The battleground of two Infections and a cancer: Human Papilloma Virus, premalignant lesions of the cervix and their interaction with Human Immunodeficiency Virus in southwestern Nigeria

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Human Papilloma Virus, premalignant lesions of the cervix and their interaction with Human Immunodeficiency Virus in southwestern Nigeria

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Title: The battleground of two infections and a cancer : Human Papilloma Virus, premalignant lesions of the cervix and their interaction with HIV in southwestern Nigeria

Abstract

Background: The highest numbers of HIV-infected women are in sub-Saharan Africa, where the natural progression of HIV disease in the absence of treatment results in death before the onset of invasive cervical cancer. With improved access to treatment, several studies outside West Africa demonstrated an increased risk of pre-invasive cervical lesions among HIV-infected women and the positive impact of treatment on the outcome. Given the various HIV strains in Nigeria and other West African countries, a different outcome may be expected. Unfortunately, limited information exists on the subject in the sub-region. **Aim:** To study the effect of HIV infection on the burden of premalignant lesions of the cervix; assess the diagnostic accuracy of direct visual inspection of the cervix; and contribute to policy formulation and the development and implementation of an effective cervical cancer prevention and control programme in Nigeria. **Method:** The studies (I-V) were conducted among adult women of known HIV status in south-western Nigerian (2011- 2012). Study I, a randomised control study among 1140 women, determined the effect of HIV infection on the test performance of direct visual inspection of the cervix in detecting cytology - diagnosed squamous intraepithelial lesions. Studies II and III utilised data generated in Study I to determine the interaction between HIV infection, antiretroviral therapy, and precancerous lesions of the cervix. Study IV, a cross-sectional study, assessed the acceptability of cervical cancer screening among 1517 HIV- positive women. Study V prospectively determined the outcome of follow- up after a positive cervical cancer screening test. **Results**: Visual inspection with Lugol's iodine was found to be inferior to visual inspection with acetic acid and inadequate as a cervical cancer screening tool in cases of severe immune deficiency (specificity of 66.9% and negative predictive value of 50.0%). The prevalence of high risk HPV and squamous intraepithelial lesions were 19.6% and 8.4%, respectively. HPV 16 (3.9%), 35 (3.5%) and 58 (3.5%) were most frequently found. HIV positive women were found to be at increased risk of high risk HPV infection (OR: 1.8; 95% CI: 1.4 - 2.2) and squamous intraepithelial lesion (OR: 5.4; 95% CI: 2.9 - 8.8). Antiretroviral drugs was found to protect against high risk HPV infection (OR: 0.4; 95% CI: 0.3- 0.5) and development of squamous intraepithelial lesions. Although only 56.2% of HIV positive women were aware of cervical cancer screening, the test was acceptable to 79.8% of them. Among the 108 women who screened positive during outreach cervical cancer screening, 47.2% defaulted from follow -up as a result of transportation and cost- related issues and an anticipated long waiting time at the referral centre. Poorly educated women (OR: 3.1, CI: 2.0 – 5.2) and those residing more than 10 km from the clinic (OR: 2.0, CI: 1.0 – 4.1) were most likely to default. **Conclusion.** Precancerous lesions of the cervix were found to be higher in HIV positive women, especially severely immunocompromised ones and those not on treatment. Cervical cancer screening is acceptable to women but default from follow - up after positive screening was high, especially among poorly educated rural women. Visual inspection with Lugol's iodine was found to be inadequate for cervical cancer screening in cases of severe immune deficiency. The current strategy needs to be changed to one that will integrate cervical cancer prevention into HIV care as well as to improve access services for poorly educated rural women.

Key words: Human papilloma virus, cervical cancer, antiretroviral drugs, HIV/AIDS, pre-cancerous lesions

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To Lilian, Uche, Ebuka and Chijoke

Women are not dying because of untreatable diseases. They are dying because societies have yet to make decision that their lives are worth saving: We have not yet valued women's lives and health highly enough

Professor Mahmoud Fathalla,
Past President, FIGO

Abstract

Background: The highest numbers of HIV-infected women are in sub-Saharan Africa, where the natural progression of HIV disease in the absence of treatment results in death before the onset of invasive cervical cancer. With improved access to treatment, several studies outside West Africa demonstrated an increased risk of pre-invasive cervical lesions among HIV-infected women and the positive impact of treatment on the outcome. Given the various HIV strains in Nigeria and other West African countries, a different outcome may be expected. Unfortunately, limited information exists on the subject in the sub-region.

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Method: The studies (I-V) were conducted among adult women of known HIV status in south-western Nigerian (2011- 2012). Study I, a randomised control study among 1140 women, determined the effect of HIV infection on the test performance of direct visual inspection of the cervix in detecting cytology - diagnosed squamous intraepithelial lesions. Studies II and III utilised data generated in Study I to determine the interaction between HIV infection, antiretroviral therapy, and precancerous lesions of the cervix. Study IV, a cross-sectional study, assessed the acceptability of cervical cancer screening among 1517 HIV- positive women. Study V prospectively determined the outcome of follow- up after a positive cervical cancer screening test.

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and squamous intraepithelial lesions were 19.6% and 8.4%, respectively. HPV 16 (3.9%), 35 (3.5%) and 58 (3.5%) were most frequently found. HIV positive women were found to be at increased risk of high risk HPV infection (OR: 1.8; 95% CI: 1.4 - 2.2) and squamous intraepithelial lesion (OR: 5.4; 95% CI: 2.9 - 8.8). Antiretroviral drugs was found to protect against high risk HPV infection (OR: 0.4; 95% CI: 0.3- 0.5) and development of squamous intraepithelial lesions. Although only 56.2% of HIV positive women were aware of cervical cancer screening, the test was acceptable to 79.8% of them. Among the 108 women who screened positive during outreach cervical cancer screening, 47.2% defaulted from follow -up as a result of transportation and cost- related issues and an anticipated long waiting time at the referral centre. Poorly educated women (OR: 3.1, CI: 2.0 – 5.2) and those residing more than 10 km from the clinic (OR: 2.0, CI: 1.0 – 4.1) were most likely to default.

Conclusion. Precancerous lesions of the cervix were found to be higher in HIV positive women, especially severely immunocompromised ones and those not on treatment. Cervical cancer screening is acceptable to women but default from follow - up after positive screening was high, especially among poorly educated rural women. Visual inspection with Lugol's iodine was found to be inadequate for cervical cancer screening in cases of severe immune deficiency. The current strategy needs to be changed to one that will integrate cervical cancer prevention into HIV care as well as to improve access services for poorly educated rural women.

Key words: Human papilloma virus, cervical cancer, antiretroviral drugs, HIV/AIDS, pre-cancerous lesions

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
APIN	AIDS Prevention Initiative Nigeria
ARV	Antiretroviral
ART	Antiretroviral therapy
ASCUS	Atypical Squamous Cell abnormality of Undetermined Significance
CC	Cervical Cancer
CCA	Cervical Cell Abnormality
CD4	Cell Differentiating 4
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma in Situ
CRF	Case Record Form
CRF02 AG	HIV Circulating Recombinant Forms 02 AG
CSIL	Cervical Squamous Intraepithelial Lesion
DNA	Deoxyribonucleic Acid
DVI	Direct Visual Inspection
FMoH	Federal Ministry of Health
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HR HPV	High Risk Human Papilloma Virus
HSIL	High Grade Squamous Intraepithelial Lesion
KM	Kilometre
LSIL	Low Grade Squamous Intraepithelial Lesion
MTCT	Mother To Child Transmission
NIMR	Nigerian Institute of Medical Research
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
RNA	Ribonucleic Acid
SIL	Squamous Intraepithelial Lesion
TB	Tuberculosis

VIA	Visual Inspection with Acetic Acid
VILI	Visual Inspection with Lugol's Iodine
WHO	World Health Organization

List of Publications

I **Ezechi OC**, Odberg Pettersson K, Gbajabiamila TA, Gab-Okafor Chidinma V, Idigbe Ifeoma E, Ujah IAO, Ostergren PO. Evaluation of direct visual inspection of the cervix in detecting cytology - diagnosed squamous intraepithelial lesions in women of known HIV status. A randomized controlled open label study (CANHIV Study). *Manuscript submitted for publication*

II **Ezechi OC**, Ostergren PO, Nwaokorie FO, Ujah IAO, Odberg Pettersson K. The burden, distribution and risk factors for cervical oncogenic Human papilloma virus infection in HIV positive Nigerian women. Virology Journal 2014, 11:5

III **Ezechi OC**, Odberg Pettersson K, Okolo CA, Ujah IAO, Ostergren PO.The association between HIV infection, antiretroviral therapy and cervical squamous intraepithelial lesions in south western Nigerian women. PLoS ONE 2014; 9(5)

IV **Ezechi OC**, Gab-Okafor CV, Ostergren PO, Odberg Pettersson K. Willingness and acceptability of cervical cancer screening among HIV positive Nigerian women. BMC Public Health 2013, 13:46 http://www.biomedcentral.com/1471-2458/13/46

V **Ezechi OC**, Odberg Petterson K, Gbajabiamila TA, Idigbe IE, Kuyoro T, Ujah IAO, Ostergren P O. Predictors of default from follow-up care in a cervical cancer screening program using direct visual inspection in southwestern Nigeria. BMC Health Services Research 2014, 14:143 http://www.biomedcentral.com/1472-6963/14/143

Contents

Introduction

The motivation to embark on this study is based on my first-hand experience as an obstetrician/gynaecologist at an HIV treatment centre that provides comprehensive HIV care, treatment, and support to over 20,000 adult Nigerians. I observed first-hand the challenges of AIDS and non-AIDS defining malignancies among the patients. It is projected that 20% to 40% of all HIV positive patients will be diagnosed with a malignancy in their lifetime (1). Cancer of uterine cervix, the end stage of Human papilloma virus (HPV) infection, is one of the malignancies and a unique cancer among women living with HIV infection (2-7).

Women aged 18 years and above constitute over 60% of the patients in our HIV treatment centre, and recent reports project that as many as 25% of all women with no evidence of cervical disease will develop premalignant lesion within 3 years of diagnosis (1,7). These grave data emphasised the urgent need to assess the determinants of cervical disease among HIV positive women, with the aim of applying acquired information to develop context specific strategies to improve access to cervical cancer prevention and control services.

Prior to the introduction of highly active antiretroviral therapy (HAART), most HIV infected women in low resource settings did not live long enough for the transformation of precancerous lesions of the cervix to cervical cancer to take place (1,8,9). However, with improved access to HAART, an increasing number of women survive long enough for cervical cancer precursors to manifest and progress to invasive cancer (1,8,9). The use of HAART by HIV positive women has the potential of impacting positively on the effect of HIV infection on cervical cancer and its precursor lesions. HAART reduces mortality among HIV infected individuals and improves longevity, which ultimately increases the duration of their exposure to HPV, thereby allowing the accumulation of genetic somatic mutations that in turn increase the likelihood that lesions of the cervix will develop (1). Conversely, the use of HAART reduces HIV

plasma viral load and leads to immune restoration, which may moderate the effect of HIV infection on the progression of HPV infection and associated precancerous lesions (1). Despite studies that have examined the effects of HAART on the course of cervical lesions in HIV positive women, the effect of HAART use on the course of cervical lesions is not conclusively known (1,7,10-15).

Additional impetus to embark on this pursuit was the adoption of cervical cancer prevention guidelines meant for the general population for HIV positive women. More importantly the guideline was not based on evidence from West African sub - region which has genetically distinct HIV strains and epidemiology (4,16,17).

Nigeria ranks second in global HIV burden and contributes 10% of the global cervical cancer burden, yet little or no information exists on the impact of HAART on cervical lesions among women benefitting from the highly successful and donor dependent HIV programme (4,17). Previous studies in Nigeria have focused on the prevalence of precancerous and invasive lesions of the cervix among HIV infected women (18-22). In addition, detailed literature search identified no study in Nigeria and West African sub - region that systematically evaluated the effect of HIV infection and its treatment on the epidemiology of cervical precancerous lesions.

The studies that make up this thesis attempt to provide answers to some of the observations noted in the course of providing care for Nigerian women living with HIV infection. It will also hopefully assist in developing context specific cervical cancer prevention strategies for HIV positive women.

Background

In the same settings of high cervical cancer burden, the HIV/AIDS pandemic has overwhelmed the health care delivery systems and has had an enormous negative impact on women (17,23). Cervical cancer rates are on the rise, paralleling the HIV epidemic (24). The observed rise in this rate has been linked to chronic deviations in the immune systems due to HIV infection (6, 24).

It was expected that the immune-reconstitution from the use of HAART would halt and reverse the global rise in cervical cancer incidence and mortality (25). Decades after the introduction of HAART, the expected change is yet to be observed. Instead, mortality from cervical cancer has been increasingly reported in low income countries, including Nigeria (26).

Cervical Cancer

The cancer of the uterine cervix (CC) originates from squamous epithelial cell in the transformation zone of the cervix in about 80% of all cases (27-29). It may occasionally arise from the glandular epithelial cells, and very rarely from other cervical cell types. When it originates in the glandular epithelial cells of the cervix, it is referred to as cervical adenocarcinoma (28,29).

Epidemiology

The morbidity and mortality associated with CC is a major global public health challenge. Every year half a million women are diagnosed with CC and another 250,000 reportedly die from the disease, making CC the second most common female cancer worldwide (16,17,30). Although there are wide variations in the burden and incidence between countries (ranging from 3 – 50 per 100,000), more than 80% of the global burden of CC and related deaths occur in low income countries of South and Central America, sub-Saharan Africa, and south and south-east Asia (30,31). CC affects women during the most productive years of their lives, leading to

misery, low productivity, and economic hardship on individual, family and societal levels (30, 31).

The wide variation in CC burden and incidence is a reflection of differences in exposure risk, state of health system, and ultimately the quality of CC prevention services. High-risk prevalence regions include East and West Africa, with a cumulative risk of 3.8%, Southern Africa (2.9%), South-Central Asia (2.6%), Middle Africa, and South America (2.5%) (32) (Table 1).

While incidence and mortality rates of CC have declined substantially in high income countries following the introduction of screening programs, CC remains the most common female cancer in sub-Saharan Africa. It is estimated that nearly 80,000 new cases and over 60,000 deaths occur yearly (33, 34). The age - standardised rate and mortality are reported to be 34.8% and 22.5%, respectively. While the North African region has the lowest incidence (6.6%), the East Africa region has the highest rate (42.7%). However, there are countries with high and low rates within each region (32). The vast majority of women who suffer from CC in sub-Saharan Africa present with late disease, which is far beyond the capacity of surgery or other treatment modalities to handle (34). Supportive and/or palliative care services are poorly developed and funded; consequently thousands of women residing in slums and rural areas suffer a miserable end of life as the cancer spreads to adjacent pelvic organs, resulting in great discomfort, pain, bleeding, fistula formation, and obstruction of the bowel and ureters (34).

