EFFICACY OF VISUAL INSPECTION WITH ACETIC ACID IN SCREENING FOR CERVICAL PRE-MALIGNANT LESIONS IN HIV INFECTED WOMEN: A CROSS SECTIONAL STUDY

Belinda S. Balandya, MD

MMed (Obstetrics and Gynaecology) Dissertation

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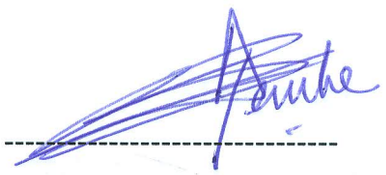
CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled: **Efficacy of Visual Inspection with Acetic acid in screening for cervical pre-malignant lesions in HIV infected women; a cross sectional study,** in partial fulfillment of the requirements for the degree of Master of Medicine in Obstetrics and Gynaecology of the Muhimbili University of Health and Allied Sciences.



Dr. Andrea B. Pembe

(Supervisor)

29-11-2010

Date

DECLARATION AND COPYRIGHT

I, Dr. Belinda S. Balandya, declare that this is my own original work, and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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Date----29/11/2010.----------

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ABSTRACT

Background:

Cancer of the uterine cervix is the most common gynaecological cancer in developing countries, including Tanzania. There is high prevalence of HIV infection in Tanzania and studies have shown an increased risk of Cervical Intraepithelial Neoplasia (CIN) in HIV infected women. Routine cytological screening by Pap smear is not feasible in most developing countries as it needs highly trained personnel, infrastructures, systems to communicate results and funding. Visual Inspection with Acetic acid (VIA) is the best alternative, as it is cost effective, with proven sensitivity and specificity in the population. The present study evaluated the performance of VIA compared with Pap smear, in the detection of premalignant lesions of the cervix in HIV infected women.

Methodology:

The study was conducted among HIV infected women attending the Care and Treatment Centre (CTC) at Muhimbili National Hospital between November 9th 2009 and February 16th 2010. A total of 316 women aged 18-70 years had a Pap smear taken for cytology, followed by spraying on the cervix with 4% acetic acid and then inspecting it. Cytology was considered negative when there was no CIN lesion reported from the Pap smear taken and positive if a CIN lesion (CIN I to III) was reported. Detection of a well-defined, opaque acetowhite lesion close to the squamocolumnar junction or close to the external cervical os constituted a positive VIA.

Results:

Out of 316 women, 132 (42.4%) women had acetowhite lesions on VIA, making the proportion of abnormal cervical lesions to be 42.4%. One hundred and one out of 312 women (32.4%) had CIN lesions detected on Pap smear. The proportion of agreement between these two tests was 0.3. The proportion of agreement was moderate in women with advanced WHO HIV clinical stage of the disease and in women not on ART (Anti Retroviral Therapy). Women with CD-4 count less than 200 cells/mm^3 had more abnormal cervical lesions.

Conclusion:

There is significant proportion of HIV positive women with premalignant lesions of the cervix. The proportion of agreement between VIA and Pap smear is 0.3. Considering the proportion of HIV women with abnormal lesions and the difficulty in logistics of doing Pap smear in low resource settings, it is recommended to introduce screening of premalignant lesions of the cervix using VIA to all HIV infected women.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ASCUS	Atypical Squamous Cells of Undetermined Significance
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma In Situ
CTC	Care and Treatment Clinic
DNA	Deoxyribonucleic Acid
DPX	Dextrene Polystyrene Xylene
HAART	Highly Active Anti Retroviral Therapy
HPV	Human Papilloma Virus
HIV	Human Immunodeficiency Virus
LSIL	Low Grade Squamous Intraepithelial Lesion
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
SIL	Squamous Intraepithelial Lesion
STIs	Sexually Transmitted Infections
VIA	Visual Inspection with Acetic Acid
WHO	World Health Organization

INTRODUCTION

Carcinoma of the cervix is a malignant growth of the uterine cervix. It is the second most common type of cancer among women, and was responsible for over 250,000 deaths in 2005, approximately 80% of which occurred in developing countries[1]. It is however the most prevalent gynaecological malignancy amongst women of developing countries. Between 2005 and 2008 it accounted for 65.7% of histologically confirmed gynaecological cancers in the Ahmadu Bello University teaching hospital, Nigeria[2] and caused 44.7% of all gynaecological cancer deaths in Lagos University Teaching hospital[3]. In South Africa, the overall lifetime risk for women to develop this cancer is estimated at 1 in 41, while for black women it is 1 in 34[4].

Most women who die from cervical cancer, particularly in developing countries, are in the prime of their life. They may be raising children, caring for their family, and contributing to the social and economic life. Their death is both a personal tragedy, and a sad and unnecessary loss to their family and their community. This is because there is compelling evidence that cervical cancer is one of the most preventable and treatable form of cancer, as long as it is detected early and managed effectively[1].

The primary underlying cause for developing cervical cancer is infection with Human Papilloma Virus (HPV) mainly types 16 and 18[5]. Majority of the HPV infections regress spontaneously, but a few persist to cause a chronic infection. Later histological changes occur in the epithelial layer of the cervix characterized microscopically by a spectrum of events progressing from cellular atypia to various grades of dysplasia before becoming

malignant. These changes may be dormant for 10-20 years, in which time the affected woman does not have any symptoms[1]. This is called the pre- invasive phase and it is the time utilized to capture the disease in its early phase by using different screening methods.

Sellors et al[6] in their article 'Colposcopy and treatment of cervical intraepithelial neoplasia' state that: The concept of cervical cancer precursors dates back to the late 19th century when areas of non-invasive atypical epithelial changes were recognized in tissue specimens adjacent to invasive cancers. The term carcinoma *in situ* (CIS) was introduced in 1932 to denote those lesions in which the undifferentiated carcinomatous cells involved the full thickness of the epithelium, without the disruption of the basement membrane. The association between CIS and invasive cervical cancer was subsequently reported. The term dysplasia was introduced in the late 1950s to designate the cervical epithelial atypia that is intermediate between the normal epithelium and CIS. It was found that there is a direct correlation between the histologic grade and progression of the disease; which led to the concept of a single, continuous disease process of normal epithelium through epithelial precursor lesions to invasive cancer. On the basis of the above observations, a new terminology; Cervical Intraepithelial Neoplasia (CIN) was introduced in 1968 to denote the whole range of cellular atypia confined to the epithelium[7]. CIN was divided into three grades; CIN 1 which corresponds to mild dysplasia, CIN 2 to moderate dysplasia and CIN 3 corresponded to both severe dysplasia and CIS.

Once the disease becomes invasive, the woman starts to experience symptoms of the disease which include abnormal vaginal discharge, intermenstrual vaginal bleeding, and post coital bleeding. In advanced disease, lower abdominal and lower back pain may be experienced. The outcome of the disease becomes worse with the spread of the cancer into the cervical stroma and the surrounding structures[7].

Epidemiological studies have identified a number of risk factors that contribute to the development of cervical cancer, including low socioeconomic class, sexual intercourse at an early age, multiple sexual partners, multiparity, long term oral contraceptive use, smoking, vitamin deficiency and sexually transmitted infections[8]. The first three epidemiological factors are proxy indicators of HPV exposure, which is implicated as a primary aetiologic agent in the development of cervical squamous cell carcinoma and its precursors[9].

Human Immunodefiency Virus (HIV) infection is a cause of a significant number of cases of immunosuppression. The natural history of malignancy may change dramatically in the presence of HIV infection[10]. HIV has been shown to cause a high incidence of chronic infections and a rise in the rate of progression of the disease from pre-invasive to invasive phase, therefore they develop invasive cancer at a younger age compared to non-HIV infected women. Because of decreased immunity to clear the cancer cells, people with HIV have a rapid spread of the cancer and subsequent death from the disease. Invasive carcinoma of the cervix is now used by the Center for Disease Control as an HIV/AIDS-defining illness and augmented category B conditions (symptomatic but not

acquired immunodeficiency disease-defining illness) to incorporate moderate or severe cervical dysplasia[11].

