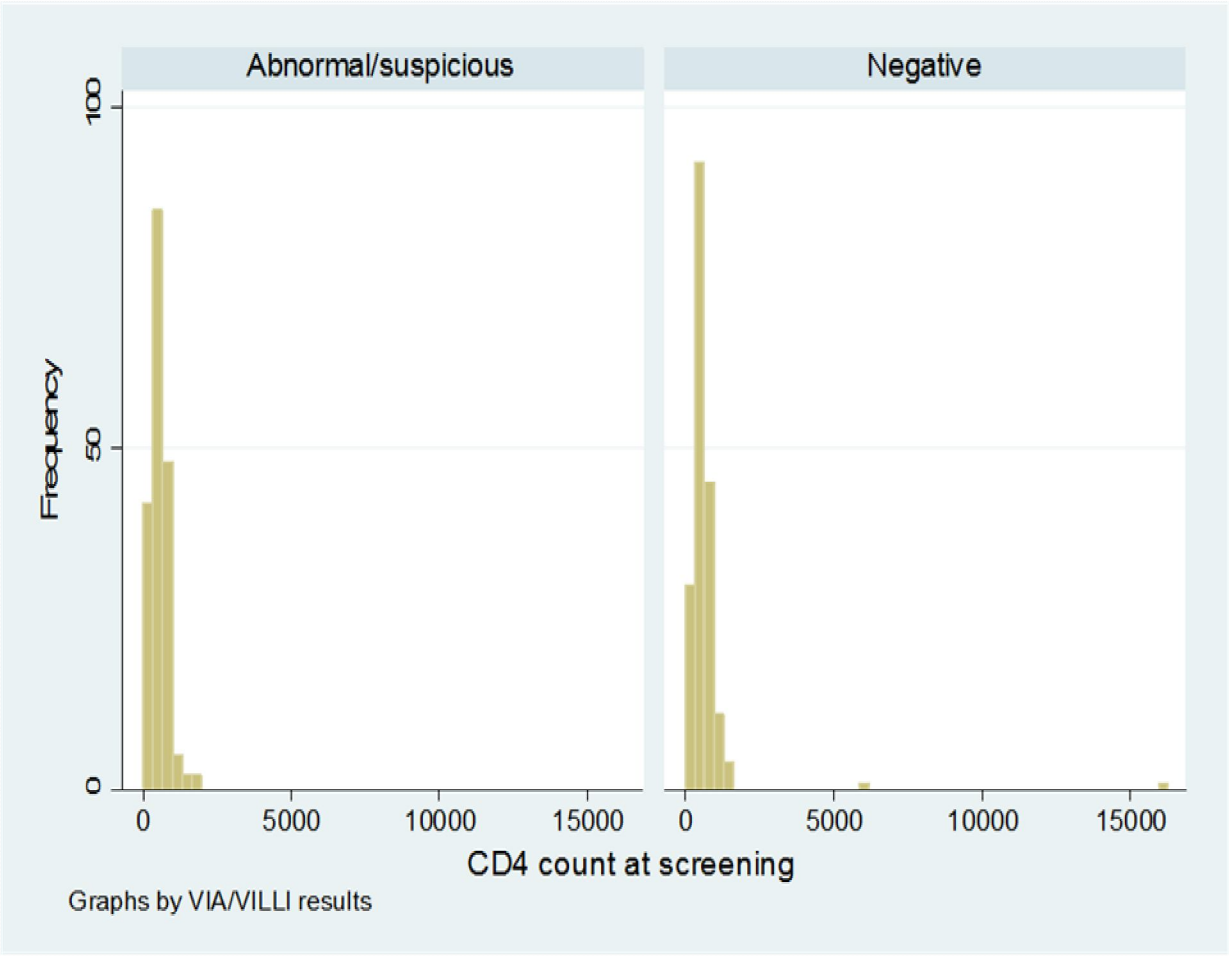
	Abnormal VIA/VILI					
	Yes (n = 184)	**No (n = 184)**	**OR**	**95% CI**		**P**
Marital status						
Widowed	27(14.7)	23(12.5)	1.00			
Married	87(47.3)	88(47.8)	0.84	0.45	1.58	0.593
Separated	20(10.9)	16(8.7)	1.06	0.45	2.52	0.886
Single	42(22.8)	47(25.5)	0.76	0.38	1.52	0.441
Unknown	8(4.3)	10(5.4)	0.68	0.23	2.01	0.488