Table 1. Age standardized incidence and mortality rates of cervical cancer by world region (Source: Globocan 2012**)**

Region/Sub- region	Incidence per 100,000	Mortality per 100,000
World	14.0	6.8
High Income countries	9.9	3.3
Low income countries	15.7	8.3
Africa		
▪ Eastern Africa	▪ 42.7	▪ 27.6
▪ Western Africa	▪ 29.3	▪ 18.5
▪ Southern Africa	▪ 31.5	▪ 17.9
▪ Middle Africa	▪ 30.6	▪ 22.2
▪ Northern Africa	▪ 6.6	▪ 3.2
Sub - Saharan Africa	34.8	22.5
South East Asia	16.3	7.9
Europe	11.2	3.8
Americas	14.9	5.9
Western Pacific	8.5	3.5

Precancerous lesions of the cervix

Squamous intraepithelial lesions

Squamous intraepithelial lesions (SIL), also known as cervical intraepithelial neoplasia (CIN) or cervical dysplasia is a term that refers to abnormal growth of epithelial cells of the cervix. It is the precursor lesion of CC and has the potential of transform into CC if left untreated. While the majority of SIL cases remain stable, or are eliminated by the host's immune system without intervention, less than 2% of cases of SIL progress to become CC when untreated (35-37).

Various terminologies have been used to classify precancerous lesions of the cervix, including the original Papanicolaou classification (38) and the World Health Organization dysplasia grading system (39). At present two systems are used for cytology reporting: the British Society for Clinical

Cytology system (40) and the Bethesda system (41). The two later classification systems use nomenclature that accurately conveys the morphological unity and malignant potential of all cervical intraepithelial neoplastic lesions if left untreated. However, the Bethesda system was used in Study III because it has a wider application, and it provides a uniform, well-defined diagnostic terminology that facilitates unambiguous communication between laboratory and clinician (38,42). The revised Bethesda system (41) segregated premalignant squamous lesions into three categories:

i. Atypical squamous intraepithelial cells (ASC)
ii. Low-grade squamous intraepithelial lesions (LSIL)
iii. High-grade squamous intraepithelial lesions (HSIL)

The need for appropriate interpretation of abnormal results and subsequent referral of clients by health workers for optimal follow-up cannot be overemphasized (38). Table 2 compares the different classification systems for precancerous lesions of the cervix to histologic classification, which is the gold standard for the diagnosis of precancerous and cancerous lesion of the cervix.

Table 2. Abnormal cervical squamous cell reporting systems (Source: Dim et al. 2012)

Cytology (Pap) Result			Histology Equivalent
Bethesda Classification	British Society of Clinical Cytology	WHO Classification	Cervical intraepithelial neoplasia (CIN)
ASC-US	Borderline		
ASC-H	-	-	-
LSIL	Mild dyskaryosis	Mild dysplasia	CIN I
HSIL	Moderate dyskaryosis	Moderate dysplasia	CIN II
	Severe dyskaryosis	Severe dysplasia	CIN III

Human papilloma virus

Human papilloma virus (HPV) infection is the commonest sexually transmitted infection globally, and most sexually active women will acquire it at some point during their lifetime (43). It is estimated that among women with normal cervical cytology, between 10% and 17% of them have HPV infection at a given point, with higher prevalence among women below 25 years of age (43).

HPV is one of the most powerful human carcinogens and has been implicated in cancers at several sites, including the uterine cervix, anus, and nasopharynx (16). Approximately 5% of all cancers have been attributed to HPV infection, of which more than 80% occur in low income countries (44). Globally, the most common HPV types detected in CC lesions were types 16 (57%), 18 (16%), 58 (5%), 33 (5%), 45 (5%), 31 (4%), 52 (3%), and 35 (2%). The vaccine- preventable types (16, 18), and 45 accounted for a greater percentage of infections detected in CC lesions, compared to cervixes with normal cytology (43-45). Increasingly, types other than the vaccine- preventable ones are being reported in regions outside Europe and North America (5, 46).

The prevalence of HPV infection in Nigeria is not precisely known, as there are only few population-based studies (47-49). Most of the studies are either hospital or clinic based (46, 50-53). The prevalence rate of high risk HPV ranged from 10.7% to 37.5%, with higher rates of 36.0% to 44.9 % (54,55) and 40.9% to 90.7% (52,53) reported among HIV positive individuals and those with cervical lesions respectively, in Nigeria.

The link between HPV, squamous intraepithelial lesions, and cervical cancer

Infection with any of the high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) is a necessary, although not sufficient, cause of almost all cases of CC (36, 56, 57). HPV types 16 and 18 alone are reported to be responsible for over 70% of all CC cases (58-60). However, reports from

sub-Saharan Africa, south East Asia and Latin America point to a more significant role for other non-HPV types 16 and 18 than has previously been considered (47, 48, 52, 61-63).

The progression from HPV infection to CC occurs in four steps, namely: HPV transmission, acute HPV infection, persistent HPV infection leading to precancerous changes, and invasive cancer (36, 56, 64, 65). Each of these steps can be reproducibly differentiated. Genital HPV infections are mostly transmitted by skin-to-skin or mucosa-to-mucosa contact. Although the rate of infection per sexual act is not precisely known, evidence suggests that it is likely to be very high considering the prevalence of HPV infection (56, 65).

Transmission across genotypes has been shown to be similar. Concurrent multiple infection with several HPV types do occur as a result of a common route of transmission. Rates of 20% to 30% have been reported (56, 66).

Following exposure to HPV, infecting viral particles reach germinal cells in the basal layer through tiny tears in the mucosa (Fig. 1). “The early human papillomavirus genes E1, E2, E4, E5, E6, and E7 are expressed and the viral DNA replicates from episomal DNA after infection. In the upper layers of the epithelium the viral genome is replicated further, and the late genes L1 and L2, and E4 are expressed**.** L1 and L2 encapsidate the viral genomes to form progeny virions in the nucleus. The shed virus can then initiate a new infection. Low grade intraepithelial lesions support productive viral replication. The progression of untreated lesions to microinvasive and invasive cancer is associated with the integration of the HPV genome into the host chromosomes (red nuclei), with associated loss or disruption of E2, and subsequent up-regulation of E6 and E7 oncogene expression” (57).

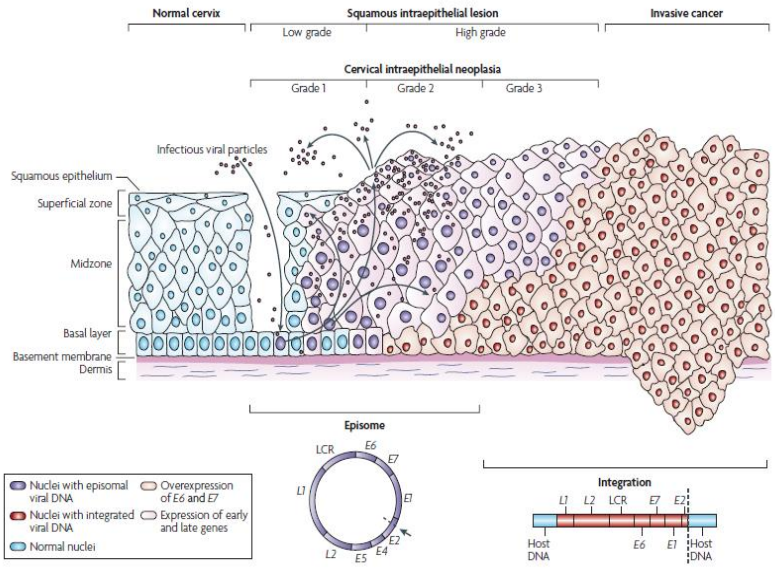


Figure 1. Human papillomavirus lifecycle and organization of its genome (Source: Woodman CBJ et al. 2007). Reprinted with permission

Penetrative sexual intercourse is not strictly necessary for transmission of HPV, as it can also be transferred to the cervix from an original infection at the introitus. Following HPV infection of the cervix, the infection may remain locally stable, regress spontaneously, or progress to develop a low grade squamous intraepithelial lesion (Fig. 2). However, most low grade squamous intraepithelial lesions do not progress, especially those that occur in young and immunocompetent women (52, 57, 64)**.**

It has been established that about 10% of all women with HPV infection will develop precancerous changes in their cervical cells, especially women aged 30 to 40 years. Of the women who do develop precancerous changes, 8% to 10% will further progress to carcinoma in situ. Invasive cancer develops over many years (or even decades) in 1% to 2% of women with premalignant lesions. A peak in risk occurs at about 35 to 55 years of age (35-37). All but the last stage may reverse to normality. Rarely, some squamous intraepithelial lesion may become cancers within a short

interval of 1to 2 years (35-37,52,59). Each HPV genotype acts as an independent infection, with differing oncogenic risks linked to evolutionary species (35-37).

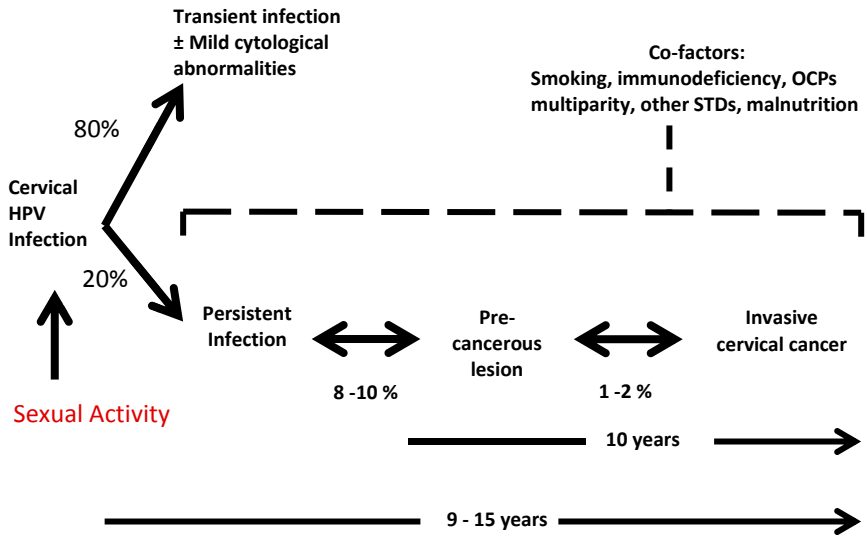


Figure 2. The progression of HPV infection to cervical cancer

Though this causal model is supported by both epidemiological and laboratory data, the actual molecular virology underlying HPV persistence, progression, and invasion is not yet fully understood (35,57).

Risk factors for cervical cancer

Research has shown that only a small fraction (1% to 2%) of all cases of high risk HPV (HR HPV) infections lead to the development of CC, implying that additional factors are necessary for HR HPV infection progression to invasive cancer (43,44,47,48,52,58,60,62,63). However, these factors are often found to be related to HPV infection.

Infection with HPV and its persistence are not only associated with age but with other factors such as social, sexual, and reproductive behaviour (35,36). The risk for HPV acquisition markedly increases with the number of lifetime sexual partners. Infection with Chlamydia trachomatis, herpes simplex virus and other sexually transmitted infections have been reported to be associated with the acquisition of HPV infection (67, 68). In the presence of HIV-associated immune deficiency, the ability of the body's immune system to control HPV infections is impaired (4, 6, 7, 61). Other risk factors for CC include a history of smoking, early sexual debut, childbirth, and multiparity (Fig. 2). Women previously treated for premalignant lesions of the cervix are also at increased risk for future development of CC (4, 6, 7, 61, 67, 68).

Prevention of cervical cancer

CC is preceded by a pre-cancerous stage and thus presents unique opportunities for prevention and control. Firstly, precancerous lesions can be prevented from occurring through behavioural change, risk reduction, and HPV vaccination of young women aged 9 to 13 years. Secondly, it can be stopped through early detection of precancerous lesions and instituting appropriate treatment (16).

According to WHO, "the core principle of a comprehensive approach to cervical cancer prevention and control is to act across the life course using the natural history of the disease to identify opportunities in relevant age groups to deliver effective interventions" (69, p. 3). However, for a society to derive the full benefit from a comprehensive CC prevention and control programme it should be implemented by a multidisciplinary team composed of staff from family planning, reproductive health, maternal health, cancer control, immunization, and adolescent health. Such an approach (Fig. 3) should include community education, social mobilization, HPV vaccination, screening, treatment, and palliative care, while at the same time ensuring that HPV vaccination does not replace CC screening,

as the available vaccine only protects against CC caused by HPV 16 and 18 only (69).

Public health education

HPV and HIV share similar transmission routes. Therefore, public enlightenment and health education focusing on risk reduction should reduce the burden of both diseases (16). Education of parents and young women who were infected with HIV through blood transfusion and mother to child to transmission (MTCT) on the importance of HPV vaccination will reduce the number of young women infected with HPV.

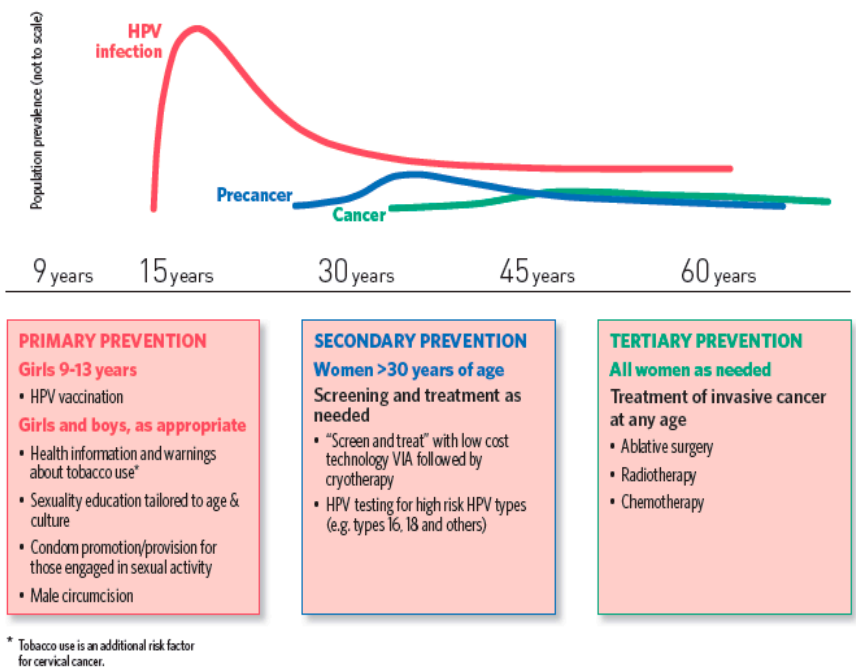


Figure 3. Overview of programmatic interventions over the life course to prevent cervical cancer (Source: WHO guidance document on comprehensive cervical cancer prevention 2013).