A number of screening methods have been used including cytology (Papanicolaou smear and liquid based cytology), visual inspection of the cervix (with Acetic acid or Lugol's Iodine) and detection of HPV deoxyribonucleic acid (DNA). Each has its own sensitivity and specificity. So far under the best conditions in developed countries or research settings, conventional cytology can detect up to 84% of precancer and cancerous lesions. However, under poor conditions its sensitivity can be as low as 38%. The specificity of the test is usually over 90%[1]. Visual inspection of the cervix with acetic acid (VIA) has been shown to have an average sensitivity for detection of precancer and cancerous lesions of almost 77%, with a range of 56% to 94%. The specificity ranges from 74% to 94% with an average of 86%[1]. To date, there is no information regarding neither the effect HIV has on sensitivity and specificity of these screening tests, nor the effect ART or clinical stage of HIV disease has on these parameters. HAART has the potential to prevent progression of HPV infections and cause regression of CIN lesions[12].

A major advantage with VIA is that it is a real-time screening test, as the outcome is known immediately after the administration of the test, so that further investigations/treatment can be planned and carried out during the same visit[13]. Cytological investigations are expensive and cannot be afforded in the low resource countries, and as a consequence a large number of women especially those living in rural settings do not have access to cervical cancer screening.

In 1997, the Tanzanian government endorsed the National Cancer Control program to establish sustainable cancer services by promoting cancer research, training personnel, improving facilities and promoting screening. In addition, The International Agency for Research on Cancer (IARC) and INCTR (International Network for Cancer Treatment & Research) are working together with national institutions in Tanzania, to evaluate the role of alternative, low-technology approaches. Beginning 2001 to March 2006, The Ocean Road Cancer Institute; Tanzania's only cancer treatment center, started screening women with VIA and VILI, aided by colposcopy. A total of 9728 women were screened by VIA, 93.6% had no cancerous lesions, 4% had precancerous lesions, and 2.4% had invasive cancer (*personal communication to the ORCI's chief in division of cancer prevention services*).

Women with CIN 1 are only followed up with cytological and colposcopic investigations because spontaneous regression occurs in 60-80%[14].

A number of procedures, majority using ablative techniques, have been used in treating CIN 2 and CIN 3. These techniques include cryotherapy, carbon dioxide laser vaporization therapy, loop electrosurgical excision and cervical conization. Hysterectomy is commonly used in women who have completed their families.

LITERATURE REVIEW

Carcinoma of the cervix remains to be an important cause of morbidity and mortality in the world and the Tanzanian female population in particular. The crude incidence rate is 40.6/ 100,000 women in Tanzania compared to 16/100,000 globally *(personal communication to the ORCI's chief in division of cancer prevention services)*. A large number of new cases, approximately 500,000; of carcinoma of the cervix are still being identified globally at different stages of the disease annually, almost 7500 of these being in Tanzania[15]. Highest incidence rates are also seen in Latin America and the Carribean, sub-Saharan Africa, and South and South East Asia; where the age-standardized incidence rate is almost 31 per 100,000[16].

Like in other East African countries, cervical cancer is also common in Kenya. This was seen in a study done at Kenyatta National Hospital where 85% of women diagnosed as having reproductive tract malignancies had invasive cervical cancer[17].

In the year 2006, the ORCI registered 955 new cases of cervical cancer[18] and in the year 2007 it received 1006 women with carcinoma of the cervix at the outpatient clinic, these being the leading disease of all other cancer admissions[19].

HIV infected people and AIDS patients develop cancer more frequently than the general population. The incidence of cancer among HIV/AIDS women is 21 fold of that of the general population. Highly statistically significant standardized incidence ratios were

found for Kaposi's sarcoma, non- Hodgkin's lymphoma and invasive cervical cancer[20]. Squamous Intraepithelial Lesions (SILs) in HIV infected women progress more rapidly, are more resistant to standard therapeutic regimens and has high recurrence rate[21]. Women infected with HIV present with cervical cancer at a younger age compared to those not infected with HIV[15]. The HIV infected women are as well at an increased risk of getting vaginal and vulval intraepithelial neoplasia. HPV prevalence in urine and cervical smear samples of HIV infected women is high and the HPV test results are highly concordant[21]. A study conducted in Brazil showed that HIV infection is strongly associated with development of CIN[22]. CIN is not an uncommon occurrence in HIV infected women in Tanzania. Squamous Intraepithelial Lesions have been shown to be the commonest cervical changes seen in HIV infected women[23].The chances are increasing with a high HIV viral load and low CD-4 counts[22], thus increasing the prevalence of CIN and cervical cancer in women with advanced stages of HIV infection.

HIV infected women are also at an increased risk of getting multiple infections with different HPV types. A study on cervical and oral Human Papilloma virus types in HIV-1 positive and negative women with cervical disease in South Africa showed that significantly more HIV infected women had multiple cervical infections than non-HIV infected women; and more oral HPV infections[24]. Immunosuppression favors cervical high-load HPV infection with oncogenic genotypes and its clinical expression in HIV-seropositive women[25].

Methods that are used in screening for cervical cancer include cytological evaluation, HPV DNA testing and visual inspection of the cervix using either dilute acetic acid or Lugol's iodine[1].Cytological evaluation using Pap smear is the method commonly used in screening for cervical cancer. Visual inspection of the cervix is cheaper and can be used in low resource countries.

In a study done on evaluation of cervical screening in rural North India, the rates of positive findings by the VIA and VILI methods were 14.2% and 15.6%, respectively, and by cytology testing the rates were 5.4% for ASCUS and 3.1% for LSIL thresholds. The rates of positive findings declined with increasing participant age for the visual methods but remained relatively stable across age groups for cytology testing. Compared with the cytology method, significantly more CIN 1 lesions were detected by visual screening; the detection rates of CIN 2 and CIN 3 lesions, however, were similar for all 3 screening methods[26]. It was also shown that VIA and VILI were suitable alternate screening tests to cytology for detecting cervical neoplasia in low-resource settings[27]. These were also the findings in Peru, where VIA was found to be useful for detection of precursor lesions for cervical cancer not only in low-resource settings but also in well-equipped health centers and cancer centers. In these non-low-resource settings, VIA had a positive predictive value comparable to the conventional Pap smear, but it was more likely to achieve earlier diagnosis, follow-up, and treatment than screening using cytological method[28].

A study done in South Africa showed that relying on only one diagnostic method for cervical cancer screening was not enough. To increase the sensitivity and specificity of

screening, it is important to combine more than one method. This is especially so in African countries because of absence of regular screening; which is the result of inherent disadvantages of the Pap smear: high cost, low sensitivity, the need for a laboratory with high human expertise and a complex screening program logistic system[13].

The sensitivity of cytology alone is not great enough for implementing as a screening test in a developing country where screening programs are often inadequate. Screening with a combination of tests, once or a few times per woman's life, is a more acceptable alternative since it allows for less screening events without sacrifying sensitivity[29].

In Nigeria, a study done in 2005 revealed that majority of gynecologists were using visual inspection, unaided (41.8%), with Lugol's iodine (37.3%), acetic acid (27.6%) and colposcopy (23.9%) in investigating cervical intraepithelial neoplasia. This study showed that colposcopy, an important step in investigation of CIN, was unpopular among Nigerian gynecologists because of non availability and lack of necessary expertise[30].

The sensitivity of cytology can range between 31 and 72%, depending on the abnormality threshold used to define positivity, with a corresponding specificity range of 94–99% and a negative predictive value range of 97–99%. In this study, VIA and VILI performed by nurses and physicians were slightly more sensitive but less specific than Pap cytology across multiple combinations of test and lesion thresholds. It was recommended that given their lower cost and easy deployment, visual inspection methods merit further assessment as cervical cancer screening methods for low-resource countries[31].

In 2002, the Malawi Ministry of Health incorporated cervical cancer screening in its National Reproductive Health Policy, and endorsed VIA as an appropriate approach to cervical cancer prevention[31]. This new program screened 7,048 women for carcinoma of the cervix using VIA; in which 778 (11%) were detected to have precancerous lesions[32].

In a study done in Tanzania on situational analysis for diagnosis and treatment of cervical cancer in mainland Tanzania, it was found that screening against cervical cancer was appallingly inadequate at all levels of health care delivery system. Apart from medical doctors at tertiary level, other medical personnel including nurses were poorly or hardly utilized for cervical cancer screening[33]. It was earmarked that if visual inspection methods were to be used, other medical personnel than medical doctors could be utilized to scale up cervical cancer screening in the country.