Marital status was not associated with abnormal VIA/VILI result (Table 3).

Table 4: CD4 count analysis as a factor for abnormal VIA/VILI at KNH CCC

	Abnormal VIA/VILI					
	Yes(n=184)	**No(n=184)**	**OR**	**95% CI**		**p**
CD4 count						
<200 cells/mm^3	18(9.8)	7(3.8)	2.74	1.12	6.73	0.028
≥200 cells/mm^3	166(90.2)	177(96.2)	1.00			
<350 cells/mm^3	47(25.5)	32(17.4)	1.63	0.98	2.7	0.058
≥ 350 cells/mm^3	137(74.5)	152(82.6)	1.00			
<500 cells/mm^3	101(54.9)	78(42.4)	1.65	1.1	2.5	0.017
≥ 500 cells/mm^3	83(45.1)	106(57.6)	1.00			

Figure 5: Relationship between CD4 count at screening and the VIA/VILI outcome of participants



Women who had a CD4 count less than 500 cells/mm^3 were more likely to have an abnormal VIA/VILI OR 1.65; p=0.017; 95CI 1.1-2.5. Those women with CD4 Counts less than 200 were also more likely to have an abnormal VIA/VILI result than those women with CD4 counts above 200 cells/mm^3 OR 2.74; p=0.028; 95CI 1.12-6.73 (Table 4 and figure 5).

Table 5: WHO AIDS staging as a factor for abnormal VIA/VILI at KNH CCC.

	Abnormal VIA/VILI					
	Yes(n=184)	**No(n=184)**	**OR**	**95% CI**		**p**
WHO staging						
Not staged	3(50.0)	3(50.0)	0.98	0.19	4.98	0.978
Stage 1	87(50.6)	85(49.4)	1.00			
Stage 2	32(53.3)	28(46.7)	1.12	0.62	2.01	0.714
Stage 3	54(50.0)	54(50.0)	0.98	0.6	1.58	0.925
Stage 4	8(36.4)	14(63.6)	0.56	0.22	1.4	0.214

Overall, WHO staging was not associated with abnormal VIA/VILI result (table 5).

Table 6: BMI as a factor for abnormal VIA/VILI screening in HIV positive females in KNH.

	Abnormal VIA/VILI					
	Yes(n=184)	No(n=184)	OR	95% CI		p
BMI category						
Underweight (<18.5)	4(80.0)	1(20.0)	4.00	0.43	36.84	0.221
Normal weight (18.5-24.9)	60(50.0)	60(50.0)	1.00			
Overweight (25-29.9)	78(52.3)	71(47.7)	1.10	0.68	1.78	0.702
Obese (≥30)	42(45.2)	51(54.8)	0.82	0.48	1.42	0.483

BMI was not found to be associated with abnormal VIA/VILI result (table 6).

Table 7: AIDS defining illness as a factor for abnormal VIA/VILI screening in HIV positive patients in KNH CCC.

	Abnormal VIA/VILI					
	Yes(n=184)	**No(n=184)**	**OR**	**95% CI**		**p**
AIDS defining illness						
No	154(83.7)	153(83.2)	1.00			
Yes	30(16.3)	28(15.2)	1.06	0.61	1.87	0.827
Missing data	0(0.0)	3(1.6)	NA			

AIDS defining illness was found not to be associated with abnormal VIA/VILI (Table 7).