In addition, educating those women and their spouses on the importance of CC screening will increase the acceptance CC prevention and control services. Despite high awareness of CC in most low income countries, including Nigeria, the actual knowledge of risk reduction strategies is very low.

The low uptake of screening is consequently not surprising (38, 70-72). Sexual and reproductive health and rights education among youth aims at delaying sexual debute, marriage, and encouraging safe sex practices as this has the potential of reducing the burden of HIV and HPV infection in endemic countries (72).

HPV vaccine

The availability of HPV vaccine offers hope for the prevention of CC, especially among young girls between the ages of 9 to 15 years, including those infected with HIV through MTCT and blood transfusion (16, 73-75). HR HPV types 16 and 18 account for approximately 75% of all cervical cancers, 55% of HSIL, and 20% to 35% of LSIL (73).

Vaccination of girls aged 9 to 15 years with either the bivalent (targets HPV 16 and 18) or the quadrivalent (targets HPV 6,11, 16 and 18) prophylactic recombinant HPV vaccines will protect them against 75% of CC due to HPV 16 and 18 (74,75). The remaining 25% of CC cases are caused by other oncogenic non-HPV 16 and 18 type virus. Thus women who received HPV 16 and 18 based vaccines should continue to be screened for cervical cancer, but at a greatly reduced frequency (74, 75).

Although HPV vaccine based on types 16 and 18 is currently recommended, geographic and regional variations, possibly due to HIV infection is increasingly been reported (47,48,50-53,62,63). If this is confirmed to be true, the current HPV vaccine based on type 16 and 18 may not be as effective as projected in those settings. West Africa sub-

region have genetically different circulating HIV types and strain compared to Europe and North America (76-78) , yet limited information exists on the interaction between HIV, HPV and their contribution to the development of invasive CC (3,18,20,46,51) .

Cervical cancer screening

CC screening is the systematic application of a test to identify abnormal cervical lesions in women who have no symptoms. Women between ages 30 and 49 years should be targeted for screening, regardless of their perception of having perfect health that might make them refrain from accepting the screening services offered (69,79). Establishing systems and methods for early detection of precancerous lesions has measurably reduced the morbidity and mortality associated with CC. However, insufficient resources, weak health systems, limited numbers of trained service providers, and competing health priorities have made universal coverage of CC screening difficult to achieve in most resource-poor settings (16,79). Since organized screening has also proven to be cost-effective, it should be promoted in order to make it available to all women (79).

Cervical cytology

The Pap smear test first introduced in 1928 by Dr. George Papanicolaou revolutionized CC prevention services, as it took advantage of the long latency period (10 to 15 years) between the occurrence of SIL and the development of invasive cancer. Pap smear-based CC programmes have had a great impact on the reduction of CC incidence in North America and Europe (39-41). However, it has found to be ineffective in low-resource settings, as it requires an efficient health care system and skilled personnel.

Direct visual inspection

Only 5% of all women in low-income countries undergo cytology-based screening due to aforementioned lack of facilities and heath care professionals (17). The few services that are available are confined to

tertiary facilities in city centres. In addition, delays in reporting cytology results make it unlikely that the women tested will ever receive their results or any treatment and follow-up care. Challenges like these prevent Pap smear-based screening programmes from being effective in most low-income countries (16, 17).

Several studies have demonstrated that direct visual inspection (DVI) with acetic acid (VIA) or Lugol's Iodine (VILI) is a sensitive alternative screening method (16, 31, 33). DVI is cheap and non-invasive, and can be carried out in any health care facility that can perform pelvic examinations. More importantly, it provides instant results, and those needing treatment for precancerous cervical lesions can begin to receive it on the same day in the same facility (17). Although the specificity and sensitivity of DVI has been shown to be acceptable for screening at the population level (16, 31, 33), its test characteristics in a predominantly HIV positive population has yet to be conclusively demonstrated.

Laudable as the recommendation of DVI is as an alternative strategy for CC screening in low-resource settings, the shortage of health care professionals, especially physicians, is a threat to its successful implementation. To overcome this challenge, the strategy of "task shifting" was introduced (80). Increasingly referred to as "task sharing", it is defined as 'delegating tasks to existing or new cadres with either less training or narrowly tailored training for the required service' (80, 81). It is primarily about the rational redistribution of tasks where health care professionals are scare with the goal of making the most efficient use of health care workers in the system. Such a strategy has been used primarily in HIV and CC screening and treatment services in the southern and eastern African regions where it has had excellent outcomes (80, 81).

HPV testing

Currently, HPV cannot be cultured in a reliable manner, and therefore its diagnosis relies on molecular techniques to detect HPV DNA in samples (82). Because there are over 100 HPV genotypes, the detection of HPV

DNA is not sufficient. An ideal test must be able to determine the various genotypes present in a specimen. Until recently, hybrid capture technology was the most widely used method of HPV detection. This technique detects nucleic acid targets directly, using the signal amplification method to provide sensitivity comparable to the target amplification method. The second generation hybrid capture is able to detect the following high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 , 59 and 68. The gold standard for HPV detection is currently the target amplification-based technology of polymerase chain reaction (PCR) (54,82). It is a standard laboratory procedure that can be adapted for both detection and typing of HPV. However, its associated costs and technological requirements often make it inappropriate for routine screening programs in resource-limited settings (43). The sensitivity of HPV DNA testing ranges from 66.1% to 83.9 %. Its false positive rates of 15.5% to 17.1% depend on whether samples are self-collected or clinician-collected (83).

Human Immunodeficiency Virus

HIV infection continues to pose a major public health challenge worldwide. Despite efforts to combat this deadly pandemic and its related comorbidities including CC (84, 85), the need for effective interventions has been since the disease first appeared in the early 1980s.

Epidemiology

According to UNAIDS, more people than ever are living with HIV, largely due to the increased access to treatment. At the end of 2012, an estimated 35.3 million people throughout the world were living with HIV, an increase of 20.1% over 2001 figures. This reflects the large number of new HIV infections and the significant expansion of access to HAART, which has helped reduce AIDS-related deaths (86, 87). The proportion of women living with HIV has remained stable at 50% globally, although more women are affected in sub-Saharan Africa. In addition, the majority (76%)

of HIV- related deaths have occurred in sub-Saharan Africa, where more than two-thirds (68%) of all adults living with HIV reside (86, 87).

Recent advances in HIV research have resulted in effective treatment protocols and a striking decrease in AIDS-related death rates in most countries. The toll in suffering and death in sub-Saharan African, however, remains enormous because of the inability to achieve universal access to treatment. This poor access to services is compounded by the absence of human and physical infrastructure needed to deploy services in rural areas. Coupled with this is a general resistance to undergoing HIV testing or receiving treatment due to the prevalence of stigma and discrimination. As a result, life expectancy in several African countries has decreased significantly, negating gains made over the past few decades (9,78,87).

Due to its large population, Nigeria is among the countries most affected by the HIV epidemic. The HIV prevalence rate among Nigerians aged 15 to 49 years is 3.4% (9, 78, 87). Nigeria has the second highest burden of HIV globally, with an estimated 3.5 million victims of the disease, about 60% of whom are women (9, 78, 87). The HIV epidemic in Nigeria is complex and varies widely by region and risk group. In some areas, it is more concentrated being driven by high-risk behaviours such as anal sex and intravenous drug use, while other regions have more generalized epidemics that are mainly sustained by multiple sexual partnerships (78, 89). Adolescents and youths are particularly vulnerable, with young girls and women at higher risk than young boys and men. The findings of the 2010 Nigerian HIV Integrated Biological and Behavioural survey shows that high risk sexual practices among commercial sex workers and men who have sex with men, intravenous drug use, trafficking of women, and unregulated blood transfusions are major risk factors that contribute to HIV transmission and spread in the country (89).

Since the introduction of ART programmes in Nigeria in 2002, over half a million HIV- infected adults have received treatment, 60% of whom are women (9, 78). Unfortunately, limited information exists on the prevalence, and distribution of HPV and CC among those women

(3,18,20,51). Following a detailed literature search, only one study was identified that evaluated the interaction between HIV infection, immunosuppression and ARV drug use on the burden and distribution of HPV infection in Nigeria (51). That study showed HIV infection associated with increased risk of any HPV, HR HPV and multiple HPV infections. However, the study did not evaluate the effect of HIV- related immunosuppression and ARV drug use on HPV infection.

Interaction between HIV, HPV, and cervical cancer

The link between HIV and CC is direct and deadly. According to Blitz and colleagues (7), women who are co-infected with HIV and HR HPV are 4 to 5 times more susceptible to CC than HIV-negative women infected with HR HPV. This has important implications for HIV programmes, especially in countries with significant HIV epidemics (3, 18, 20). HPV has been shown to be responsible for different malignancies at a variety of anatomical sites, including the uterine cervix (57).

While the body's immune system eliminates most HPV infections over time in individuals with intact immune function, HPV tends to persist in immune compromised individuals. This probably a result of the body's inability to control the expression and replication of HPV in people with compromised immune systems (90, 91). It is, therefore, likely that HIV-infected individuals will be at increased risk of infection and persistence of HPV, compared to HIV negative women. Many studies both in sub Saharan Africa, and globally, have reported higher incidences of HPV infection, precancerous lesions and CC in HIV-positive women as compared to the general population (90-93). In the presence of HIV infection and its associated immune-suppression, the body is unable to eliminate HPV, leading to the persistence of oncogenic HPV genotypes. The persistence of oncogenic HPV infection in cervical cells ultimately results in the development of precancerous lesions and invasive carcinoma. HIV - infected women are also more likely to have a recurrence of precancerous lesions after treatment than are HIV- negative women (94). The increased

incidence of other HPV- associated cancers in HIV- positive women and men confirms the role of an intact immune system in the control of HPV infection and CC (90-93).

Based on the above, it was expected that improved access to HIV treatment would result in the reduction of HPV infection and cervical cancer incidence in regions with a high HIV burden. However, such a change is yet to be seen. Therefore the anticipated positive effect of immune reconstitution on HPV and CC following HIV treatment has been questioned (3, 18, 93, 95). The findings in current literature on the effect of HAART on the incidence of HPV and associated lesions, and cancers are sharply divided (5, 6, 12-15, 94, 96). While some studies have indicated that the incidence of HPV, precancerous lesions and CC have decreased with the use of HAART (13,96), others have shown no association between ARV drug use, HPV infection, and related diseases (5,6,14,15). These studies were conducted outside the West African sub – region, a setting with different HIV types, strains and clades (5, 7, 13-15 94, 96).

Integration of cervical cancer services into HIV programmes

Since CC is a common cause of death among HIV positive women, advocacy for the integration of CC screening into HIV treatment programmes is crucial and has been proven to increase the uptake of screening, early diagnosis, treatment, and reduction in default from follow- up care (97-99). Additionally, such screening reduces HIV-associated discrimination and transportation costs that result from referring HIV positive women to CC screening services outside the HIV treatment clinics (97,98). So far, integrated services is not in practice in most resource-limited settings, including Nigeria, as there is an unsubstantiated fear that its introduction may disrupt the already successful donor-supported HIV programmes (97,98). However, experience from Zambia has shown that low cost CC prevention services

integrated into HIV treatment programmes ensure early detection of CC in high-risk HIV-infected women in a cost effective way. In addition, it provides opportunities for other sexual and reproductive health care and services for women (99,100).

The Nigerian context

The Federal Republic of Nigeria is a country of diverse traditions and cultures, including a variety of wildlife, landscapes, and climates. It is the most populous country in Africa, and has one of the fastest growing populations worldwide. It is a political and economic powerhouse in sub-Saharan Africa. However, its over 167 million inhabitants experience everything from abject poverty and suffering to enormous wealth and comfort. Nigeria's landscape ranges from tropical rainforests in the south to dry savannah lands and desert in the north (Fig. 4). It is a nation of great potential and diversity, much of it untapped and underutilized (101).

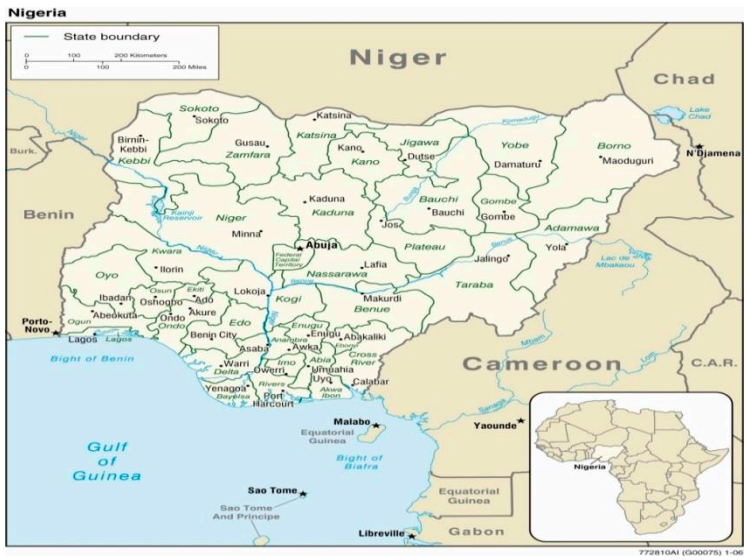


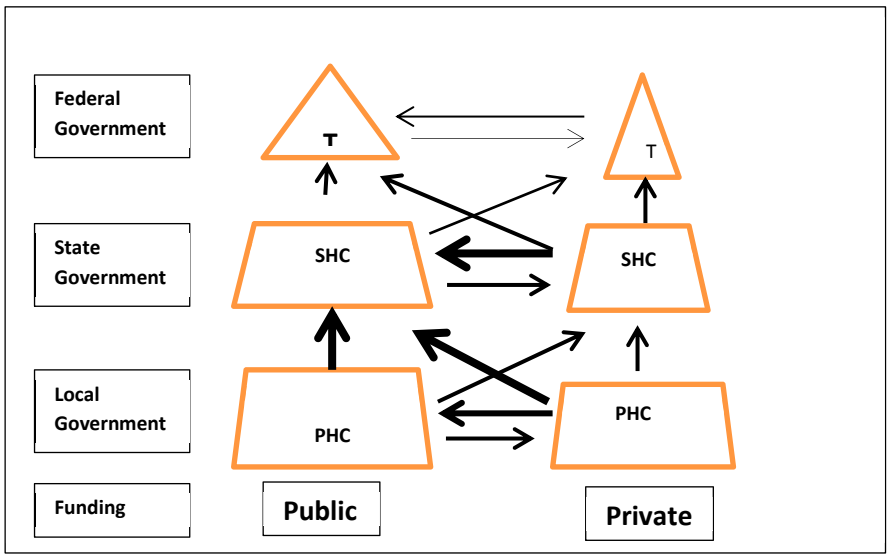
Figure 4. Map of Nigeria (Source: CIA Fact book). Reprinted with permission

Health care delivery system

The provision of health care in Nigeria is a joint function of the federal, state, and local governments. The primary health care system is managed by 774 local government areas (LGAs) authorities, with support from their respective State Ministry of Health and a parallel private health care system. Private health care facilities are operated by the private for profit, faith based and other non-governmental organisations (102). Primary health care operates at the village, district, and community levels. The State Ministry of Health manages the secondary health care system. Private health care providers also operate at this level of care. The state governments also provide primary care services. Tertiary health care service is mainly provided by the federal government at teaching and specialist hospitals throughout the country, with some state governments and a small number of private organisations also providing care at this level (102).