Currently in Tanzania, it is not a routine procedure to do screening for cervical cancer in HIV infected women. Since several studies have shown an increase in cervical cytological abnormalities in HIV infected as compared to non-HIV infected women, it is important to do screening at least in the first visit if conditions do not allow a regular screening program.

PROBLEM STATEMENT AND RATIONALE OF THE STUDY

Carcinoma of the cervix has an advantage of having a long period of 10 to 20 years before the precancerous lesions develop into malignancy. During this period screening for the precancerous lesions can be done and those detected can be treated to halt progression to cancer[1]. The occurrence of carcinoma of the cervix is increasing because of the HIV pandemic. HIV infected women are more likely to have a persistent HPV infection and therefore the occurrence of CIN/invasive cancer is increased as compared to non-HIV infected women[23]. HIV induced immunosuppression leads to impaired cell-mediated immunity, with the consequence of inadequate clearance of HPV infections, and spontaneous regression of low-grade CIN lesions occur rarely[34]. With the increase in the availability of the HAART, many HIV infected women live long enough to develop CIN and cervical cancer.

The highest burden of HIV/AIDS is in Sub-Saharan Africa, where majority of the women have no access to cervical cancer screening. Routine screening for cervical cancer in HIV infected women is not commonly done even when they present with signs and symptoms of cervical cancer. This is mainly because of the feasibility and logistics of using Pap smear. Recently, newer methods including VIA and VILI have been proven to yield almost equal results. The newer methods also have an advantage of getting the results immediately with a possibility of earlier treatment. They are also cheaper and can be undertaken in low resource settings.

This study is designed to assess the efficacy of screening for precancerous lesions among HIV infected women using VIA. In addition, it will shed light on reliability of VIA in the different clinical stages of HIV infection. The results of this study can be used to introduce and organize screening programmes for cervical cancer in Tanzania among HIV infected women.

OBJECTIVES

Broad objective

To assess the efficacy of Visual Inspection of cervix with Acetic acid in screening for cervical pre-malignant lesions in HIV infected women attending care and treatment clinic (CTC) at Muhimbili National Hospital.

Specific objectives

1. To determine proportion of women with abnormal cervical changes on visual Inspection of the cervix with Acetic acid.

2. To determine proportion of women with cervical pre-malignant lesions using Pap smear.

3. To determine factors associated with occurrence of abnormal cervical changes in VIA and Pap smear.

4. To find factors associated with the proportion of agreement between visual inspection with acetic acid and Pap smear.

METHODOLOGY

Study design

A cross- sectional study design.

Study setting

The study was done at MNH HIV/AIDS CTC. MNH is a tertiary, Consultant and University teaching hospital; located in Dar es Salaam, Tanzania. It provides medical services to a population of about 4 million residents of Dar es Salaam region. The hospital receives referrals from nearby Municipal hospitals (Temeke, Ilala and Mwananyamala) and District hospitals from Coast region surrounding Dar es Salaam region. In actual practice, it also provides primary and secondary care for majority of Dar es Salaam residents.

MNH HIV/AIDS CTC

The MNH HIV/AIDS CTC is situated at the ground floor of the new OPD building. Doctors who attended to these patients were from the department of Internal Medicine. Patients who attend this clinic are the ones who have been diagnosed to have been infected with the HIV; including those who have started antiretroviral therapy, those who have not yet started therapy but are attending for evaluation and follow-up.

The clinic operates on all the five working days (Monday to Friday from 9am to 5pm). It receives an average of 60 patients per day, of whom two thirds are females at different ages.

Study population

Women attending the HIV/AIDS CTC at MNH.

Study sample

This consisted of women attending MNH HIV/AIDS CTC who gave consent to participate in the study.

Sample size estimation

Sample size was calculated using the formula below:

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

where;

N= Minimum sample size required

Z= Percentage of normal distribution = 1.96 (using 95% Confidence Interval)

P= Proportion of study units with CIN 38.3% was be used[25]

d= Maximum error allowed. In this study the maximum error used will be 5%.

Using the above formula, the estimated sample size was 363.

Sampling

Convenient sampling method was used where all eligible women were asked to participate in the study.

Data collection

Between November 9, 2009 and February 16th, 2010 data were collected by the principal investigator and a research assistant. The research assistant approached individually women attending the HIV/AIDS CTC to explain the purpose of the study and asked for consent for them to participate. Only those who consented were enrolled in the study. Women who had hysterectomy and those who were pregnant were excluded from the study. Those who were menstruating were advised to come for testing after finishing menstruation.

A structured questionnaire was used to collect data on socio-demographic characteristics, risk factors for development of CIN and cervical cancer. Other information on WHO clinical stage of the disease, use of HAART and its type were taken from the women's files. At the MNH HIV/AIDS CTC the WHO clinical stage of HIV disease is normally assessed every time the woman attends the clinic, using a special form (appendix). Therefore, it is the clinical stage of HIV disease at the day the Pap/ VIA was done. Later an examination was performed in the side room of ward 33 of the maternity block.

After thorough explanation of the procedure to the woman, she was placed in lithotomy position. First a Pap smear was taken using a cytobrush, the cells were transferred on to a labeled dry glass slide and then fixed in 50% Ether and alcohol. Then, the cervix was sprayed with dilute acetic acid (4%) for visual inspection (VIA). Colour change was noted and documented on the questionnaire. The smears were sent for cytological reading at the Central Pathology Laboratory of MNH after labeling.

VIA and the taking of a Pap smear was not done to women who had any signs suggestive of an active infection. Instead medicines were prescribed and they were advised to come at a later date for examination.

For those who had no recent CD-4 counts (within 3 months) and HIV viral load results, blood was taken in room 18 of the new OPD and taken to the laboratory (normal procedure). Unfortunately only few viral load results could be found because a good amount of the tests were not done (because of prohibitive cost), and therefore viral load was not included in the data analysis.

At the laboratory, the smears were hydrated in graded alcohol from 95%, 85% and 70%, followed by immersion in water. The smears were then stained with Harris Haematoxylin for 5 minutes. Later they were dehydrated in ascending graded alcohol from 70% to 95% and stained with orange G for 3 minutes, rinsed in 95% alcohol, followed by staining with EA 36 for three minutes and then dehydrated in absolute alcohol. Thereafter the smears were placed in xylene to remove alcohol and then mounted in DPX and glass cover slips applied, ready for microscopic reading. This is known as the Papanicolaou method of cervical smear preparation before its reading [35]. All these procedures were performed by a single experienced cytotechnician, working at the MNH CPL.

All the smears were read by a single Consultant Cytopathologist, who was blinded to the colour change of the cervix after application of dilute acetic acid.

Data analysis

Data collected was entered using EpiData software and analyzed using the Statistical Package for Social Science (SPSS) version 13. Numerical variables were summarized into tables while categorical variables were compared among women with normal or

abnormal VIA and Pap smear using Chi square test. Two sided P value was used to assess the level of significance. A p-value less than 0.05 was considered to be significant.

The proportion of agreement between VIA and cytology by Pap smear method was calculated by the Cohen's kappa. The results are presented as proportion of agreement and 95% confidence intervals (CI).

Ethical consideration and clearance

Ethical clearance was sought from the MUHAS ethical committee and permission to conduct the study at MNH from the Executive Director of MNH. The purpose of the study and its importance was communicated to women approached for the study. Women who agreed and consented had the liberty to withdraw from the study at any time.

Women found to have CIN 1 and the one with squamous metaplasia were counseled and advised to have a repeat Pap test after 6 months. Those who were found to have CIN 2 and CIN3 were referred to ORCI for colposcopy. Women diagnosed to have cervicitis, cervical cancer and other gynaecological diseases were treated according to hospital treatment protocols.

RESULTS

Three hundred and thirty four women were approached to participate in the study. Eight (2.4%) women refused to participate. Of the 326 women who agreed to participate, 4 refused VIA and Pap smear to be done and 6 were excluded, thus remaining with 316 women for VIA and Pap smears (Figure 1). The 2 women with invasive cancer had been attending the clinic, one for 2 years and another for 5 years, and were on HAART.