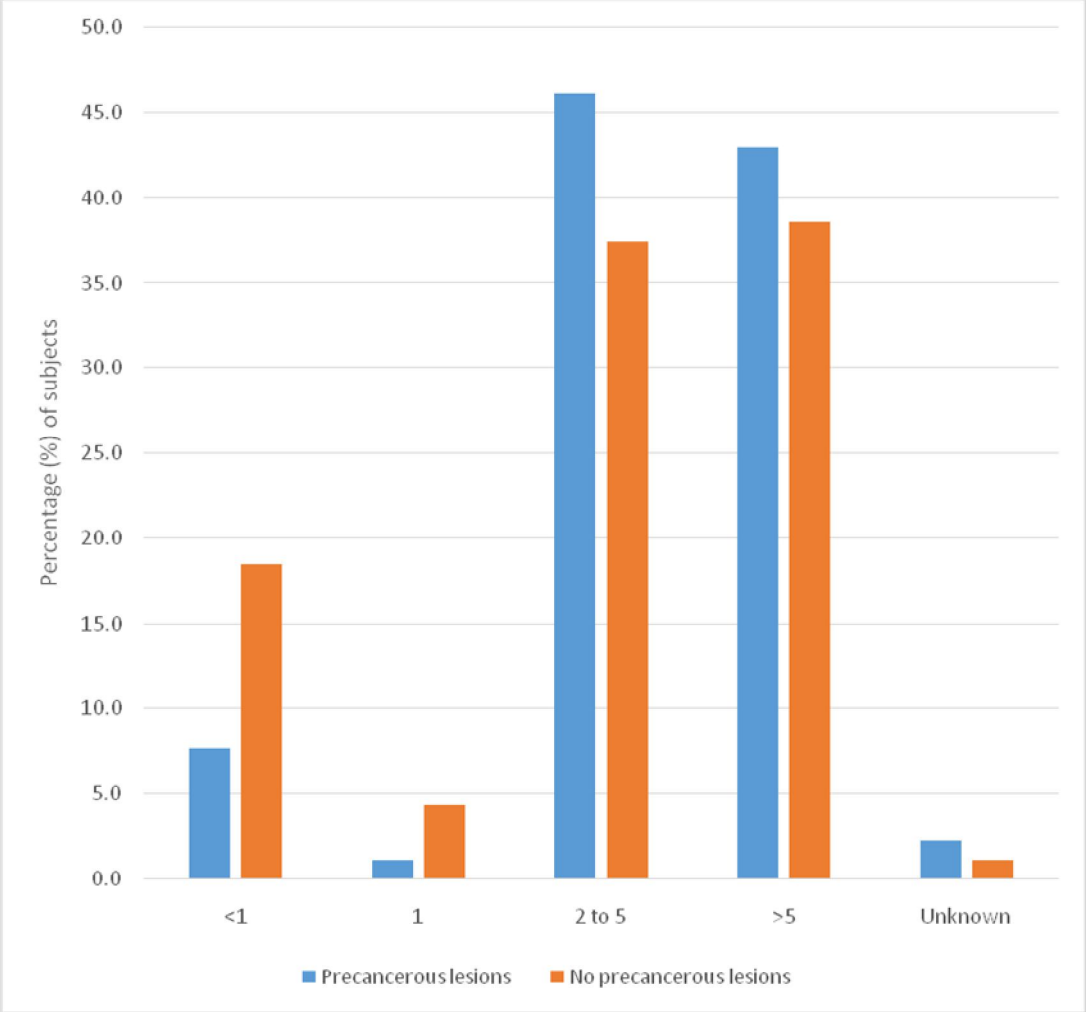
Table 8. ARV REGIMEN as a factor for abnormal VIA/VILI screening in HIV positive patients in KNH CCC.