Patients are referred from the primary to the secondary and from the secondary to the tertiary system. Cross referrals occur from the private to the public system and from the public to the private system, particularly during industrial strikes and labour unrest (Fig. 5).

In principle, the Nigerian health system is decentralised into a three tier structure with responsibilities at the federal, state, and local government levels. However because these responsibilities are not clearly delineated, the funding, responsibility and types of care provided is left to the discretion of each tier. With this lack of clarity in roles and responsibility, there is an almost total absence of effective linkages and referrals, which has a significant impact on the quality of care provided and the country's health indicators (Table 3).



Key: PHC: Primary health care; SHC: Secondary health care; THC: Tertiary Health care.

Figure 5. Schematic diagram showing relationships within Nigerian health system (Sizes of boxes and thickness of arrows reflects sizes of each system and volume of referrals)

Table 3. Nigerian Health Indicators (Source: UNICEF 2012).

Indicators	Score
Total population	168,833,800
Under 5 mortality rate	124 per 1,000 live births
Life expectancy at birth	52.1years
Total adult literacy rate	51.1%
Adult HIV prevalence	3.1%
Number of people living with HIV/AIDS	3,400,000

Women living with HIV (% of population living with HIV)	1,700,000(50%)
Total fertility rate	6
Gross national income per capita (US$)	1430
Population below international poverty line	54.4%
Public spending on health as a % of GDP	2
Contraceptive prevalence	17.5%
Skilled attendance at birth	48.7%
Adjusted maternal mortality ratio	630 per 100,000 live births

Status of cervical cancer in Nigeria

CC is the second most common malignancy of women in Nigeria. Its age standardized incidence is estimated at 29.0 per 100,000 (52). While this figure has declined over time in high- and medium-income countries since the introduction of screening programmes, the reverse is the situation in Nigeria, where the incidence of CC has paralleled the HIV epidemic (17). The rise in the CC rate has been linked to chronic immune deficiency due to HIV infection (6,24).

Although CC is the most common female genital cancer and a major cause of mortality among women, (3, 18, 20-23) there has been no nationwide study to determine the incidence and mortality of CC in Nigeria. Available regional and hospital-based studies report incidence rates ranging from 16.7 to 30 per 100,000 women (16, 85,100). Unlike other cancers, CC mostly affects women between 30 and 50 years old, when they are still raising their families and contributing to the national development. The death of such women is a family, community, and national tragedy (16).

Babarinsa, Adewole, and colleagues in Ibadan, Nigeria, reported an increasing trend in CC incidence and death that they attributed to poor awareness, inadequate screening facilities, and lack of an organized national screening programme (85,88).

It is estimated that about 10,000 new cases of CC are diagnosed each year. In 8 out of 10 cases these women present with advanced disease, hence the disproportionately high mortality (16, 17,103). The annual incidence and mortality are expected to rise to 19,000 and 15,000, respectively by 2015 (16). Data from various cancer registries in Nigeria shows that CC has accounted for 10% to 20% of all cancer cases in the past 5 to10 years (103). Despite the burden and mortality associated with CC in Nigeria, screening for this disease is abysmally low at less than 10% (70, 71,104).

CC is also a common malignancy among HIV positive women and is categorised as one of the AIDS-defining conditions (2,4,6,15). In HIV positive women, CC becomes not only a life defining event but a disease that affects their quality of life and survival (2). Hospital- based studies among HIV-positive women in Nigeria showed a high prevalence of SIL ranging from 17.8% to 29.0%, that is, much higher than the 6.9% prevalence found among the HIV negative population (3, 21, 22). Recognizing that cervical cancer is a major cause of morbidity and mortality among HIV-positive women, and that it is a disease that is largely preventable, in 2010 Nigerian HIV treatment guidelines began recommending routine screening for CC among HIV positive women (78). Unfortunately, only 4.0% of all HIV positive women surveyed in south eastern Nigeria were aware of CC screening (89), indicating that a majority of the at-risk population do not benefit from CC screening programmes. To achieve universal coverage for all HIV positive women, the national HIV programme has adopted CC screening as a standard of care and is making significant efforts to integrate it into existing HIV care services (78).

Cervical cancer prevention and control services

In 2010, the Federal Ministry of Health (FMoH) Nigeria, recognizing the contribution of CC to the ill health and death among Nigerian women, launched a National Cervical Cancer Control Policy that provided uniform guidelines for CC prevention and control corresponding to global best practices (16). This policy seeks to ensure that Nigerian women have access to services that will reduce the incidence of invasive cancer and its impact on health and development (16**).**

The National Cervical Cancer Control Programme is managed by a national coordinator at the FMoH. However, because of Nigeria's federal system, the coordinator is only responsible for activities at federal institutions and facilities (101). The 36 states of the federation and the federal capital territory also have coordinators who are responsible for CC control activity at state levels.

The policy recommends VIA or VILI for CC screening at the primary health care and community level. Women who screen positive at this level are to be referred to a secondary facility for confirmation and management. Women presenting directly at a secondary level facility are to be managed using a single- visit approach (16). The management of overt cancer is the responsibility of the tertiary health institutions at the federal and state levels. Pap smear- based screening is available at most teaching hospitals, but in consequence the long waiting time between screening and obtaining results many clients are lost to follow up. Loop Electrosurgical Excision Procedure (LEEP) is available at a very few university teaching and some private tertiary hospitals. For treatment of overt cervical cancer, extended hysterectomy and radiotherapy is only available at some university hospitals that are centers of excellence in medical oncology (16, 107).

Challenges to the implementation of national cervical cancer policy

The national policy, although it appears excellent on paper, is invalid for Nigerian women who are confronted by a weak health system, deficient infrastructure, a poor referral system, and inadequately trained personnel.

Four years after the introduction of the national policy, routine CC control and prevention activities remain almost none-existence. Those services that do exist are often adhoc community outreach programmes organized mainly by NGOs, women groups and academic institutions. Such outreach programmes are often uncoordinated, and either a corporate social responsibility or a research project, rather than a full-fledged national-level programmes. This may partly account for the low screening coverage in Nigeria. At the secondary and tertiary levels, essential diagnostic and treatment options are almost nonexistence. In addition, the referral system is weak and those women who screen positive during the rare screening opportunities available are seldom linked to the few confirmatory and treatment centers (38, 79). The situation is worse for HIV positive women, who are faced by the double challenge of discrimination and a lack of integrated service.

In summary , the overburdened health care system in Nigeria is struggling with challenges that are common to many low -income countries: poor community awareness and lack of knowledge about CC and screening, limited resources and infrastructure, weak referral systems, and the unavailability of HPV vaccines within the national immunization programmes (17, 38).

Rationale of the study

HIV infection continues to be a major public health challenge and a leading cause of death among Nigerian women. Nigeria ranks second among all countries worldwide in the total number of people infected with HIV (9, 16). In addition, CC is a major a cause of death of Nigerian women (16, 17) and is on the rise, paralleling the HIV epidemic (16, 78).

While studies from high-income countries have shown a definite association between HIV on one hand, and CC and its precursor lesions on the other hand (2,10,90,108), studies from sub-Saharan Africa show conflicting findings (4,6,7). Such discrepancies may be attributable to differences in the HIV strains and clades circulating among the population in which the studies were conducted (109).

In Nigeria and other West African countries, CRF02-AG HIV-1 strain represents between 50% to 70% of the circulating HIV strains, which differs distinctly from the strains circulating in other regions (108,110,111). With the recognized differences in transmission, virulence, and pathogenicity of HIV types and strains, it is possible that the outcome of interactions between HIV, HPV and CC in the West African sub-region may differ from reported outcomes in other regions of the world (9, 16,111-113).

It is assumed that the mechanism, through which HIV increases the risk of CC, is related to the immune deficiency resulting from HIV infection (1, 20,108). Chronic immune deficiency results in the inability of the cervical cells to control and clear high-risk HR HPV infections (10, 90). As the use of HAART restores the immune deficiency caused by HIV infection (114), it is expected that its use may alter the course of HPV-related cervical lesions. Despite research studies that have examined the effects of HAART on the course of cervical lesions in HIV positive women, the impact of HAART remain somewhat unclear (7, 10, 11, 13-15). The inconsistency in

findings may be a reflection of the viral diversity as well as the timing and duration of HAART among the various studies (12,115).

In 2010, the Nigerian National Cervical Cancer Control Policy added CC screening using DVI and the immunisation of girls aged 9 to 15 years with an HPV vaccine as additional strategies for the prevention of CC (16). However, these major policy decisions were not based on in-country research and were mainly carried out among women of unknown HIV status (116-122) casting doubt on the effectiveness of the strategy being implemented. In addition, similar to the experience in tuberculosis field, DVI may be less sensitive and specific in severely immuno-compromised compared to immune-competent populations (123).

In our studies we aimed to develop such in-country evidence on the interaction between HPV, HIV, and CC that has hitherto been lacking, but is necessary for developing evidence-based CC control policies for the country.

Research hypothesis

Targeting persons at increased risk of CC after they have been sexually exposed is a key factor for an effective CC prevention and control strategy in high disease-burdened countries because HPV, the causative agent of CC, is sexually transmitted (124). DVI was recommended by WHO and adopted by Nigeria and other countries with a similar high burden of CC as the strategy of choice because of its' ability to detect early precancerous lesions of the cervix (16, 17, 19, 23) and its low technological requirement and its cost effectiveness (17, 19). It has further been adopted for use in screening HIV positive women for precancerous lesions of the cervix without consideration of its sensitivity, specificity, as well as positive and negative predictive values in the presence of immune deficiency caused by HIV infection (16, 78). It is hypothesised that HIV infection and its treatment will impact on the burden of precancerous lesions, the test performance of DVI and the uptake of CC screening services.

Aim

General Objective

To study the effect of HIV infection on the burden of premalignant lesions of the cervix; assess the diagnostic accuracy of direct visual inspection of the cervix; and contribute to policy formulation and the development and implementation of an effective cervical cancer prevention and control programme in Nigeria.

Specific Objectives

1. To assess the effect of HIV infection on the test characteristics of direct visual inspection of the cervix in detecting cytology-diagnosed squamous intraepithelial lesions

2. To determine the types and distribution of HPV and associated factors among HIV positive southwestern Nigerian women

3. To study the prevalence, patterns, and associated factors of cervical dysplasia among HIV positive southwestern Nigerian women

4. To assess the acceptability and predictors of cervical cancer screening among HIV positive south-western Nigeria women

5. To determine the rate of default from follow- up after positive direct visual inspection screening and the predictors of such default among southwestern Nigerian women

Material and Methods

Study settings

Nigerian Institute of Medical Research (NIMR), Yaba Lagos

NIMR is an agency of the Federal Ministry of Health and the foremost medical research institution in the country, with the responsibility for conduct of research into diseases of public health importance. In 2002, when the Government of Nigeria introduce the ART programme, NIMR was one of 25 pioneering centres charged with providing comprehensive HIV care, treatment, and support to the Nigerian population, as well as conducting back-up research for the treatment programme. The majority (65%) of patients at the NIMR HIV treatment centre are from the southwestern Nigerian states of Lagos and Ogun and the rest are from the neighbouring states of Oyo, Edo, Ondo, and Ekiti. A few individuals come from neighbouring West African countries (125). All services at the centre are provided free of charge. In addition, NIMR hosts a CC screening clinic that serves patients of the HIV clinic and the Nigerian public- at- large.

Lagos and Ogun States' Communities

In June 2011, NIMR initiated a community-based cervical cancer screening outreach programme as a corporate social responsibility in two southwestern states: Lagos and Ogun. The two states are contiguous and together they comprise approximately 13 million people, 48.8% of whom are women (126). While three urban communities (Surulere, Ikeja and Gbagada) and four rural ones (Mushin, Iju, Ikorodu and Egbeda belong to Lagos State, the only remaining urban community (Sagamu) and two communities rural (Ifo and Ibafo) belong to Ogun State(Fig.6). The average distance from the aforementioned communities to NIMR ranges from 2 to 70 kilometres. The population in the rural communities is largely of low socioeconomic status and is poorly educated in comparison to the urban population. Within the urban communities, however, pockets of slums

exist in which conditions are similar to or worse than those in the rural areas. The community components of the studies were conducted in these communities.