Figure 1: Flow chart on recruitment of women for the study

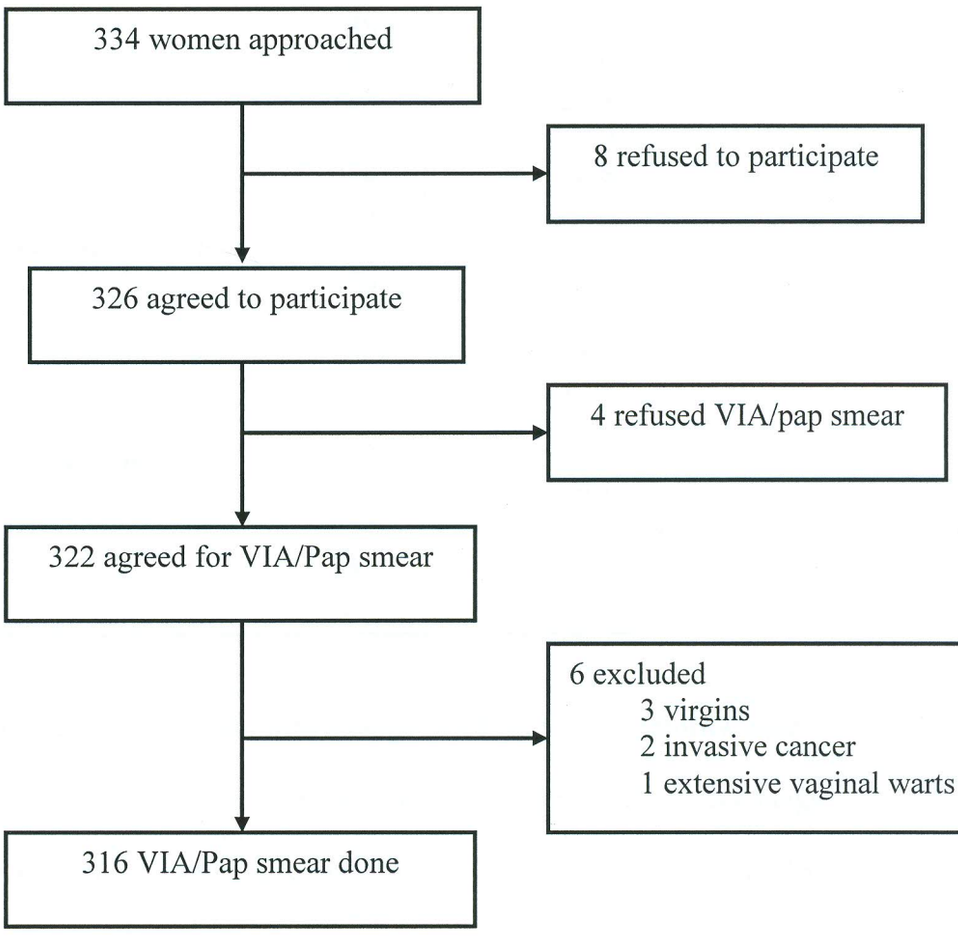


Table 1: Socio-demographic characteristics of the women (N=316)

Characteristic	Number	Percent
Age (years)		
<25	12	3.8
25-34	92	29.1
35-44	131	41.5
>45	81	25.6
Marital status		
Single	92	29.1
Married	108	34.2
Divorced	41	13.0
Widowed	75	23.7
Parity		
0	38	12.0
1	66	20.9
2	80	25.3
3	60	19.0
≥4	72	22.8
Age at first sexual intercourse (years)		
<15	26	8.2
15-19	153	48.4
20-24	105	33.2
≥25	32	10.1
Lifetime number of sexual partners		
1	54	17.1
2	77	24.4
3	82	26.0
≥4	103	32.5

The median age of the women was 38 years with a range of 18-70 years. Majority were married (34.2%) and had delivered. The median number of deliveries was 2, with a range of 0-10. Almost half (48.4%) of the women had their first sexual intercourse between 15-19 years; with the median age at first sexual intercourse being 19 years (Range: 12-30). Most (32.5%) of the women had four or more lifetime sexual partners (Table 1).

Table 2: Women's clinical stage of HIV, CD-4 counts, use of anti retro viral drugs and duration since diagnosis of HIV (N=316)

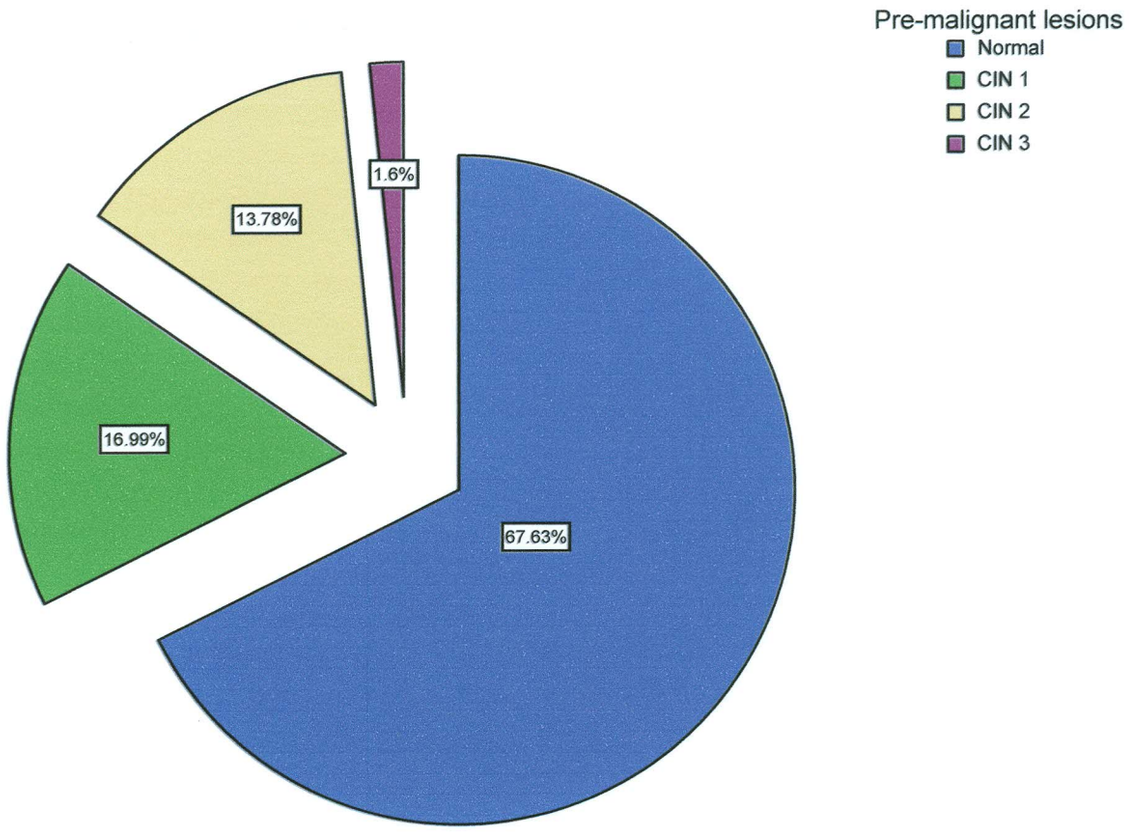
Characteristic	Number	Percent
Clinical stage of HIV		
1	5	1.6
2	286	90.5
3	20	6.3
4	5	1.6
CD-4 count (cells/mm^3)[a]		
<200	55	17.7
200-350	89	28.6
≥351	167	53.7
Type of HAART [b]		
Triomune 30	88	31.3
Combivir and Nevirapine	68	24.2
Combivir and Efavirenz	113	40.2
Second line drugs	12	4.3
Duration of use of HAART (years)		
<1	58	20.6
1-2	52	18.5
≥3	171	60.9
Duration since diagnosis of HIV infection (years)		
<2	85	26.9
2-4	89	28.2
>4	142	44.9

[a]CD-4 count results were not available for 5 women.
[b]35 women were not started on antiretroviral drugs.

Table 2 shows that majority (90.5%) of the women were in WHO HIV clinical stage 2 and had CD-4 count of 351 cells/mm3 or more. More than three quarters (88.9%) of the women were using HAART, and among them 40.2% were using Combivir and Efavirenz.