	Abnormal VIA/VILI					
ARV REGIMEN	**Yes (n=184)**	**No (n=184)**	OR	95 % CI		P value
AZT+3TC+NVP	10(5.4)	22(12)	1			
AZT+3TC+EFV	10(5.4)	10(5.4)	2.20	0.70	6.96	0.18
TDF+3TC+NVP	30(16.3)	34(18.5)	1.94	0.79	4.75	0.146
TDF+3TC+EFV	53(28.8)	69(37.5)	1.69	0.74	3.87	0.215
TDF+3TC+LPVr	9(4.9)	8(4.4)	2.48	0.74	8.31	0.142
UNDOCUMENTED	72(39.1)	41(22.3)	3.86	1.67	8.95	0.002

Most patients with abnormal VIA/VILI had missing documentation on ARV regimen. (Table 8)

Figure 6: Duration of ARV therapy in female patients screened for abnormal VIA/VILI in KNH CCC (n=184)

Figure based on %



Most participants with abnormal VIA/VILI had used ARV therapy for between 2 and 5 years 85/184 (46.2%) whereas majority of participants with no abnormalities on VIA/VILI screen had used ARV therapy for more than 5 years 71/184 (38.6%), figure 3.

Table 9: ARV therapy as a factor for abnormal VIA/VILI in HIV infected females in KNH CCC

	Abnormal VIA/VILI					
	Yes(n=184)	**No(n=184)**	**OR**	**95% CI**		**p**
ARV treatment						
None	0(0.0)	9(100.0)	NA			
Yes	152(50.0)	152(50.0)	1.00			
Unknown	32(58.2)	23(41.8)	1.39	0.78	2.49	0.265

ARV regimen was found not to be associated with abnormal VIA/VILI result (table 8).

DISCUSSION

The main findings of this study are that, 19 % and 13% of HIV infected women screened for cervical cancer using VIA/VILI had a suspicious lesions for cancer and positive VIA/VILI result respectively, with overall abnormal VIA/VILI result of 32%. For this study both suspicious for cancer and positive result were regarded as abnormal VIA/VILI result. The age group 50-59 years was protective for abnormal VIA/VILI result. A CD4 cell count of $\leq$500mm^3 was associated with a higher likelihood of abnormal VIA/VILI result. Of note, was that women with a lower CD4 cell count were even more likely to have an abnormal VIA/VILI result. The following were not associated with an abnormal VIA/VILI result: marital status, WHO clinical staging, body mass index, AIDS defining illness and ART.

The prevalence of precancerous lesions at the Nazareth hospital in Nairobi Kenya, was found lower (26.7%) compared to the KNH CCC, where the abnormal VIA/VILI result was very high (32%). The Nazareth study did not stratify by HIV status [42]. The positivity rate of VIA/VILI is accepted to be between 5%-10% in the general population, which is also lower than our study, understandably because our study population is HIV infected. For this reason, it's plausible that the prevalence of abnormal VIA/VILI is high in our population. This is because, HIV infection is associated with multiple and persistent infection of HPV, the aetiological cause of cancer of the cervix. It is important in this case to have more stringent screening for HIV infected patients in our setting, because cervical precancerous lesions progress faster to invasive cancer of the cervix in HIV infected patients [5].

This study found that, participants within the age group of 50-59 years were protected from abnormal VIA/VILI. This contradicts the current knowledge on cervical cancer incidence related to age, which does not follow the pattern of increasing incidence with age seen for most cancers. There are two peaks in the age-specific incidence rates: the first in women aged 30-34 (at 20 per 100,000 women) and the second in women aged 80-84 (at 13 per 100,000 women). The earlier peak is related to many women becoming sexually active in their late teens/early 20s [45,46] giving rise to an increase in human papillomavirus (HPV) infection [47]. Our findings were not consistent with this trend and needs to be explored further.