The studies that resulted in Papers I-III were conducted at the CC screening clinic at NIMR, and 10 communities in Lagos and Ogun states (Fig. 6). Paper IV was a product of study conducted at the HIV treatment centre at NIMR before the integration of CC into the centre's HIV services. Paper V resulted from a feasibility study in the 10 communities between October and December 2011, to determine the appropriateness of conducting the main study in the setting

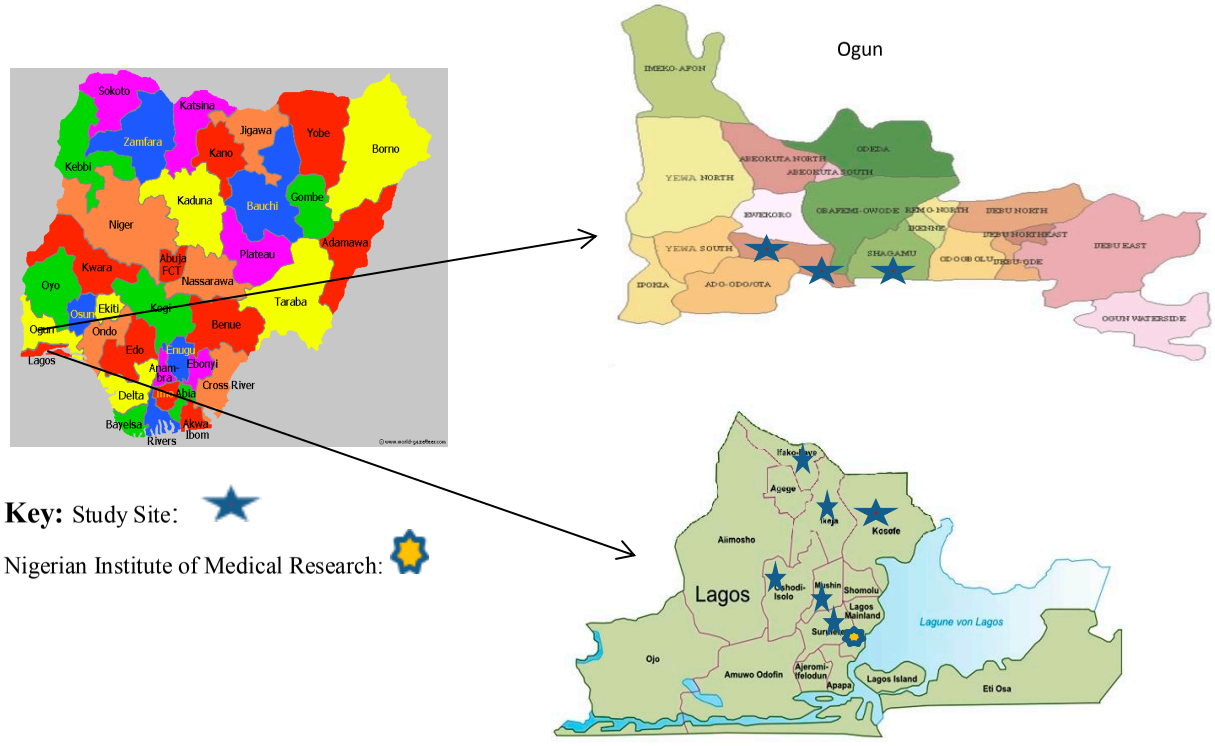


Figure 6. Map of Nigeria showing the states of Lagos and Ogun with location of study site.

Study design

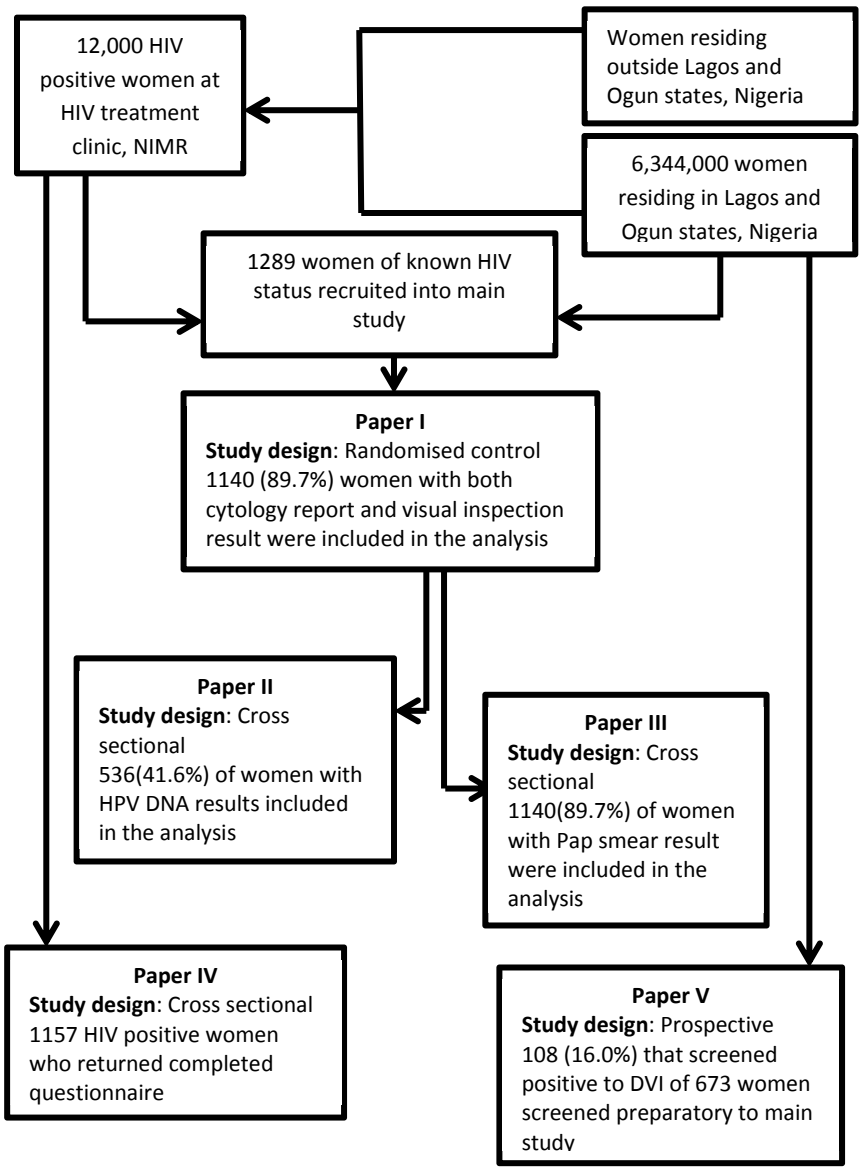


Figure 7. Participant selection and design of studies I – V

Study population

Included in the studies that comprise this thesis were adult women who presented themselves for CC screening at the NIMR clinic or at the venues of the community CC outreach screening programmes between October 2011 and December 2012 (Studies I – III and V). Also included were HIV positive women who presented themselves at the NIMR HIV Treatment Centre between the 1st and 30th of April 2011 for drug refills, physician consultations, or routine laboratory tests (Study IV).

Inclusion and exclusion criteria: Women eligible to participate in the studies were those who were a) ages 18 years and above, b) aware of their HIV status or willing to undergo HIV counseling and testing, c) consented to undergo a pelvic examination and provide cervical smear or swab samples for investigation, d) and agreeable to being interviewed to complete case record forms (Studies I – III and V) and a study questionnaire (IV). They were also required to give a contact address or phone number. Women were excluded if they refused to undergo HIV testing, had overt CC, were unwilling to sign an informed consent form, or had a history of allergy to acetic acid or Lugol's iodine. Also excluded were women who were ill, were more than 20 weeks pregnant, had given birth less than 12 weeks previously, had a history of treatment of cancerous lesions, or had undergone a total hysterectomy.

Study procedure and sample collection

Prior to being screened, the participants in Studies I–III and V were informed about CC screening and its importance, including the required follow-up appointment and all study related procedures. After signing the informed consent form and having provided relevant information, participants were subjected to a thorough pelvic examination. Further, a Pap smear and a sample for microbiological examination (when indicated) were collected, and DVI using either VIA or VILI was conducted. Physicians and midwives, who had received competency-based training prior to the study, performed all the examinations.

The women were placed in a modified lithotomy position, and the cervix was exposed with the help of a disposable Cusco bivalve speculum and examined. Cervical cell scrapings were collected by an Ayres spatula and a cytobrush. The smear was prepared by spreading the specimen uniformly across a pre-labelled glass slide, and the tip of the cytobrush was placed into a transport medium and sent to the molecular biology laboratory for storage at 2º to 8ºC. The cytology smear was immediately fixed using a commercial fixator containing 95% ethyl alcohol. The slides were consequently batched and transported for analysis.
After collecting the cervical smear, the same examiner performed VIA or VILI, depending on a predetermined group allocation by a computer-generated randomization list prepared and held by the main investigator using a free online random number generator (http://randomnumbergenerator.intemodino.com/en/).

VIA procedure and interpretation

After collection of the samples from the Pap smear and microbiological tests, VIA was performed by generously applying freshly prepared 5% acetic acid to the entire cervix with a cotton swab. After 1 minute, the cervix was illuminated with a bright lamp and visually examined ('naked eye' examination). The VIA findings were recorded using the following criteria:

VIA negative:

- No acetowhite lesions
- Acetowhitening on endocervical polyps, nabothian cysts
- Prominent white line like acetowhitening of the squamous junction
- Faint, translucent, ill-defined, irregular acetowhite lesions on the cervix
- Definite, angular, geographic, acetowhite lesions far removed from the squamocolumnar junction

VIA positive:

- Opaque, dense, dull, definite, well-defined acetowhite lesions touching the squamocolumnar junction or close to the external os
- Large, circumferential, well-defined, thick, dense acetowhite lesions
- Acetowhite lesions on clinically visible ulceroproliferative growth of the cervix

VILI procedure and interpretation

After collection of the samples from the Pap and microbiological tests, VILI was performed by generously applying Lugol's iodine to the entire cervix with a cotton swab. The cervix was illuminated with a bright lamp and visually examined. The findings of VILI were recorded using the following criteria:

VILI negative:

- Homogeneous mahogany brown or black staining of the cervix while columnar epithelium does not change colour and remains pale
- Patchy, indistinct, ill-defined, colourless or partially brown areas in the transformation zone
- Scattered, irregular, ill-defined non-iodine uptake areas on the cervix, with or without extension to the vagina
- Thin, yellow, non-iodine uptake areas with angular or digitating margins resembling geographical areas, located far removed from the squamocolumnar junction

VILI positive:

- Well-defined, dense, thick, bright, mustard yellow or saffron yellow non-iodine uptake areas touching the squamocolumnar junction
- Circumferential, well-defined, thick, dense, yellow lesions occupying a large portion of the cervix
- Ulceroproliferative growth of the cervix turning yellow

For Study IV a semi-structured questionnaire containing both closed and open-ended questions was used for data collection. The questionnaire was pre-tested among 25 patients for comprehensibility, appropriateness of language, sensitivity of questions, and average duration of administration. The feedback received after this process was used to modify and finalize the study questionnaire.

Laboratory investigations

HIV test

HIV testing was conducted according to Nigerian national HIV counselling and testing guidelines on all women before enrolling in the study. Diagnosis was based on positive test results using a double enzyme-linked immunosorbent assay algorithm.

Viral load and CD4 cell count test**:** These tests were conducted at the NIMR Human Virology Laboratory. Whole blood from the HIV positive women was used to perform a CD4 assay using the Cyflow Counter and Kits (Partec, Germany) according to the manufacturer's instructions. The viral load assay was performed using the Roche Amplicor HIV-1 monitor test (version 1.5) according to the manufacturer's instruction.

Cytology analysis

The cytology samples were analysed at the Department of Pathology, University of Ibadan, Nigeria, according to the Bethesda system. The cytopathologists who performed the cytological analyses were blinded to the participants' HIV status. A senior pathologist read all tests originally classified as abnormal and 15% of those classified as normal. All slides were pre-coded with the participant's study number before samples were taken. In the event of disagreement between the cytopathologist and senior pathologist report, the slides were sent to another senior pathologist for an independent review. For all such cases, that review constituted the final diagnosis.

DNA extraction and detection

All samples were subjected to centrifugation at 200 rpm for 10 minutes. Precipitated cells were digested using a digestion buffer containing protein kinase. HPV DNA was extracted with phenol-chloroform, re-suspended in 100 µl elution buffer, and stored at −20°C prior to molecular analysis. The quality and integrity of the DNA sample for Polymerase Chain Reaction (PCR) was verified by amplification of a 268 base pair region of the human B-globin gene. Specimens with negative internal control amplification were excluded. All the B-globin positive samples were amplified with HPV4A ACE PC (Inqaba, South Africa). To detect the genotype of the 13 high risk HPV strains, samples were amplified using the specific primers HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

Data management

Sample size calculation

Studies I and III

Sample size was calculated to demonstrate the assumed superiority of VILI to VIA in terms of sensitivity, specificity, positive, and negative predictive value in detecting cervical squamous intraepithelial lesions (CSIL) diagnosed by cytology in women of known HIV status. To this effect a minimum of 530 participants for each arm was sufficient to achieve 80% power at a 5% significance level (one-sided). It was assumed that the proportion of cases correctly detected by VILI would be 90% and that the maximum dropout rate in the study would be 5%. Thus, 580 participants were randomly allocated to each arm.

Study II

Sample size was calculated using the formula: $N = Z\alpha^2 P\ (1\text{-}P)/d^2$, where $Z\alpha$ is the Z statistic for a 95% confidence level, N is the sample size, p is the prevalence of HR HPV: 18.3% and d is the precision (127). Based on this

calculation, the screening of 229 women aged 18 years and above was considered sufficient for identifying women with HR HPV in each arm.

Study IV

The sample size was determined using a Raosoft sample size calculator (http://www.raosoft.com/samplesize.html). Approximately 9,000 women were part of the programme when the sample size was determined. Based on the most conservative response distribution of 50%, and allowing a 2.5% margin of error at a 95% confidence interval, the required sample size was calculated to be 1,313. The sample size was increased by 15% in anticipation of non-responses as in a previous study at the Centre. A final minimum sample size of 1,510 was obtained.

Study V

The sample size was calculated using the formula: $N= Z\alpha 2P (1-P)/d2$, where $Z\alpha$ is the Z statistic for a 95% confidence level, N is the sample size, p is the visual inspection with Lugol's iodine's positive rate, and d is the precision (127). Based on this calculation, following up 72 women aged 18 years and above who tested positive to VILI was considered sufficient to identify defaulters. However, the sample size was increased by 25% in anticipation of non-acceptance to be followed up after testing positive to VILI. A final minimum sample size of 90 was obtained.

Data collection

Studies I – III

Information on socio-demographic, sexual, and reproductive characteristics and on HIV treatment history was collected by trained midwives and physicians using a case record form (CRF) developed by the main investigator. The laboratory information (HIV status, CD4 cell count, HIV plasma viral load, and cytology) were extracted from the participant's laboratory results and entered into the relevant portion of the CRF by trained staff. The information entered was cross-checked with the laboratory results to avoid entry error.

Study IV

A pre-tested semi-structured questionnaire containing both closed and open-ended questions specifically designed for this study by main investigator was used for data collection. Information on socio-demographic characteristics, knowledge about CC and of its screening, previous screening history and personal perception of the risk of developing CC was obtained from respondents. The questionnaire further asked about the willingness of the respondent to accept CC screening if offered. Those who answered in the affirmative were asked to register their names with a counselor as indication of acceptance. The questionnaires were administered in English by research assistants. For those with low literacy, the interview was conducted by research assistants who spoke their local dialect.