Forty four point six percent of the women had been diagnosed to have HIV for four years or more, and were on HAART. The median number of use of HAART was 3 years, and that of duration of HIV diagnosis being 4 years.

Figure 2: Pie chart showing occurrence of pre-cancer lesions.



The proportion of women with abnormal cervical changes using VIA in this group was 134/316 (42.4%) and when Pap smear was used the proportion of women with CIN was 101/312 (32.4%) (Four Pap smear results were not available for interpretation). Few (1.6%) women had CIN 3 (Figure 2).

Other diagnoses made on the Pap smear included acute cervicitis (24 women), chronic cervicitis (15 women), *Trichomonas vaginalis* cervicitis (4 women) and one woman was diagnosed to have squamous metaplasia.

Table 3: Sociodemographic characteristics of women with normal and abnormal VIA (N=316)

Characteristics	Total	VIA		P-value
		Abnormal n (%)	Normal n (%)	
Age group				
<25	8	4 (50.0)	4 (50.0)	
25-34	96	43 (44.8)	53 (55.2)	
35-44	131	48 (36.6)	83 (63.4)	
≥45	81	39 (48.1)	42 (51.9)	0.349
Parity				
0	38	13 (34.2)	25 (65.8)	
1	66	29 (43.9)	37 (56.1)	
2	80	36 (45.0)	44 (55.0)	
3	60	23 (38.3)	37 (61.7)	
≥4	72	33 (45.8)	39 (54.2)	0.721
Age at first intercourse				
<15	10	6 (60.0)	4 (40.0)	
15-19	169	69 (40.8)	100 (59.2)	
20-24	105	43 (41.0)	62 (59.0)	
≥25	32	16 (50.0)	16 (50.0)	0.515
Lifetime number of sexual partners				
1	54	28 (51.9)	26 (48.1)	
2	77	35 (45.5)	42 (54.5)	
3	82	37 (45.1)	45 (54.9)	
≥4	103	34 (33.0)	69 (67.0)	0.101

Higher proportion of women with abnormal smears were in the age group of <25 years (50%) and in the age group ≥45 years (48.1%). Women who were para 4 and above also had a higher proportion of abnormal smears (45.8%); as well as those who had first sexual intercourse below 15 years of age (60.0%).

Table 4: Sociodemographic characteristics of women with normal and abnormal Pap smears (N=312)

Characteristics	Total	Pap smear result		P-value
		Normal n (%)	Abnormal n (%)	
Age group				
<25	12	6 (50.0)	6 (50.0)	
25-34	88	62 (70.5)	26 (29.5)	
35-44	131	102 (77.9)	29 (22.1)	
≥45	81	41 (50.6)	40 (49.4)	0.001
Parity				
0	38	24 (63.2)	14 (36.8)	
1	65	48 (73.8)	17 (26.2)	
2	78	58 (74.4)	20 (25.6)	
3	59	41 (69.5)	18 (30.5)	
≥4	72	40 (55.6)	32 (44.4)	0.221
Age at first intercourse				
<15	26	18 (69.2)	8 (30.8)	
15-19	150	104 (69.3)	46(30.7)	
20-24	104	73 (70.2)	31 (29.8)	
≥25	32	16 (50.0)	16 (50.0)	0.315
Lifetime number of sexual partners				
1	54	33 (61.1)	21 (38.9)	
2	75	50 (66.7)	25 (33.3)	
3	80	56 (70.0)	24 (30.0)	
≥4	103	72 (69.9)	31 (30.1)	0.208

Higher proportion of women with abnormal smears were in the age group of <25 years (50%) and in the age group ≥45 years (49.4%). Women who were para 4 and above also had a higher proportion of abnormal smears (44.4%); as well as those who had first sexual intercourse at the age group above 23 years (41.3%) and age group 15-18 years (32.5%).

Table 5: WHO clinical stage of HIV, CD-4 count, use of antiretroviral drugs and duration since diagnosis of HIV infection in women with normal and abnormal VIA (N=316)

Characteristics	Total	VIA		P-value
		Abnormal n (%)	Normal n (%)	
Clinical stage of HIV				
Stage 1	5	1 (20.0)	4 (80.0)	
Stage 2	286	119 (41.6)	167 (58.4)	
Stage 3	20	11 (55.0)	9 (45.0)	
Stage 4	5	3 (60.0)	2 (40.0)	0.386
CD-4 count [a]				
<200	55	25 (45.5)	30 (54.5)	
200-350	89	38 (42.7)	51 (57.3)	
>350	167	69 (41.3)	98 (58.7)	0.864
HAART				
Using	281	119 (42.3)	162 (57.7)	
Not using	35	15 (42.9)	20 (57.1)	0.954
Type of HAART [b]				
Triomune 30	88	41 (46.6)	47 (53.4)	
Combivir and nevirapine	68	27 (39.7)	41 (60.3)	
Combivir and Efavirenz	113	45 (39.8)	68 (60.2)	
2nd line	12	6 (50.0)	6 (50.0)	0.699
Duration of use of ARVs (years) [b]				
<1	57	20 (35.1)	37 (64.9)	
1-2	54	24 (44.4)	30 (55.6)	
≥3	170	75 (44.1)	95 (55.9)	0.462
Duration since diagnosis of HIV infection (years)				
<2	85	32 (37.6)	53 (62.4)	
2-4	89	40 (44.9)	49 (55.1)	
>4	142	62 (43.7)	80 (56.3)	0.573

[a] CD-4 count results were not available for 5 women.
[b] 35 women were not started on antiretroviral drugs.

Table 5 above shows that the likelihood of a woman to have an acetowhite lesion on VIA increases with an increase in the WHO clinical stage of HIV disease, being high (60.0%) in those with stage 4 disease. It is also higher (45.5%) in those with CD-4 counts less than 200 cells/mm^3 as compared to those with higher CD 4 counts.

Though not statistically significant, women who were on second line HAART had more acetowhite lesions on VIA (50.0%), followed by those who were using Triomune 30 (46.6%) as compared to those who were on other treatment regimens.

Use of ARVs for less than a year is associated with a less probability of having an acetowhite lesion on VIA (35.1%) contrast from those who have used ARVs for one year or more.

Table 6: WHO clinical stage of HIV, CD-4 count, use of antiretroviral drugs and duration since diagnosis of HIV infection in women with normal and abnormal Pap smears (N=312)

Characteristics	Total	Pap smear result		P-value
		Normal n (%)	Abnormal n (%)	
Clinical stage of HIV				
Stage 1	5	5 (100.0)	0 (0.0)	
Stage 2	282	191 (67.7)	91 (32.3)	
Stage 3	20	12 (60.0)	8 (40.0)	
Stage 4	5	3 (60.0)	2 (40.0)	0.849
CD-4 count [a]				
<200	54	34 (63.0)	20 (37.0)	
200-350	89	61 (68.5)	28 (31.5)	
>350	165	112 (67.9)	53 (32.1)	0.873
HAART [b]				
Using	278	187 (67.3)	91 (32.7)	
Not using	34	24 (70.6)	10 (29.4)	0.849
Type of HAART				
Triomune 30	87	58 (66.7)	29 (33.3)	
Combivir and nevirapine	68	44 (64.7)	24 (35.3)	
Combivir and Efavirenz	111	78 (70.3)	33 (29.7)	
2nd line	12	7 (58.3)	5 (41.7)	0.380
Duration of use of ARVs (years)				
<1	57	44 (77.2)	13 (22.8)	
1-2	53	34 (64.2)	19 (35.8)	
≥3	168	109 (64.9)	59 (35.1)	0.190
Duration since diagnosis of HIV infection (years)				
<2	83	55 (66.3)	28 (33.7)	
2-4	89	62 (69.7)	27 (30.3)	
>4	140	94 (67.1)	46 (32.9)	0.785

[a] CD-4 count results were not available for 4 women.
[b] 34 women were not started on antiretroviral drugs.

Though not statistically significant, women who were on ARVs had more abnormal pap smears (32.7%) as compared to those who were not yet on treatment. The chances were higher in those women on second line regimen.

Use of ARVs for less than a year is associated with a less probability of having an abnormal smear (22.8%) contrast from those who have used ARVs for one year or more. It appears that the likelihood of a woman having a precancerous lesion increases with an increase in the clinical stage of HIV disease, and is higher in those with CD-4 counts less than 200 cells/mm^3

Table 7: The proportion of agreement between VIA and Pap smear in relation to WHO clinical stage of HIV, CD-4 count, use of HAART, and type of antiretroviral drug used.