A lower CD4 cell count was also found to be associated with an abnormal VIA/VILI result. A low immunity is the possible reason. Low immunity is the pathogenesis in HIV infection that leads to a high likelihood of infection with multiple and persistent oncogenic HPV sub-type infections. It is the multiplicity and persistence of HPV infections that lead to cervical intraepithelial neoplasia [5]. The role of ARV is debatable, and in this study the lack of documentation of regimen types meant that the association with abnormal VIA/VILI and ARVs could not be evaluated.

The limitation of this study is that the participants were screened on a voluntary basis which may have excluded those with different socio demographic and clinical characteristics and missing data due to its retrospective nature. Despite these, the study has strength in its design of an unmatched case control, which allowed analysis of all possible associations and the pragmatic nature implying that the findings are more likely a true reflection of routine practice. The results will be used to strengthen existing policy of cervical cancer screening among HIV infected women.

CONCLUSION

Our study revealed a high prevalence of abnormal VIA/VILI among the study participants, surprisingly lower abnormal VIA/VILI prevalence for participants aged 50-59 and a high abnormal VIA/VILI risk for participants with low CD4 count less than 500 cells/mm^3.

RECOMMENDATION

1. Better technical support and operating procedures at the Comprehensive care Clinic (CCC) to ensure that all relevant data is captured and entered for better monitoring and evaluation 2. Better referral system for abnormal VIA/VILI cases that allows for prompt colposcopy and/or LEEP to avoid delays in management and resulting progression of disease.

REFERENCES

1. D. M. Parkin, F. Bray, J. Ferlay, , et al. “Global cancer statistics, 2002,” Ca-A Cancer Journal for Clinicians, vol. 55, no. 2, pp. 74–108, 2005.
2. Centres for Disease Control and Prevention, “MMWR. Recommendations and Reports 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults,” January 2011, http://www.cdc.gov/mmwr/preview/mmwrhtml/ 00018871.htm.
3. International AIDS Vaccine Initiative & PATH, “HPV Vaccine Adoption in Developing Countries: Cost and Financing Issues,” 2007, http://www.iavi.org/, http://www.path.org/publications/.
4. World Health Organization, “Control of cancer of the cervix uteri: review article based on a report of a WHO meeting, November 1985, Geneva,” Bulletin of the World Health Organization, vol. 64, pp. 607–618, 1986.
5. Walboomers JM, Jacobs MV, et al. (1999). "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide". J. Pathol. 189 (1): 12–9.doi:10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F. PMID 10451482.
6. M. Maiman, R. G. Fruchter, E, et al.“Human immunodeficiency virus infection and cervical neoplasia,” Gynecologic Oncology, vol. 38, no. 3, pp. 377–382, 1990.
7. Sankaranarayanan R, Shyamalakumary B, Wesley R, et al. Visual inspection with acetic acid in the early detection of cervical cancer and precursors. Int J Cancer 1999; 80:161.
8. P. Ghosh, G. Gandhi, et al. ‘Visual inspection of cervix with Lugol’s iodine for early detection of premalignant & malignant lesions of cervix’. Indian J Med Res 136, August 2012, pp 265-271.
9. Ministry of Public Health and Sanitation and Ministry of medical services, ‘National Cervical Cancer Prevention Program Action Plan 2011-2015’ August 2011.
10. Kjaer SK, van den Brule AJ, et al. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. BMJ 2002; 325:572.
11. Wright TC, Cox JT et al. Human papillomaviruses — natural history of infections. In: Clinical uses of human papillomavirus (HPV) DNA testing, American Society for Colposcopy and Cervical Pathology, 2004.
12. Rodríguez AC, Schiffman M, , et al. et al, Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst 2008; 100:513.
13. Schlecht NF, Kulaga S, , et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001; 286:3106.