Study V

In addition to collecting socio-demographic and reproductive characteristics as for study I – III**,** women who screened positive to VILI or VIA were followed up prospectively. The phone number and home address of these women were collected for tracking purposes. In the case of a missed follow-up appointment (two occasions), the woman is called on the phone to ascertain why the appointment was missed and another appointment scheduled. Those who could not be reached via phone were traced to their homes by female research assistants. The reasons for default from follow-up care were obtained from those who could be tracked. This information was entered into the relevant sections of the study record form.

Study variables

- Age at first intercourse: The age of the participants at first penetrative vaginal intercourse
- Age: Age at the last birthday
- Atypical squamous cell abnormality of undetermined significance (ASC-US): Cellular abnormalities that are more marked than those

attributable to reactive changes but that quantitatively or qualitatively fall short of a definitive diagnosis of LSIL. Not being included as SIL and being distinguished from ASC cannot exclude high grade squamous intraepithelial lesions (ASC-HSIL)

- CD4 cell count: The body immunity level measured as the number of CD4 T lymphocytes per mm3 of blood by Cyflow Counter and Kits
- Cervical cell abnormality (CCA): Any atypical squamous cells, LSIL, HSIL or invasive cancer found by cytology
- Current use of contraceptive: Use of a modern contraceptive method at the time of study.
- Distance from community to clinic: Approximate distance (kilometer) between residence of women and the NIMR clinic
- Default from follow-up: Screening positive and failing to keep rescheduled second appointment or could not be reached
- Duration of ARV drug use: Time elapsed since commencement of ART
- Educational status: Educational level completed
- High grade squamous intraepithelial lesion: Encompassing moderate and severe dysplasia, carcinoma in situ /CIN 2 and CIN 3, with features suspect for invasion
- High risk Human Papilloma Virus: The 13 oncogenic HPV strains of 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59 and 68
- HIV treatment status: Use of ART by the HIV positive women for more than 3 months.
- Lifetime sexual partners: Total number of participants' sexual partners at the time of recruitment
- Low grade squamous intraepithelial lesion: Encompassing HPV/mild dysplasia/CIN 1
- Marital status: Marital status (married, divorced, single or widowed) at the time of study.
- Occupation: The main income generating activity/work engaged in by the women.
- Parity: Number of previous births after 28 weeks of gestation.
- Previous cervical cancer test: Women who had previously tested for cervical cancer irrespective of method of screening.

- Squamous Intraepithelial Lesion: Encompasses all cases of LHSIL and HSIL
- Type of community: Communities were classified into urban and rural depending on government's classification of the community
- Viral load: HIV RNA copies per mL of plasma as measured by Roche Ampliclor HIV-1 monitor test (version 1.5).

Outcome measures

Based on the study-specific objectives the following outcome measures were used:

1. High risk HPV positivity, defined as the presence of any of the 13 oncogenic HPV strains (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68)
2. SIL and HSIL or their equivalent of VIA or VILI positivity
3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)
4. Acceptance of CC screening test by respondents
5. Default from follow-up care after a positive screening by either VIA or VILI

Data analysis

Data analysis was performed using the SPSS statistical package, version 19.0 (SPSS Inc., Chicago, IL). The specific analysis performed for each study is summarised in Table 4. Details of each paper are in the appendix.

Table 4. Summary of type of analysis performed for each study

Paper	Study objectives	Data analysis
I.	To assess the effect of HIV infection on the test characteristics of direct visual inspection of the cervix in detecting cytology-diagnosed	Descriptive and analytic statistics. Test characteristics: Sensitivity, specificity, positive, and negative

	squamous intraepithelial lesions	predictive values
II.	To determine the types and distribution of HPV and associated factors among HIV positive southwestern Nigerian women	Descriptive statistics, bivariate and multivariate logistic regression
III.	To study the prevalence, patterns, and associated factors of cervical dysplasia among HIV positive southwestern Nigerian women	Descriptive statistics, bivariate and multivariate logistic regression
IV.	To assess the acceptability and predictors of cervical cancer screening among HIV positive southwestern Nigeria women	Descriptive statistics, bivariate and multivariate logistic regression
V.	To determine the rate of default from follow- up after positive direct visual inspection screening and the predictors of such default among southwestern Nigerian women	Descriptive statistics, bivariate and multivariate logistic regression

Ethical considerations

Approval for the study was obtained from the Institutional Review Board, Nigerian Institute of Medical Research, Lagos, Nigeria. Written informed consent was obtained from each woman invited to participate in the study after they had been given detailed information about it. Impartial witnesses who were not members of the research team assisted in the process low of securing the consent of those women whose literacy levels were low.

Results

A total of 3,715 women were invited to participate in the studies. Of these, 3,330 (89.6%) were found eligible and participated (Studies I – III: 1140: Study IV: 1,517; Study V: 673). Over sixty per cent (62.9%) of the participants in the study were recruited through the NIMR site. There were no statistically significant differences between the women recruited at NIMR and community sites, except for their HIV status. Only 18.2% of the women recruited from community sites were HIV positive, compared to 89.0% from the NIMR site ($p < 0.001$)

Characteristics of the study participants

The sociodemographic characteristics of the 3330 study participants, by site of recruitment are shown in Table 5. The majority of the participants were Christian (55.4%), married (62.6%) and belonged to the major southern ethnic groups of Yoruba and Igbo (65.5%). Only 15.3% were of northern ethnic extraction. Their ages ranged from 18 to 81 years with a median age of 37 years [IOR: 31–47]. Over two-thirds (70.3%) were between 30 and 49 years, had completed at least a secondary education (79.7%), and had delivered at least one child (79.9 %). More than half lived in a rural community (57.8%). The age at first penetrative vaginal intercourse ranged from 9 to 38 years, with a mean of 20.6 ± 4.2 years. Only 9.3% of the women had their sexual debute before age 15. The total number of lifetime sexual partners among participants ranged from 1 to 10, with a mean of 3.1 ± 2.7. Approximately 70% of the participants reported having had at least 2 sexual partners. The majority of the 3,330 women (2,088, 62.7%) that participated in the study were HIV infected.

Table 5. Sociodemographic characteristics of study participants by site of recruitment

Characteristics (Number in parenthesis)	Number of Participants			*p* value
	Total (%) n = 3,330	NIMR Site (%) n = 2,096	Community Sites (%) n = 1,234	
Age (3,323)				
< 20	33 (1.0)	21 (1.0)	12 (1.0)	
20 – 29	588 (17.7)	357 (17.1)	231 (18.7)	
30 – 39	1269 (38.2)	787 (37.6)	482 (39.1)	0.18
40 – 49	1067 (32.1)	702 (33.6)	365 (29.6)	
≥ 50	366 (11.0)	222 (10.6)	144 (11.7)	
Parity(3,323)				
Nulliparous	668 (20.1)	398 (19.1)	270 (21.9)	
Para 1 – 4	2,172 (65.4)	1,389 (66.5)	783 (63.5)	0.12
≥ Para 5	483 (14.5)	302 (14.5)	181 (14.7)	
Religion (3,330)				
Christianity	1,846 (55.4)	1,146 (54.9)	700 (56.6)	
Islam	1,454 (43.7)	922 (44.2)	532 (42.5)	0.73
Others	30 (0.9)	19 (0.9)	11 (0.9)	
Marital status (3,304)				
Unmarried	952 (28.8)	605 (29.1)	347 (28.2)	
Married	2,068 (62.6)	1,304 (62.8)	764 (62.3)	0.39
Widowed	284 (8.6)	168 (8.1)	116 (9.5)	
Ethnic group (3,260)				
Major southern	2,136 (65.5)	1,336 (65.2)	800 (66.1)	
Northern Tribes	498 (15.3)	311 (15.2)	197 (15.4)	
Southern minority	603 (18.5)	383 (18.7)	220 (18.2)	0.21
Others	23 (0.7)	19 (0.9)	4 (0.3)	
Educational status (3,297)				
Less than secondary	669 (20.3)	435 (21.0)	234 (19.1)	
Secondary and above	2,628 (79.7)	1,638 (79.0)	990 (80.9)	0.20
Employment status (3,325)				
Unemployed	516 (15.5)	335 (16.0)	181 (14.7)	
Employed	2,809 (84.5)	1,755 (84.0)	1,054 (85.3)	0.29
Area of residence (3,329)				
Urban	1,405 (42.2)	897 (42.9)	508 (41.1)	

Rural	1,924 (57.8)	1,196 (57.1)	728 (58.9)	0.32
Age first intercourse (3,069)				
< 15	255 (8.3)	174 (9.0)	81 (7.1)	
≥ 15	2,814 (91.7)	1,755 (91.0)	1,059 (92.9)	0.07
Total life time sexual partners (3,081)				
1	875 (28.4)	553 (28.5)	322 (28.1)	
2 and above	2,206 (71.6)	1,384 (71.5)	822 (71.9)	0.81
HIV status (3,330)				
Positive	2,088 (62.7)	1,863 (89.0)	225 (18.2)	
Negative	1,242 (37.3)	230 (11.0)	1,012 (81.8)	<0.001

HIV-related characteristics of the 2,088 HIV positive participants

HIV-related characteristics of the 2,088 HIV positive participants in the studies are shown in Table 6 below. Their CD4 cell count ranged from 10 to 1663 cells/mm^3, with a median of 504 [IQR: 336–691]. The majority (85.0%) of the women had CD4 cells counts above 200 cells/mm^3. Plasma HIV viral load levels ranged from undetectable (< 20 copies) to 677,693 copies/ml, with a median of 200 [IQR: 200–2293]. Over 60% of the women had viral loads of less than 1000 copies/ml. While the majority (67.9%) had been on ART for periods ranging from 3 months to 9 years at the time of recruitment, the remaining 32.1% were not.

Table 6. HIV-related characteristics of 2,088 HIV-infected participants in Studies I – V

Characteristics (Number in parenthesis)	Number of participants (%)
Duration of HIV disease (2,088)	
< 13 months	618 (29.6)
13 – 36 months	505 (24.2)
> 36 months	965 (46.2)
Antiretroviral drug use(2,088)	

On drugs	1,418 (67.9)
Not on drugs	670 (32.1)
CD4 cell Count (2,058)	
< 200	309 (15.0)
≥ 200	1,749 (85.0)
Viral load (2,018)	
< 1000	1,214 (60.2)
1000–9999	287 (14.2)
≥ 10,000	517 (25.6)

Study I

Test performance of direct visual inspection of cervix in the presence of HIV infection

The test performance of VIA and VILI was compared using two threshold cut off points (low threshold: SIL; high threshold: HSIL) of cytology-diagnosed cervical cell abnormality. The result of the test performance is shown in Table 7.

Using a low threshold reference standard, the test performance of VIA was similar to that of VILI except for VIA's significantly higher PPV of 91.5% compared to 77.7% for VILI (p = 0.001) when the HIV status of the respondents was not taken into consideration. Repeating the same analysis while considering HIV status showed that VILI performance among HIV positive women was significantly ($p < 0.05$) inferior across all 4 test characteristics, as compared to VIA. The sensitivity, specificity, PPV, and NPV of VILI was 77.2%, 71.2%, 52.4%, and 88.4%, respectively compared to the sensitivity (81.9%), specificity (93.1%), PPV (86.4%), and NPV (97.1%) of VIA. Among HIV negative women, VIA and VILI performance was similar except for PPV, where the VIA score of 89.3% was statistically higher than the 63.4% for VILI (p = 0.0001).

Analysis using a high threshold reference cut-off for cytology-diagnosed HSIL and masked HIV status, VIA and VILI performed similarly except for PPV, where the VIA score of 87.8% was significantly higher than the 22.0% score for VILI (p = 0.000). Comparing the test characteristics of VIA and VILI in HIV positive participants only, VIA performed better in sensitivity (90.3% vs. 82.4%), specificity (93.1% vs. 71.3%) and PPV (75.3% vs. 19.9%) as compared to VILI. VILI's higher NPV score of 97.8%, compared to VIA's score of 94.5% (p = 0.68), was not statistically significant. The performance of the two tests was similar in HIV negative women, except for the PPV score, in which the VIA score of 85.9% was significantly higher than VILI's score of 61.5% (p = 0.000).

Further analysis to determine the effect of HIV immune deficiency on VIA and VILI test characteristics showed that VIA performed better in all four test characteristics among HIV positive women with CD4 cell counts below 200 cells/mm^3. VILI performance was 70% or less across all test characteristics (sensitivity 70.0%, specificity 66.9%, PPV 46.7%, and NPV 50.0%) in comparison to the test characteristics of VIA, which were all above 70% (sensitivity 71.3%, specificity 88.2%, PPV 83.3%, and NPV 88.2%). Among the HIV positive cohort with a CD4 cell count above 200 cells/mm^3, though the VILI test scores were above 70% except for positive predictive value of 57.5%, VIA tests scores were consistently superior (p < 0.05) (Table 7).

Table 7. Test performance of Visual Inspection with acetic acid and with Lugol's iodine in detecting cervical squamous intraepithelial lesions diagnosed by cytology.

Test performance	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (%)	Negative Predictive Value (%)
SIL cut-off point				
• ALL				
○ VIA	82.7 (80.9 – 89.3)	99.2 (93.1 – 100.0)	91.5 (86 – 3-97.2)	98.1 (93.2 – 100.0)
○ VILI	87.5 (70.3 – 89.9)	99.2 (89.1 – 99.4)	77.7 (72.1 – 85.8)	99.6 (93.5 – 100.0)
• HIV Positive s only				
○ VIA	81.9 (79.3 – 96.1)	93.1 (90.3 – 100.0)	86.4 (79.5 – 91.3)	97.1 (91.9 – 100.0)
○ VILI	77.2 (64.9 – 84.2)	71.2 (63.9 – 87.4)	52.4 (48.9 – 58.3)	88.4 (81.2 – 95.5)
• HIV negatives only				
○ VIA	90.0 (86.7 – 98.9)	97.2 (86.7 – 100)	89.3 (82.3 – 96.3)	99.6 (95.3 – 100.0)
○ VILI	86.7 (84.3 – 94.1)	94.0 (81.4 – 98.6)	63.4 (55.9 – 76.1)	98.3 (95.1 – 100.0)
HSIL cut-off point				
• ALL				
○ VIA	93.3 (89.2 – 98.9)	99.2 (89.8 – 100.0)	87.8 (73.4 – 93.7)	97.8 (90.9 – 100.0)
○ VILI	92.9 (83.4 – 98.3)	89.6 (81.49 – 1.9)	22.0 (20.9 – 23.4)	99.7 (96.3 – 100.0)
• HIV Positives only				
○ VIA	90.3 (83.2 – 99.3)	93.1 (87.1 – 100)	85.3 (73.6 – 87.3)	94.5 (91.6 – 97.6)
○ VILI	82.4 (71.9 – 93.2)	71.3 (65.3 – 79.9)	19.9 (17.7 – 22.1)	97.8 (93.5 – 99.8)
• HIV negatives only				
○ VIA	96.0 (95.8 – 100.0)	99.7 (87.5 – 100.0)	85.7 (81.7 – 90.7)	98.9 (95.2 – 100.0)
○ VILI	80.0 (72.5 – 92.3)	80.0 (67.8 – 94.9)	61.5 (55.3 – 67.8)	99.1 (95.6 – 100.0)
• CD4 cell count < 200*				
○ VIA	71.3 (61.2 – 85.1)	88.2 (76.3 – 93.1)	83.3 (79.3 – 90.1)	88.2 (82.4 – 95.1)
○ VILI	70.0 (68.8 – 88.3)	66.9 (57.8 – 74.9)	46.7 (41.9 – 50.3)	50.0 (44.8 – 54.6)
• CD4 cells count ≥ 200*				
○ VIA	93.8 (84.5 – 99.1)	98.5 (86.9 – 100.0)	93.4 (89.5 – 98.7)	99.5 (96.3 – 100.0)
○ VILI	87.5 (75.9 – 94.5)	73.4 (65.1 – 83.9)	57.5 (53.4 – 61.9)	98.9 (94.9 – 100.0)

SIL: Squamous Intraepithelial Lesion; HSIL: High Grade Squamous Intraepithelial Lesion *Data only for HIV positive participants

Study II

Effect of HIV infection and antiretroviral drugs on the burden of high risk HPV.