Characteristic	Pap smear result	VIA		Proportion of agreement	95% Confidenc Interval
		Abnormal	Normal		
Clinical stage of HIV					
Stage 1-2	Normal	50	146	0.3	0.2-0.3
	Abnormal	68	23		
Stage 3-4	Normal	9	6	0.6	0.4-0.7
	Abnormal	5	5		
CD-4 count (cells/mm^3)[a]					
≤350	Normal	28	67	0.3	0.2-0.4
	Abnormal	34	14		
>350	Normal	29	83	0.3	0.2-0.3
	Abnormal	39	14		
HAART [b]					
Using	Normal	47	140	0.2	0.2-0.3
	Abnormal	70	21		
Not using	Normal	12	12	0.6	0.4-0.7
	Abnormal	3	7		
Type of HAART [b]					
Triomune 30	Normal	19	39	0.3	0.2-0.4
	Abnormal	22	7		
Combivir and nevirapine	Normal	9	35	0.2	0.1-0.3
	Abnormal	18	6		
Combivir and Efavirenz	Normal	17	61	0.2	0.1-0.3
	Abnormal	26	7		
2nd line	Normal	2	5	0.3	0.1-0.6
	Abnormal	4	1		

[a]CD-4 count results were not available for 4 women.
[b]34 women were not on antiretroviral drugs.

The proportion of agreement between VIA and Pap smear is 0.3 (Fair), with a wider 95% confidence interval in early (CIN 1) cervical disease as compared to late cervical disease (CIN 2&3); 0.2-0.4 Vs 0.2-0.3 respectively.

Table 7 shows the proportion of agreement between VIA and Pap smear is less in women with early WHO HIV clinical stage of the disease (stages 1 and 2) as compared to those in stages 3 and 4 (0.3 and 0.6 respectively). It is also less in those women who were on HAART (0.2) contrast from those who were not using HAART (0.6).

Women who were on Triomune 30 and those on second line HAART had a better proportion of agreement between VIA and Pap smear as compared to other modalities of treatment, with a wider range of 95% Confidence Interval seen in those on second line regimen (0.1-0.6). No difference is seen with regard to the level of CD 4 count.

DISCUSSION

This study was designed to explore the performance of VIA in screening for pre-malignant lesions of the cervix in HIV infected women. Pap smear method of screening, being the mostly used method, was used in order to compare the two tests. With the increase in the availability of ARVs, majority of women with HIV live long enough to develop CIN and carcinoma of the cervix. This being the most at risk group in development of CIN and cervical cancer, effective screening methods that are affordable are needed in order to reduce the burden of this disease in developing countries.

The proportion of women with abnormal cervical changes in the present study among women attending HIV/AIDS CTC at MNH was 42.4% using VIA. These results are higher than the findings of 18.7% in Calcutta and suburbs in eastern India where the HIV status of the participating women was not known[36], and the findings of 17.8% women volunteers from the population in Free State Province of South Africa where majority of the women were black[29]. The results in our study are probably high because our study was conducted among HIV infected women. When Pap smear was used the proportion of women with CIN was 32.4%. In 1996/97, Kapiga et al found the prevalence of CIN to be 2.9% in HIV infected women who were followed up to 3-6 months after having delivered[37]. Studies have shown pre-malignant lesions of the cervix to be more common among HIV infected than non-HIV infected women[38, 39]. The results in this study are similar with results among HIV infected women attending gynaecology clinic at Kilimanjaro Christian Medical Centre in the Nothern part of Tanzania [38]; and higher than the findings of 22.7% among attendees of Ampath HIV clinic at Webuye District

Hospital in Kenya [40]. A study in Brazil found the prevalence of CIN to be 42.9% among HIV infected women [39], while Moodley et al. reported almost half of the women initiating ART in Cape Town, South Africa had low or high grade Squamous Intraepithelial Lesion [41]. Since a significant number of the women attending the HIV CTC during the study period were not included in the study, it is possible that the proportion of women with CIN in this study is underestimated. This proportion may increase further with the increase in sample size. Pre-malignant lesions of the cervix in HIV infected women are very common and there is a need to do regular screening and provide appropriate treatment to all HIV infected women.

In this study it was found that the proportion of agreement between VIA and Pap smear in HIV infected women was 0.3 (30%). A fair number of HIV infected women with CIN can be picked by VIA. The reason for having only a fair proportion of agreement may be accounted for by the fact that VIA picks up other lesions like cervicitis that are not premalignant. This proportion of agreement was moderate (0.6%) in women with advanced cervical disease (CIN 2 & 3) and in women who were not using HAART. Laktabai in the study conducted in Kenya reported the degree of agreement between VIA and Pap smear in HIV infected women to be 76.2% for HSIL and 52.0% for LSIL[40]. VIA has been shown to have an average sensitivity for detection of precancer and cancerous lesions of almost 77%, with a range of 56% to 94%[1] in a population where the HIV status of the women was not checked. In this group of women, the sensitivity of VIA (for CIN 2 or higher) was 83.3 % in Kenya [42]; and in Kinshasa the results differed depending on who

did the test, it was 71.1% when done by a physician and 55.5% when done by a nurse[31]. Therefore VIA can be used in screening for CIN in low resource settings, especially so in HIV infected women who need it the most.

The age groups with more abnormal cervical changes in our study when VIA or Pap smear was used included those who were less than 25 years and those 45 years and above. The young age group is the one with high HPV infection rates of high risk types, with a peak prevalence of 25-30% in women under 25 years of age[1]. In Tanzania, it was found that the strongest risk factors for the presence of any HPV-DNA were young age and HIV infection [43]. Co- infection with HIV is among the risk factors that lead to chronic HPV infections and rapid progression to CIN and cervical cancer[1]. Being HIV infected probably led to their faster progression to CIN[34]. This means the criteria for screening women who are at least 30 years and above[1, 44] needs to be reviewed; it should be done earlier in HIV infected women because they are more at risk. This may mean starting programmes to ensure that all HIV infected women are screened for cervical cancer when attending HIV clinics, or all women found to have CIN or cervical cancer in different screening programmes to be given an opportunity for HIV screening. Both options may give good results, especially in lower health facilities. The fact that 2 women in our study who had been attending the clinic for long were found to have invasive cervical cancer, shows that gynaecological services in HIV CTCs is poorly done or not at all. Increasing availability of ARVs will make many HIV infected women have a chance of using HAART and reduce the progression of CIN. The age group 45 years and above,

HPV infection may have occurred 10-20 years earlier and not regressed, and have now progressed into CIN. In this later group, the risk is probably as that in the non HIV population; but in Kenya, HIV infected women who presented with invasive cervical cancer were significantly younger than non-HIV infected women[17]. This fact furthermore cements the importance of cervical cancer screening in HIV infected women.

Women who were on ART for more than 1 year had more abnormal cervical changes on VIA and abnormal Pap smears as compared to those who had used ART for less than a year. It is possible that ARVs have an effect on the cervical cells that leads to their increased ability to become detected by the VIA. Our findings were different from what was seen in Kenya, where no significant differences were seen among women with positive and negative screening results in use of ART[40]. It has been shown that women initiating HAART have a high prevalence of HPV DNA that decline over 96 weeks of HAART[45]. One would expect a regression of CIN lesions [12] with the reconstitution of immunity as evidenced by an increase in CD-4 count after starting HAART, but it has also been shown that there is no clearance of HPV in cervical samples of women started on ARV[46]. This is especially true for HPV 16, the type that is responsible for more than 50% of invasive cervical cancer, suggesting that it might be somehow immune to the body's defense mechanisms[47]. Some studies have shown little or no impact of HAART on the incidence of cervical cancer [48] and it is still unclear whether the natural history of cervical cancer is affected by ART [46]. Therefore it is possible that HAART does not cause regression of CIN lesions, as there is no documented reduction in the burden of

cervical cancer from the time these drugs started to be used almost ten years ago. To date there are neither enough studies to address the effect of HAART on VIA nor on the natural history of cancer of the cervix. However, from our findings, majority of the women who attend the HIV CTC can benefit from VIA because women who were on HAART constituted 88.9%.