14. Moberg M, Gustavsson I, Gyllensten U, et al. Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma in situ. Int J Cancer 2004; 112:854.
15. Wallin KL, Wiklund F, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. N Engl J Med 1999; 341:1633.
16. Bory JP, Cucherousset J, et al. Recurrent human papillomavirus infection detected with the hybrid capture II assay selects women with normal cervical smears at risk for developing high grade cervical lesions: a longitudinal study of 3,091 women. Int J Cancer 2002; 102:519.
17. Koshiol J, Lindsay L, et al. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008; 168:123.
18. Mohammed, I. Tanui et al, "guidelines on antiretroviral therapy in Kenya", National Aids and STI control programme (NASCOP), Ministry of Health, Kenya. 3rd edition, pp. 33-35, December 2005.
19. D. J. Jamieson, A. Duerr, et al., "Characterization of genital human papillomavirus infection in women who have or who are at risk of having HIV infection," American Journal of Obstetrics and Gynecology, vol. 186, no. 1, pp. 21–27, 2002.
20. H. D. Strickler, R. D. Burk, et al., "Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women," Journal of the National Cancer Institute, vol. 97, no. 8, pp. 577–586, 2005.
21. B. Ledergerber, A. Telenti, et al. "Risk of HIV related Kaposi's sarcoma and non-Hodgkin's lymphoma with potent antiretroviral therapy: Prospective cohort study," British Medical Journal, vol. 318, no. 7201, pp. 23–24, 1999
22. L. P. Jacobson, T. E. Yamashita et al., "Impact of potent antiretroviral therapy on the incidence of Kaposi's sarcoma and non-Hodgkin's lymphomas among HIV-1— infected individuals," Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, vol. 21, no. 4, pp. S34– S41, 1999.
23. J. M. Palefsky, et al. "Cervical human papillomavirus infection and cervical intraepithelial neoplasia in women positive for human immunodeficiency virus in the era of highly active antiretroviral therapy," Current Opinion in Oncology, vol. 15, no. 5, pp. 382–388, 2003
24. I. Heard, J. M. Palefsky, et al. The impact of HIV antiviral therapy on human papillomavirus (HPV) infections and HPV-related diseases," Antiviral Therapy, vol. 9, no. 1, pp. 13–22, 2004.
25. C. S. Rabkin, R. J. Biggar, et al. "Cancer incidence trends in women at high risk of human immunodeficiency virus (HIV) infection," International Journal of Cancer, vol. 55, no. 2, pp. 208–212, 1993.
26. X. W. Sun, L. Kuhn, T. , et al. "Human papillomavirus infection in women infected with the human immunodeficiency virus," New England Journal of Medicine, vol. 337, no. 19, pp. 1343– 1349, 1997.