One hundred and one out of 515 women (19.6%) studied were found to be infected with at least one HR HPV type; 28 (5.4%) had multiple HPV infections ranging from 2 to 5 types (median: 3). As shown in Fig. 8, the HPV types most frequently identified were HPV 16 (3.9%), HPV 35 (3.5%), HPV 58 (3.5%), HPV 31 (3.3%), HPV 18 (2.3%), HPV 52 (2.3%), and HPV 51 (1.9%). The vaccine preventable HR HPV genotypes 16 and 18 infections were detected in 32 (31.7%) of the 101 women found to be infected with HR HPV.

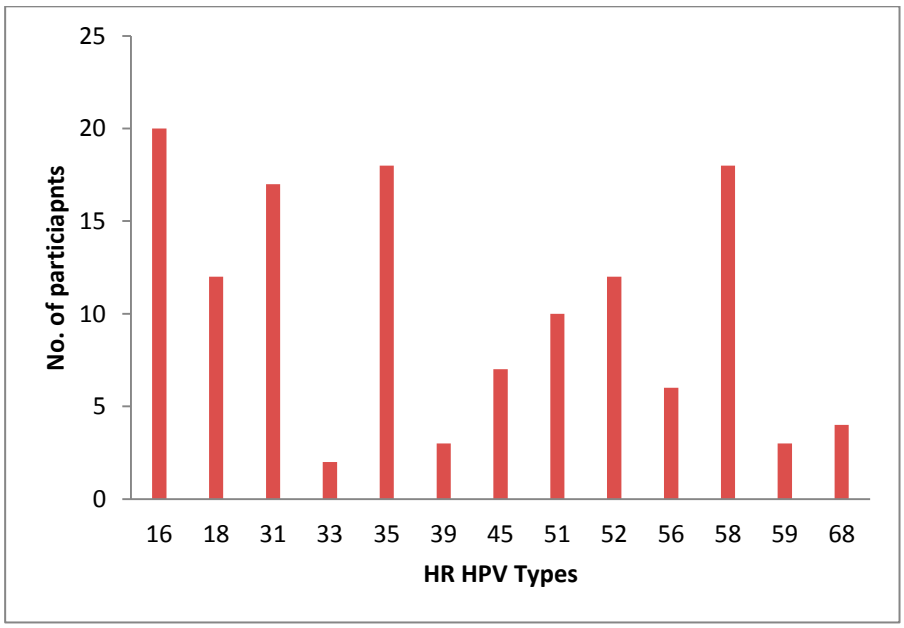


Figure 8. Distribution of high risk Human papillomavirus types in 515 cervical samples

Table 8 shows the distribution of high risk HPV types by HIV status, Odds ratios and 95% confidence interval(95% CI), after adjustment for age, ART status, and lifetime sexual partner. The prevalence of HR HPV among HIV positive women (24.5%) was significantly higher than the prevalence of 15.9% in HIV negatives (OR = 1.7; 95% CI: 1.1 – 2.7). The multiple infection rate of 8.2% among HIV positive women was higher than the 3.9% in HIV negative women (OR = 2.6; 95% CI: 1.3 – 5.3). A similar trend was observed for HPV 16 (OR = 3.1; 95% CI: 1.3 – 6.3) and HPV 35 infections (OR = 3.6; 95% CI: 1.4 – 6.9), but not in other 11 HR HPV types studied. There were no differences (p = 0.54) in the prevalence of HPV 16 and 18 between the HIV positive (31.5%) and HIV negative women (31.9%).

The association between HIV related variables and HR HPV infection is shown in Table 9. After controlling for confounders of age, HIV status, ARV drug use, type of community, number of lifetime sexual partners and marital status in a three model multivariate logistic regression analysis, HIV positive status, degree of immune status and ARV drug use retained an independent association with HR HPV infection. Compared to HIV negatives, HIV positive women were found to have almost twice the risk of HR HPV (OR: 1.7; 95% CI: 1.4 – 2.2). HIV positive women with severe immune deficiency (CD4 < 200 cells/mm3) were found to be at an increased risk of HR HPV infection compared to those with a CD4 cell count above 200 cells/mm3 (OR: 2.4; 95% CI: (1.7 – 5.9). HIV positive women with a CD4 cell count above 500 cells/mm3 were found to be at reduced risk for HR HPV compared to those with a CD4 count below 500 cells/mm3 (OR: 0.7; 95% CI: 0.5 – 0.8). Among HIV positive women, the use of ARV drugs was found to lower the risk of HR HPV infection two fold (OR: 0.4; 95% CI: 0.3 – 0.5).

Table 8. Distribution of high risk Human Papillomavirus (HR HPV) genotypes among participants by HIV status

HR HPV status	All patients n = 515 (%)	HIV positive n = 220 (%)	HIV negative n = 295 (%)	*P* value	Odds ratio (95% CI)
HR HPV negative	414 (80.4)	166 (75.4)	248 (84.1)		1(ref)
HR HPV positive	101 (19.6)	54 (24.6)	47 (15.9)	0.01	1.9 (1.2 – 2.9)
HPV 16	20 (3.9)	14 (5.4)	6 (2.6)	0.02	3.3 (1.1 – 9.7)
HPV 18	12 (2.3)	3 (1.4)	9 (3.10	0.3	0.4 (0.1 – 1.8)
HPV 31	17 (3.3)	12 (5.5)	5 (1.7)	0.03	3.1 (1.1 – 11.1)
HPV 33	2(0.4)	1 (0.5)	1 (0.3)	0.9	1.3 (0.0 – 49.3
HPV 35	18 (3.5)	13 (5.9)	5 (1.7)	0.02	3.4 (1.2 – 11.9)
HPV 39	3 (0.6)	1 (0.5)	2 (0.7)	0.6	0.7 (0.1 – 9.4)
HPV 45	7 (1.4)	3 (1.4)	4 (1.4)	0.6	1.0 (0.2 – 5.4)
HPV 51	10 (1.9)	5 (2.3)	5 (1.7)	0.9	1.4 (0.3 – 5.4
HPV 52	12 (2.3)	6 (2.7)	6 (2.0)	0.8	1.4 (0.4 – 4.8)
HPV 56	6 (1.2)	2 (0.9)	4 (1.4)	0.5	0.7 (0.1 – 4.3)
HPV 58	18 (3.5)	9 (4.1)	9 (3.5)	0.6	1.4 (0.5 – 3.9)
HPV 59	3 (0.6)	1 (0.5)	2 (0.7)	0.6	0.7 (0.02 – 9.4)
HPV 68	4 (0.8)	1 (0.5)	3 (1.0)	0.4	0.4 (0.02 – 4.8)
Multiple infection	28 (5.4)	18 (8.2)	10 (3.9)	0.03	2.5 (1.1 – 6.1)

C.I : Confidence Interval

Table 9. HIV related-variables independently associated with high risk Human papillomavirus infection after adjustment for potential confounders in a multivariate logistic regression analysis

HIV related Variables (Number in parenthesis)	Initial model[α] OR (95% CI)	2nd model[ȝ] OR (95% CI)	Final model[ɗ] OR (95% CI)
HIV status (515)			
Negative	1 (ref)	1 (ref)	1 (ref)
Positive	1.7 (1.3 – 2.5)	1.7 (1.3 – 2.5)	1.7 (1.4 – 2.2)
Antiretroviral drug use (220)			
Not ARV drugs	1 (ref)	1 (ref)	1 (ref)
On ARV drugs	0.4 (0.3 – 0.9)	0.4 (0.3 – 0.9)	0.4 (0.3 – 0.5)
CD4 cell count (220)			
< 200	2.9 (1.3 – 7.1)	2.5 (1.5 – 6.1)	2.4 (1.7 – 5.9)
200 – 499	1 (ref)	1 (ref)	1 (ref)
≥ 500	0.6 (0.3 – 1.0)	0.6 (0.3 – 0.9)	0.7 (0.5 – 0.8)
Viral load (220)			
<1000	0.5 (0.2 – 1.9)	0.6 (0.4 – 1.3)	0.6 (0.3 – 1.4)
1000-9999	1 (ref)	1 (ref)	1 (ref)
≥ 10,000	3.1 (1.1 – 9.9)	3.1 (0.9 – 8.7	2.7 (0.6 – 8.1

α: adjusted for age; ȝ: adjust for HIV status and antiretroviral drug use; ɗ: in addition to a and c adjusted for type of community, life time sexual partner and marital status.

Study III

Effect of severe immune deficiency of HIV, and antiretroviral drugs use on cervical cell abnormalities.

The distribution of cervical cell abnormalities by HIV status among the women in our study is shown in Table 10. The prevalence of SIL was 8.5%, with a higher prevalence of 14.3% in HIV positive compared to 3.3% in HIV negative women. The prevalence of specific cervical abnormalities were consistently higher in HIV positive women compared to their HIV negative counterparts. The increased prevalence of cervical abnormalities in HIV positive women were retained after controlling for potential confounders of age, marital status, age at first intercourse, and number of lifetime sexual partners.

Table 11 shows the association between HIV infection, ART and premalignant lesions of the cervix. Of the 96 abnormal smears with SIL, the majority were found among HIV positive women (79.2%), as against 20.8% among HIV negative women. The increased risk of SIL observed among HIV positive women persisted after controlling for potential confounders in three model multivariate logistic regressions (aOR:5.4; 95% CI:2.9 – 8.8). HIV positive women also had a seven times higher risk of acquiring HSIL compared to HIV negative women (aOR:5.7; 95% CI:2.4 – 10.1)**.** HIV positive women with a CD4 cell count of less than 200 cells/mm^3 were found to be at increased risk of squamous intraepithelial abnormality. The increased risk observed was retained after adjustment for potential confounders in the three models, i.e., SIL (aOR: 1.9; 95% CI: 1.1 – 5.9) and HSIL (aOR: 5.7; 95% CI: 1.1-7.2), compared to those with a CD4 cell count above 200 cells/mm^3. HIV positive women who were not on ART were found to be at increased risk of SIL (aOR: 2.1; 95% CI: 1.4 – 3.5) and HSIL (aOR: 2.6; 95% CI: 1.1 – 6.4), compared to those on ART. High HIV viral load lost independent association with both SIL (aOR: 1.9; 95% CI: 0.8 – 3.7) and HSIL (aOR: 2.7; 95% CI: 0.7 – 5.7) after adjustment was made in the final model for ART use (see table 11).

Table 10. Distribution of cervical cell abnormalities by HIV status among the 1,140 participants

		Cervical epithelial cell abnormality					
		Negative (%)	Positive (%)	Type of cervical cell abnormality			
				ASCUS (%)	SIL (%)	LSIL (%)	HSIL (+ IC) (%)
All women (n = 1,140)		920 (80.7)	220 (19.3)	121 (10.7)	96 (8.5)	67 (5.9)	31 (2.7)
HIV status	Negative	551 (59.9)	50 (22.7)	30 (24.8)	20 (20.8)	14 (20.9)	6 (19.4)
	Positive	369 (40.1)	170 (77.3)	91 (75.2)	76 (79.2)	53 (79.1)	25 (80.6)
Odds ratio (95% CI)	Crude	1 (ref)	5.2 (3.6-7.4)	4.0 (2.5-6.2)	4.9 (2.9 -8.4)	5.8 (3.1-11.1)	5.8 (2.2-16.4)
	Adjusted*	1 (ref)	5.3 (3.3-6.9)	3.9 (2.2-6.7)	4.9 (2.7-7.1)	5.8 (3.4-9.6)	5.9 (3.3-10.3

ASCUS: Atypical squamous cell abnormality of undetermined significance; SIL: Squamous intraepithelial lesion; LSIL: Low grade squamous intraepithelial Lesion; HSIL: High grade squamous intraepithelial Lesion; IC: Invasive carcinoma

*Adjusted for age, marital status, age at first intercourse, and number of lifetime sexual partners

Table 11. Multivariate logistic regression analysis of association between HIV-related variables and both squamous intraepithelial lesions (SIL) and high grade squamous intraepithelial lesions (HSIL)