Women with advanced WHO HIV clinical stage and those with CD-4 count less than 200 cells/mm^3 had more abnormal cervical changes when VIA was used, and also more CIN lesions when Pap smear was used; in contrast from those with WHO HIV clinical stage 1 and 2, and those with higher CD-4 counts. It is possible that the high proportion of abnormal cervical changes and CIN is because they had significant immunosuppression that pushed them into advanced HIV disease and low CD 4 counts; and therefore they might have progressed faster to developing CIN from chronic HPV infections. These findings contribute to support a critical role of immunological status over the development of CIN; because women in these categories have lower cell mediated immunity and suffer the consequence by getting opportunistic infections which push them to stage 3 and 4 WHO HIV clinical stage of the disease. Our results are similar with results among HIV infected women attending gynaecology clinic at Kilimanjaro Christian Medical Centre where CD-4 cell count < 200/mm^3 was associated with higher prevalence of SIL [38], and a significantly higher prevalence of CIN was also found among women with a CD-4 lymphocyte count less than 300 cells/ mm^3 (62.1%) as compared to those with a greater count (24.2%) in a study done in Brazil [22]. Our findings were

different from what was seen in Kenya, where no significant differences were seen among women with positive and negative screening results in WHO HIV clinical stage[40]. This is not what was seen in Finland where the risk of CIN was not associated with a decrease in CD-4 count [49]. To date, there are also no enough studies showing the effect WHO HIV clinical stage of the disease may have on VIA, which makes comparison with other studies very difficult. Since HAART can raise the woman's immunity in terms of the CD-4 count and a reduction in the HIV viral load; the progression to CIN may also be reduced as is the occurrence of opportunistic infections, and in so doing reducing carcinoma of the cervix.

Determining the HIV viral load could have been a better parameter assessing the status of the HIV infected women and the detection of CIN by VIA. But that was not possible because of the cost of a single HIV viral load test, which couldn't be afforded neither by the investigator nor by the hospital. Other studies have showed a high prevalence of CIN lesions in women with high HIV viral load [22], therefore this study could have added to the available information. Another limitation of this study is the absence of colposcopy at MNH. If colposcopy could have been done, these women would have had an advantage of being offered the 'see and treat' approach that is one of the big advantages of VIA over cytology. This added to the drawbacks of this study because these women were referred to ORCI for colposcopy and therefore these colposcopic findings could not be part of this study. Colposcopy has an advantage of magnifying the lesions that could not be seen by the naked eyes in VIA, and therefore increase the chances of picking up pre-malignant

cervical lesions. Some studies have also shown an increase in the false negative rate of cervical cytology in HIV infected women, and recommended colposcopy to be done as part of routine management in HIV infected women [50]; even though at times women who have negative colposcopic findings do progress into a much severe dysplasia or invasive cervical cancer [51]. Despite all these controversies, it could have been more informative if all the women who had a colour change of the cervix in VIA and an abnormal Pap smear could have a colposcopy done immediately and the results analyzed. Another limitation of this study is the fact that histological testing of the cervices of these women was not done, which is the gold standard in screening; and therefore could have been used to calculate the sensitivity and specificity of VIA and Pap smear.

CONCLUSION

There is a significant number of HIV infected women with abnormal cervical changes, the proportion being higher in those with low CD-4 counts and with advanced WHO HIV clinical stage of the disease. There is a fair (0.3) proportion of agreement between VIA and Pap smear in HIV infected women, and this parameter is affected by the use of ARVs and by the WHO HIV clinical stage of the disease. Even though differences exist in the proportion of agreement between VIA and Pap smear depending on the patient (use of ARVs, WHO HIV clinical stage of the disease); considering the proportion of CIN in HIV infected women, it is worthy it to use VIA as the screening method of choice in these women in low resource settings.

RECOMMENDATIONS

There is a need to start screening women in HIV/AIDS CTCs. VIA can be used in screening these women, as much as it is used in HIV negative women, but in this group it is even more important because they have a significantly higher risk of developing CIN and therefore a higher chance of developing cervical cancer earlier as compared to HIV negative women. VIA, the cost effective method of choice, can help in reducing the cervical cancer burden in our community. There is also a need for larger follow-up studies that will determine incidence rate and progression of pre-malignant lesions in HIV infected women; and also to address the effect of ARV drugs on VIA and on the natural history of carcinoma of the cervix. Studies on the degree of agreement between VIA and Pap smear in HIV infected and non HIV infected women are also needed.

REFERENCES

1. WHO. Comprehensive Cervical Cancer Control: A guide to essential practice. 2006

2. Oguntayo OA, Zayyan M, Ismail H, et al. Carcinoma of the cervix: a tragedy of our time. 2008.

3. Obodo K, Anorlu RI, Makwe CC. Gynaecological deaths in a tertiary hospital in Lagos: a 6 year review. 2007.

4. Sitas F, Madhoo J, Wessie J. Incidence of histologically diagnosed cancer in South Africa, 1993-1995. In: National Cancer Registry, editor. Johannesburg: South African Institute of Medical Research; 1998.

5. Berek JS. (Ed.) Novak's Gynecology, Philadelphia, Lippincott Williams & Wilkins. 2002.

6. Sellors JW, Sankaranarayanan R: Colposcopy and Treatment of Cervical Intraepithelial Neoplasia. 2002; 15.

7. Berek JS. (Ed.) Novak's Gynecology, Philladelphia, Lippincott Williams & Wilkins. 2002

8. Bosch XF, Quao Y-L, Castellsague X. The epidemiology of Human papilloma virus infection and its association with cervical cancer. *Int J Gynaecol Obstet.* 2006; 94: S8-S21.

9. Zielinski GD, Rozendaal L, Voorhorst FJ, et al. HPV testing can reduce the number of follow-up visits in women treated for cervical intraepithelial neoplasia grade 3. *Gynecol Oncol.* 2003; 91: 67-73.

10. Jean-Philippe Spano DA, Jean-Luc B, Dominique F. AIDS and non-AIDS related malignancies: a new vexing challenge in HIV- positive patients: Part II. Cervical and anal squamous epithelial lesions,lung cancer, testicular germ cell cancers and skin cancers. *Eur J Int Med.* 2002; 13: 227-232.

11. CDC: 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. IN PREVENTION, C. F. D. C. A. (Ed. MMWR-Morb Mortal Wkly Rep.) 1992

12. Minkoff H, Ahdiel L, Massad LS. The effect of highly active antiretroviral therapy on cervical cytologic changes associated with oncogenic HPV among HIV-infected women. *AIDS.* 2001; 15: 2157-2164.

13. Cronje HS. Screening for cervical cancer in developing countries. *Int J Gynaecol Obste.t* 2004; 84: 101-8.

14. Falls RK. Spontaneous resolution rate of grade 1 cervical intraepithelial neoplasia in a private practice population. *Am J Obstet Gyneco.l* 1999; 181: 278-82.

15. Parkin DM, Bray F, Ferlay J. Global cancer statistics 2002. *CA Cancer J Clin.* 2005; 55: 74-108.

16. Parkin DM, Bray FI, Devessa SS. Cancer burden in the year 2000. The global picture. *Eur J Cancer.* 2001; 37 (supplement 8): 554-566.

17. Gichangi P, De vuyst H, Estambale B, Rogo K, et al. HIV and cervical cancer in Kenya. *Int J Gynaecol Obstet.* 2002; 76: 55-63.

18. Board of Trustees. Annual Activities and Financial Report for 2006. Dar es Salaam, Ocean Road Cancer Institute. 2007

19. Board of Trustees. Activities and Financial Report Year Ended 2007. Dar es Salaam, Ocean Road Cancer Institute. 2007

20. Galceran J, Marcos-Gragera R, Soler M, et al. Cancer incidence in AIDS patients in Catalonia, Spain. *Eur J Cancer.* 2007; 43: 1085-91.

21. Palefsky J. HPV infection and HPV-associated neoplasia in immunocompromised women. *Int J Gynecol Obstet.* 2006; 94: S56---S64.

22. Matos YF, Costa HL, Faundes AE. Prevalence of cervical squamous intraepithelial lesions in women with HIV. *Int J Gynaecol Obstet.* 2003; 83: 63-4.