27. Batman G, Oliver AW, et al. “Lopinavir up-regulates expression of the antiviral protein ribonuclease L in human papillomavirus-positive cervical carcinoma cells,” Antivir Ther2011; 16(4):515-25. doi: 10.3851/IMP1786.
28. Horo A, Jaquet A, et al. IeDEA West Africa Collaboration. Cervical cancer screening by visual inspection in Côte d’Ivoire, operational and clinical aspects according to HIV status. BMC Public Health 2012;12:237. doi: http://dx.doi.org/10.1186/1471-2458-12-237 PMID:22443255
29. Maiman M, Fruchter RG, et al. ‘Prevalence, risk factors, and accuracy of cytologic screening for cervical intraepithelial neoplasia in women with the human immunodeficiency virus. Gynecol Oncol 1998; 68:233–9. doi: http://dx.doi.org/10.1006/ gyno.1998.4938 PMID:9570972
30. Moon TD, Silva-Matos C, et al. Implementation of cervical cancer screening using visual inspection with acetic acid in rural Mozambique: successes and challenges using HIV care and treatment programme investments in Zambézia Province. J Int AIDS Soc 2012;15:17406. doi: http://dx.doi.org/10.7448/IAS.15.2.17406 PMID:22713260
31. Mabeya H, Khozaim K, Liu T, et al.Comparison of conventional cervical cytology versus visual inspection with acetic acid among human immunodeficiency virus-infected women in Western Kenya. J Low Genit Tract Dis 2012;16:92–7. doi: http://dx.doi. org/10.1097/LGT.0b013e3182320f0c PMID:22126834
32. Firnhaber C, Mayisela N, Mao , et al. Validation of cervical cancer screening methods in HIV positive women from Johannesburg South Africa. PLoS One 2013;8:e53494. doi: http:// dx.doi.org/10.1371/journal.pone.0053494 PMID:23326441
33. Ahdieh L, Muñoz A, Vlahov D, et al. Cervical neoplasia and repeated positivity of human papillomavirus infection in human immunodeficiency virus-seropositive and -seronegative women. Am J Epidemiol 2000; 151:1148.
34. Vernon SD, Holmes KK, , et al. Human papillomavirus infection and associated disease in persons infected with human immunodeficiency virus. Clin Infect Dis 1995; 21 Suppl 1:S121.
35. Jamieson DJ, Duerr A, Burk R, et al. Characterization of genital human papillomavirus infection in women who have or who are at risk of having HIV infection. Am J Obstet Gynecol 2002; 186:21.
36. Shields TS, Falk RT, et al. A case-control study of endogenous hormones and cervical cancer. Br J Cancer 2004; 90:146.
37. García-Closas R, Castellsagué X, et al. The role of diet and nutrition in cervical carcinogenesis: a review of recent evidence. Int J Cancer 2005; 117:629.
38. Zelmanowicz Ade M, Schiffman M,, et al. Family history as a co-factor for adenocarcinoma and squamous cell carcinoma of the uterine cervix: results from two studies conducted in Costa Rica and the United States. Int J Cancer 2005; 116:599.

39. Hemminki K, Li X, , et al. Familial risks in invasive and in situ cervical cancer by histological type. Eur J Cancer Prev 2001; 10:83.
40. Megan J. Huchko,Elizabeth A. Bukusi et al. 'Building capacity for cervical cancer screening in outpatient HIV clinics in Nyanza province of western Kenya, International Journal of Gynecology and Obstetrics 2011.
41. International Agency for Research on Cancer. A practical manual on visual screening for cervical neoplasia. Lyon, France: IARC; 2003.
42. Rahatgaonkar W. VIA in cervical cancer screening. Journal of Dental and Medical Sciences (IOSRJDMS) ISSN: 2279-0861 Volume 1, Issue 1 (July–August 2012), PP 01-04
43. Ajenifuja KO. et al A population-based study of visual inspection with acetic acid (VIA) for cervical screening in rural Nigeria. Int J Gynecol Cancer. 2013 Mar;23(3):507-12.
44. Memiah P, Mbuthia C et al 'Prevalence and Risk Factors Associated with Precancerous Cervical Cancer Lesions among HIV-Infected Women in Resource-Limited Settings', AIDS Research and Treatment, Volume 2012 (2012),
45. Levi F, Lucchini F, Negri E, et al. Cervical cancer mortality in young women in Europe: patterns and trends. European J of Cancer 2000; 36(17):2266-2271.
46. Yang BH, Bray FI, Parkin DM, et al. Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost. Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost.
47. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed December 2013.