23. Mtango LM. Cervical cytological changes in HIV infected patients attending care and treatment clinic at Muhimbili National Hospital. Dar es Salaam, Muhimbili University of Health and Allied Sciences. 2007

24. Marais DJ, Passmore JA, Denny L, et al. Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. *J Med Virol.* 2008; 80: 953-9.

25. Heard I, Tassie JM, Schmitz V, et al. Increased risk of cervical disease among Human Immunodeficiency Virus-infected women with severe immunosuppression and high Human Papillomavirus load. *Obstet Gynecol.* 2000; 96: 403-9.

26. Bhatla N, Gulati A, Mathur SR, et al. Evaluation of cervical screening in rural North India. *Int J Gynaecol Obstet.* 2009; 105: 145-9.

27. Sankaranarayanan R, Wesley R, Thara S, et al. Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. *Int J Cance.r* 2003; 106; 404-8.

28. Jeronimo J, Morales O, Horna J, et al . Visual inspection with acetic acid for cervical cancer screening outside of low-resource settings. *Rev Panam Salud Publica.* 2005; 17; 1-5.

29. Cronje HS, Cooreman BF, Beyer E, et al. Screening for cervical neoplasia in a developing country utilizing cytology, cervicography and the acetic acid test. *Int J Gynaecol Obstet.* 2001; 72; 151-7.

30. Omigbodun AO & Ayinde OA. The management of abnormal cervical smears by Nigerian gynecologists. *Int J Gynaecol Obstet.* 2005; 88: 340-1.

31. Sangwa-Lugoma G, Mahmud S, Nasr SH, et al. Visual inspection as a cervical cancer screening method in a primary health care setting in Africa. *Int J Cancer.* 2006; 119: 1389-95.

32. Kyei A, Nhkoma G. Cervical Cancer Prevention in Malawi. Malawi, JHPIEGO. 2009

33. Ngwalle EW, Mgaya HN, Mpanju-Shumbusho W, et al. Situational analysis for diagnosis and treatment of cervical cancer in mainland Tanzania. *East Afr Med J.* 2001; 78; 60-4.

34. Chirenje ZM. HIV and cancer of the cervix. *Best Pract Res Clin Obstet Gynaecol.* 2005; 19: 269-76.

35. Basu PS, Sankaranarayanan R, Mandal R, et al. Visual inspection with acetic acid and cytology in the early detection of cervical neoplasia in Kolkata, India. Int J Gynecol Cancer. 2003; 13:626-32.

36. Diamantis A, Magiorkinis E, Androutsos G: What's in a name? Evidence that Papanicolaou, not Babes, deserves credit for the PAP test. *Diagn Cytopathol.* 2009.

37. Kapiga SH, Msamanga GI,Spiegelman D, et al. Risk factors for cervical squamous intraepithelial lesions among HIV-1 positive women in Dar es Salaam, Tanzania. *Int J Gynaecol Obste.t* 1999; 67: 87-94.

38. Obure J, Olola O, Swai B, et al. Prevalence and severity of cervical squamous intraepithelial lesion in a tertiary hospital in northern Tanzania. *Tanzania Journal of Health Research.* 2009; 11: 163-169.

39. Matos YF, Costa HL, Faundes AE. Prevalence of cervical squamous intraepithelial lesions in women with HIV. *Int J Gynaecol Obstet.* 2003; 83(1):63-4.

40. Laktabai JK. Prevalance of premalignant cervical lesions: Comparing visual inspection with Pap smear in women attending HIV care clinic at the Webuye District Hospital, Kenya. Moi University, Kenya. 2009

41. Moodley JR, Constant D, Hoffman M, et al. Human papillomavirus prevalence, viral load and pre-cancerous lesions of the cervix in women initiating highly active antiretroviral therapy in South Africa: a cross-sectional study. *BMC Cancer.* 2009;10: 1186/1471-2407-9-275.

42. De Vuyst H, Claeys P, Njiru S,et al. Comparison of pap smear, visual inspection with acetic acid, human papillomavirus DNA-PCR testing and cervicography. *Int J Gynaecol Obste.t* 2005; 89: 120-6.

43. Ter Meulen J, Eberhardt HC, Luande J, et al. Human Papilloma Virus (HPV) infection, HIV infection and cervical cancer in Tanzania, east Africa. *Int J Cance.r* 1992; 51: 515-21.

44. Sherris J, Wittet S, Kleine A, et al. Evidence -based alternative cervical screening approaches in low-resource settings. *Int Perspect Sex Reprod Health.* 2009; 35: 147-54.

45. Fife KH, Wu MS, Squires KE, et al. Prevalence and Persistence of Cervical Human Papillomavirus Infection in HIV-Positive Women Initiating Highly Active Antiretroviral Therapy. *J Acquir Immune Defic Syndr.* 2009; 51: 274-282.

46. Heard I, Palefsky JM, Kazatchkine. The impact of HIV antiviral therapy on human papillomavirus (HPV) infections and HPV-related diseases. *Antivir Ther.* 2004; 9: 13-22.

47. Strickler HD, Palefsky JM, Shah KV, et al. Human papillomavirus type 16 and immune status in Human Immunodeficiency Virus-seropositive women. *J Natl Cancer Inst.* 2003; 95:(14):1062-71.

48. Heard I, Potard V, Costagliola D. Limited impact of immunosuppresion and HAART on the incidence of cervical squamous intraepithelial lesions in HIV positive women. *Antivir Ther.* 2006; 11:1091-6.

49. Lehtovirta P, Paavonen J, Heikinheimo O. Risk factors, diagnosis and prognosis of cervical intraepithelial neoplasia among HIV-infected women. *Int J STD AIDS.* 2008;19(1):37-41.

50. Maiman M, Tarricone N, Vieira J, et al. Colposcopic evaluation of Human Immunodeficiency Virus-seropositive women. *Obstet Gynecol.* 1991 ;78(1):84-8.

51. Massad LS, Evans CT, Strickler HD, et al. Outcome after negative colposcopy among Human Immunodeficiency Virus-infected women with borderline cytologic abnormalities. *Obstet Gynecol.* 2005; 106(3):525-32.

APPENDICES

APPENDIX 1: CONSENT FORM-ENGLISH VERSION

Efficacy of Visual Inspection with Acetic acid in screening for cervical pre-malignant lesions in HIV infected women;

A cross sectional study.

Introduction

My name is _____. I am a researcher from Muhimbili University of Health and Allied Sciences. I am conducting a research on one of the common diseases that affect women, but the likelihood of this disease is increased in people who are HIV infected. This is cancer of the uterine cervix. The purpose of the study is to determine how many HIV infected women are affected by this disease. The findings will help to put recommendations in order to improve care of the HIV infected women and therefore catch the disease early.

How to participate in this study

You are asked to participate in this study because you are one among many women who are infected with HIV and you are at an increased risk of getting carcinoma of the cervix. If you are willing to participate in this study, you will be interviewed for 10 minutes. The interview will be conducted only once and at the end I will examine you and later take specimens from your cervix. I will try to be as gentle as possible and it is my hope that you won't feel any pain, apart from minor discomfort.

Confidentiality

Everything will remain confidential and will be used only for research purposes. The research team will compile a report that will contain information about all other HIV infected women with a problem like yours, without mentioning your name.

Risks

I do not expect that any harm will happen to you as a result of participating in the study, except for the minor discomfort during collection of specimen.

Right to participate in the study

Taking part in this study is completely of your choice. You have the right to participate or decide otherwise without giving any reason for your decision. Once you have decided to participate you are also free to terminate your participation at any time.

Benefits of participating in this study

If you agree to participate in this study you will help us find the magnitude of this problem in our community, and enable the hospital and the country in general to establish proper management plans.

Who to Contact

If you have any questions about this study you are free to contact, the principal investigator, Dr. Belinda Balandya (0753 354005).

If you ever have questions about your rights as a participant, you may call Prof. E. F. Lyamuya, Chairman of the college Research and Publications Committee, Box 65001, Dar es Salaam. Tell 2150302-6.

If you agree to this interview, please sign this consent form.

I ..... have read and understood the contents of this consent form and my questions have been sufficiently answered. I therefore consent for the interview for this study.

Signature of the interviewee ..... Date .....

Signature of the interviewer ..... Date .....