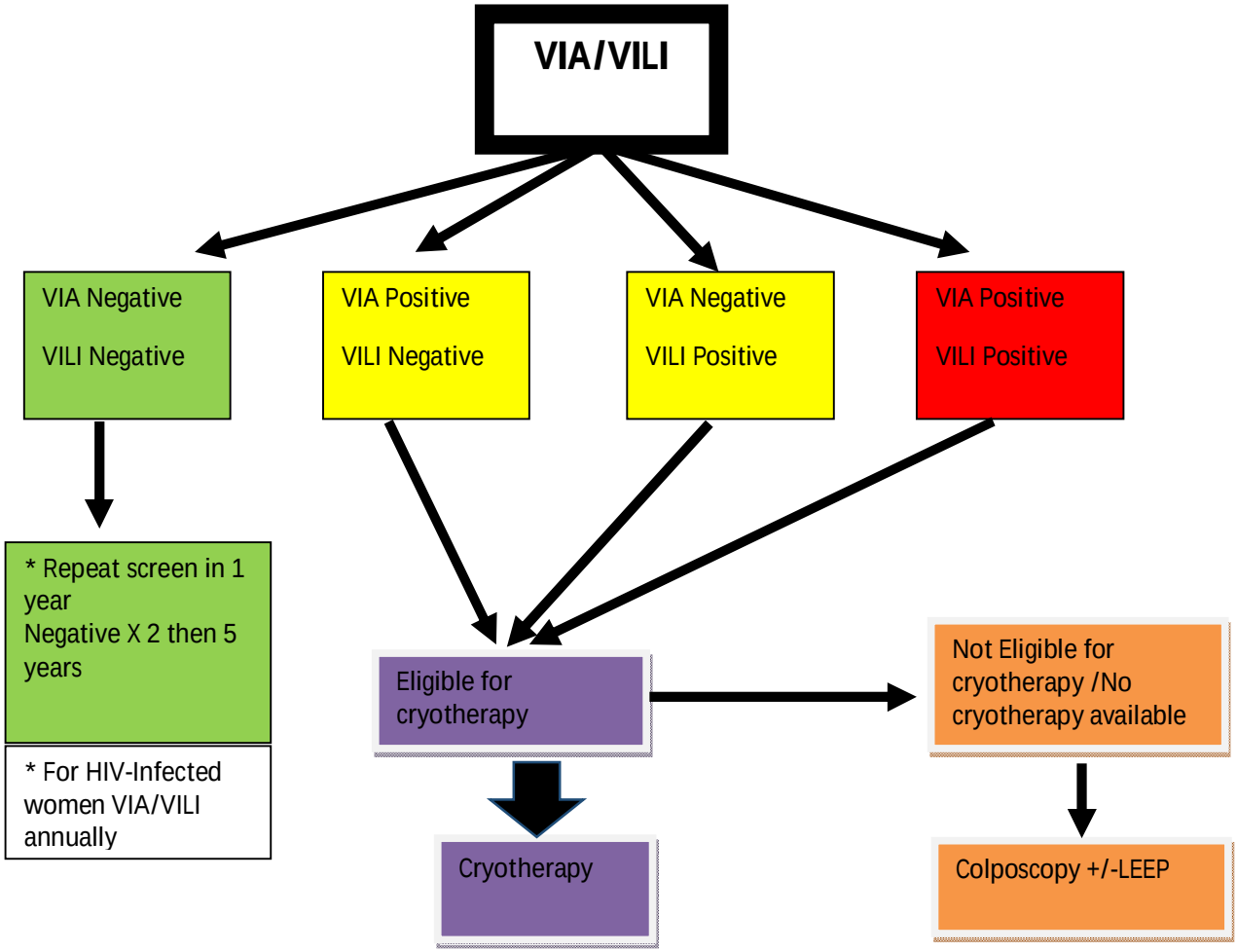
APPENDICES

Appendix 1: Data abstraction form

PATIENT NUMBER:
VIA/VILLI results o Abnormal o Normal
Marital status o Widowed o Married o Separated o Single o Unknown
Age o ……. o unknown
Body mass index (BMI) o ……. o Unknown
WHO stage of HIV disease o ……. o Unknown
ARV Regimen used o ……. o unknown

CD4 count at screening o …… o unknown
Duration of ARV use o ……. o Unknown
Number of AIDS defining illnesses o …….. o Unknown

Appendix 2 - KNH protocol on VIA/VILI



Please note: *Patients who have had VIA/VILI done and not eligible for cryotherapy or cryotherapy is unavailable should undergo colposcopy +/- LEEP and* ***NO*** *Pap smear should be done for these patients* before colposcopy