Exposure variables and confounders	Crude ratio OR (95% CI)		Model 1 OR (95% CI)		Model 2 OR (95% CI)		Model 3 OR (95% CI)	
	SIL	**HSIL**	**SIL**	**HSIL**	**SIL**	**HSIL**	**SIL**	**HSIL**
HIV positive status	5.8(3.3-10.0)	6.1(2.1-18.7)	5.6(3.2- 9.3)	6.0 (2.0-16.3)	5.4(3.2- 9.1)	5.8 (2.6-12.3)	5.5(3.3-9.9)	5.6(3.1-11.7)
Age[a] and marital status[b]	-	-	5.2(3.1-9.1)	5.6 (2.6-11.3)	5.1(3.0-8.9)	5.1 (2.2-10.5)	5.1(3.4-8.1)	5.1 (2.2-10.5)
Age at first intercourse[c]	-	-	-	-	4.7(1.9-9.5)	5.7 (2.1-9.1)	4.8(2.1-9.3)	5.7 (2.1-9.9)
Life time sexual partner[d]	-	-	-	-	-	-	5.4(2.9-8.8)	5.7 (2.4-10.1)
Antiretroviral drug use*	2.0(1.3 - 3.7)	2.7(1.1 - 6.6)	1.9(1.0-3.3)	2.7(1.2-6.8)	2.0(1.0-3.7)	2.5(0.8-6.7)	2.0(1.2-3.5)	2.6(1.0-6.7)
Age and marital status	-	-	2.0(1.2-3.5)	2.6(1.3-6.7)	2.0(1.3-3.6)	2.7(0.8-6.8)	2.0(1.1-3.3)	2.6(1.0-6.9)
Age at first intercourse	-	-	-	-	1.8(1.2-3.9)	2.7(0.9-6.9)	2.1(1.4-3.4)	2.7(1.1-6.3)
Life time sexual partner	-	-	-	-	-	-	2.1(1.4-3.5)	2.6(1.1-6.4)
CD4 cell count < 200*	3.2(1.5- 6.6)	4.0(1.2-12.6)	3.5(2.1-7.5)	4.1(3.5-11.6)	3.7(2.0-7.1)	4.6(3.2-10.1)	2.0(1.0-6.1)	4.9(3.0-12.1)
Age at first intercourse	-	-	3.3(2.0-6.5)	4.2(2.8-10.9)	3.1(1.9-9.3)	4.7(0.9-11.1)	2.7.1(1.7-9.1)	5.1(3.4-10.1)
Life time sexual partner	-	-	-	-	3.0(0.9-10.1)	4.2(0.9-9.4)	2.8.1(0.9-9.2)	5.0(3.1-11.3)
Antiretroviral drug use[e]	-	-	-	-	-	-	1.9(1.1-5.9)	5.7(1.1-7.2)
Viral load > 1000 copies/ml*	2.0(1.2-3.4)	2.8(1.1-6.8)	2.0(1.0-3.2)	2.8(1.1-6.6)	1.8(1.1-3.1)	2.8(1.2-6.9)	1.9(1.1-3.6)	2.8(1.1-4.0)
Age at first intercourse	-	-	1.9(1.1-3.1)	2.8(1.1-6.8)	1.9(1.0-3.4)	2.7(1.0-6.6)	1.8(0.9-3.4)	2.6(1.0-4.5)
Life time sexual partner	-	-	-	-	1.9(1.0-3.5)	2.7(1.2-6.4)	1.9(1.1-3.6)	2.7(1.0-5.3)
Antiretroviral drug use	-	-	-	-	-	-	1.9(0.8-3.7)	2.7(0.7-5.7)

[a]Age groups: 18–35 and ≥ 35 years (ref); [b]Marital status: Married and unmarried (ref); [c]Age at first intercourse: < 20 (ref) and ≥ 20 years ; [d]Lifetime sexual partner: 1 (ref) and ≥ 2 ; [e]Antiretroviral drug use: Yes (ref) and No

* HIV positive women only

Study IV

Acceptability of cervical cancer screening among HIV positive women in southwestern Nigerian

A total of 1,517 women were interviewed for this study. Of which 853 (56.2%) were aware of CC and 523 (34.5%) were aware of CC screening. However, a greater proportion of these women (1,210; 79.8%) were willing to undergo CC screening. Only 143 women (9.4%) reported having previously had a screening for CC. The common reasons for refusing CC screening among the 307 women (20.2%) who were not willing to screen were the anticipated cost of the screening (35.2%), religious convictions (14.0%), needing to obtain their partner's approval (12.4%), and long waiting times in clinics (12.7%).

Table 12 summarizes the results of the multivariate logistic regression analysis. After controlling for potential confounding variables (see Paper IV), HIV positive women who had had at least a secondary education were 1.4 times more likely to accept screening compared to those with less than a secondary education (OR = 1.4; 95% CI:1.03 – 1.84). Also having no living child (OR: 1.5; 95% CI: 1.1 – 2.0), newly diagnosed HIV (OR: 1.5; 95% CI: 1.1-2.0) and those aware of cervical cancer screening tests (OR: 1.5; 95% CI: 1.2 – 2.0) were more likely to accept screening than their counterparts. Religion, ethnicity, marital status, and having previously had CC screening experience were not found to be associated with cervical screening test acceptance.

Table 12: Associations between acceptances of cervical cancer screening, sociodemographic status of the respondents, cervical cancer awareness, self-risk assessment and history of previous cervical cancer screening

Variables	Accepted cervical cancer screening		95% CI Crude OR	95% CI Adjusted OR
	Yes	No		
Age (years)				
< 25	54	12	0.89 (0.46 – 1.71)	0.87 (0.46 – 1.68)
25-34	572	149	1.0 (Ref)	1.0 (Ref)
≥ 35	584	146	1.04 (0.81 – 1.35)	1.04 (0.80 – 1.34)
Tribal Group				
Yoruba	414	109	1.01 (0.84 – 1.48)	1.10 (0.49 – 2.48)
Igbo	505	127	1.0 (Ref)	1.0 (Ref)
Northern tribes	113	23	0.97 (0.66 – 1.40)	0.97 (0.67 – 1.42)
Southern minority tribes	176	46	0.71 (0.36 – 1.40)	0.78 (0.29 – 2.08)
Religion				
Christianity	676	173	1.0 (Ref)	1.0 (Ref)
Islam	532	132	0.99 (0.77 – 1.27)	0.93 (0.68 – 1.28)
Educational level completed				
< Secondary	206	54	1.3 (0.87 – 1.87)	1.2 (0.83 – 1.80)
Secondary	605	171	1.0 (Ref)	1.0 (Ref)
>Secondary	399	82	1.4 (1.03 – 1.84)	1.3 (1.02 – 1.83)[a]
Marital status				
Married	725	198	1.0 (Ref)	1.0 (Ref)
Not married	485	109	0.82 (0.63 – 1.07)	0.90 (0.68 – 1.18)
Living children				
Yes	875	243	0.69 (0.51 – 0.93)	0.72 (0.53 – 097)[b]
No	335	64	1.0 (Ref)	1.0 (Ref)
Duration of HIV disease				
< 13 months	318	99	1.49 (1.11 – 2.02)	1.5 (1.1 – 1.98)[c]
13-36months	331	91	1.0 (Ref)	1.0 (Ref)
≥ 13months	561	117	1.32 (0.97 – 1.79)	1.31 (0.97 – 1.78)
Aware of cervical cancer(CC)				
Yes	706	147	1.53 (1.19 – 1.96)	1.49 (1.15 – 1.95)[d]
No	504	160	1.0 (Ref)	1.0 (Ref)
Ever tested for CC				
Yes	118	25	1.22 (0.78 – 1.91)	1.23 (0.71 – 1.22)
No	1092	282	1.0 (Ref)	1.0 (Ref)
Self-risk assessment of CC				
High	381	102	0.92 (0.71 – 1.21)	0.93 (0.71 – 1.22)
Low	829	205	1.0 (Ref)	1.0(Ref)

[a]adjusted for age, duration of HIV and awareness of cervical cancer; [b]adjusted for age, marital status and duration of HIV; [c]adjusted for age, educational status, duration of HIV and awareness of cervical cancer; [d]adjusted for age, educational status and time elapsed since HIV diagnosis.

Study V

Outcome of community cervical cancer screening programme

One hundred and eight out of 673 women (16.1%) who screened positive at direct visual inspection during a community CC outreach screening programme were prospectively followed to determine the default rate, reasons for default, and predictors of default. Fifty-one women out of the 108 (47.2%) who screening positive failed to keep their scheduled follow-up appointment. Fifteen defaulters (29.4%) were completely lost to follow up as both their phone numbers and home addresses were incorrect and therefore could not be traced.

The reasons for default among the 36 women who could be traced were transportation expense and treatment cost (48.6%), time constraints (25.7%), 'I believe nothing is wrong with me' (11.4%), 'The clinic is too far from my house' (8.6%), and 'I am sick' (5.7%).

Table 13 summarizes the variables associated with default in a multivariate logistic regression analysis after controlling for confounders of educational level, work status, type of community, and current contraceptive use. Women who had less than a secondary education (OR = 3.1, 95% CI: 2.0 – 5.2), who lived more than 10 km from the clinic (OR: 2.0, 95% CI: 1.0 – 4.1), and had no previous CC screening experience (OR: 3.5, 95% CI: 3.1 – 8.4) were found to be at increased risk of default.

Table 13. Association between default from follow-up care after screening positive for precancerous lesion of the cervix and sociodemographic and reproductive characteristics of the women

Characteristics	Defaulter (%) n= 51	Non-defaulter (%) n= 57	Crude OR (95% CI)	Adjusted OR(95% CI)
Age (years)				
<40	25 (49.0)	20 (35.1)	1.8(0. 8– 4.2)	1.2 (0.8 – 6.9)
≥40	26 (51.0)	37 (64.9)	1 (ref)	1 (ref)
Parity				
≤2	15 (29.4)	18 (31.6)	2.0 (0.8 – 5.6)	1.1 (0.5 – 8.1)
>2	33 (70.6)	35 (68.4)	1 (ref)	1 (ref)
Education status				
< Secondary	19 (37.5)	10 (17.5)	2.9(1.1 – 7.7)	3.1 (2.0 – 5.2)[a]
≥ Secondary	31 (62.7)	47 (82.5)	1 (ref)	1 (ref)
Marital Status				
Not married	14 (27.5)	9 (15.8)	2.0 (0.7 – 5.6)	1.3 (0.8 – 6.9)
Married	37 (72.5)	47 (84.2)	1 (ref)	1 (ref)
Work status				
Not working	27 (52.9)	19 (33.3)	2.3 (1.0 – 5.4)	1.2 (0.8 – 8.1)
Working	23 (47.1)	37 (66.7)	1 (ref)	1 (ref)
Type of Community				
Urban	10 (19.6)	23 (40.4)	1 (ref)	1 (ref)
Rural	41 (80.4)	34 (59.6)	2.8 (1.1 – 7.3)	1.6 (0.9 –9.1)
Distance of residence to clinic				
< 10 km	11 (21.6)	25 (43.9)	1 (ref)	1 (ref)
≥ 10km	40 (78.4)	32 (56.1)	2.8 (1.1 – 7.3)	2.0 (1.0 –4.1)[b]
Contraceptive use				
Using	10 (19.6)	22 (38.6)	1 (ref)	1 (ref)
Not using	41 (80.4)	35 (61.4)	2.6 (1.0 -6.8)	1.4 (0.3 – 9.7)
Previous CC screening				
Screened	5 (9.8)	17 (29.8)	1 (ref)	1 (ref)
Never Screened	46 (90.2)	40 (70.2)	3.9 (1.2 –13.4)	3.5 (3.1 – 8.4)[c]
HIV status				
Positive	3 (5.9)	4 (7.0)	0.8 (0.1-4.7)	0.8 (0.2-5.1)
Negative	48 (94.1)	53 (93.0)	1 (ref)	1 (ref)

CC: Cervical cancer. [a]adjusted for work status; [b]adjusted for type of community and work status; [c]adjusted for educational status and current contraceptive use.

Discussions

Summary of main findings

Women living with HIV infection and especially those with severe immune deficiency were found to be at a higher risk of HR HPV infection and CSIL compared to women who are HIV negative.

The use of ARV drugs among HIV positive women was found to reduce the risk of both HR HPV infection and CSIL.

The use of VILI as a cervical CC screening tool in HIV positive women was not only inferior to VIA but inadequate as a screening tool in severely immune-deficient HIV positive women

A large majority of HIV positive women accepted CC screening despite low awareness and previous screening experience.

The present opportunistic outreach CC screening programme is associated with high default rate, reportedly because of anticipated cost of confirmatory tests and a long waiting time at referral facilities. Poorly educated women residing in rural areas were particularly at risk of default.

General discussion

The burden of HR HPV infection and CSIL among southwestern women of known HIV status

Reports of cervical HPV prevalence and SIL vary widely among different studies and across geographic regions. Higher prevalence rates have been reported in sub-Saharan Africa and among HIV positive women (15, 47, 49, 63). The 19.6% prevalence of HR HPV infection in our study is similar to the 19.7% reported in a population-based study in Ibadan by Thomas and colleagues (47), and also within the range of 14.7 to 21.6% found by

three other studies from Nigeria that evaluated the prevalence of HR HPV in communities (15,48,49). However, it is lower than the 20.1% to 28.8% range found in previous studies of other sub Saharan Africa countries (128-136). The observed differences may be due to the use of different sampling techniques or HPV detection methods. While some of the studies employed hybrid capture II techniques for detection of HPV, others used the polymerase chain reaction method as we did (128-131, 133-136). A more plausible explanation for the difference observed may be the lower prevalence of HIV in Nigeria (3.1%) compared to most southern and east African countries. Available scientific evidence shows a consistently higher HPV prevalence in countries where HIV levels are high, even within sub-Saharan Africa (63,137,138). In the presence of HIV infection and associated immune deficiency, the risk of HPV infection and persistence increases approximately 3 to 5 fold (135,137,138). HPV 16 was present in 19.8% of all women with positive HPV results, followed by HPV 35, 58, and 31. This figure is higher than 12.3% previously reported in sub Saharan Africa studies, but similar to 18.4% found in Asia, and lower than rates of 21.4%, and 25.5% for HIV positive women from North America and Europe, respectively (21, 22,47,48,53,61,62,92,139,140). While HPV 16 and 18 were the most common genotypes detected in Europe and North America (139,140), HPV 52 and 16 were the commonest in East Africa (61-63,139). In southern Africa, although HPV 16 and 18 remained predominant, HPV 16 lost its position as the most common form to HPV 18. (63,137). Two recent studies, one a multi-country study involving two southern Nigerian sites, and the other a hospital-based study in Abuja, north central Nigeria, reported very divergent findings. While the multi-country study reported HPV 16 and 18 as the most common HPV types identified in invasive CC (53), the Abuja study report that oncogenic HPV types 35, 52, 56, and 68 may be more important risk factors for cervical pre-cancer and cancer among women in Nigeria (51). The two studies and ours lends support the view that HR HPV genotypes other than HPV 16 and 18 may play a more significant role in the development of CC in sub-Saharan Africa than previously thought (92).

The SIL rate of 8.5% found in our study is higher than the 5% to 6.8% previously reported in Lagos, Okene, and Olufadi, Nigeria (21,49,141), but lower than the 10.7% to 15.6% reported from South Africa, Swaziland, and Zimbabwe (142-144). The higher rates of SIL found in our study and the southern African studies cited may be attributable to the large number of HIV positive women in those areas. While over 60% of the women we studied were HIV positive, the HIV prevalence in the aforementioned southern African countries are among the highest globally. In the presence of HIV infection and the immune deficiency associated with it, the body is unable to eliminate HPV, leading to the persistence of oncogenic HPV genotypes and ultimately the development of pre-cancerous lesions and invasive carcinoma (90).

HIV infection increases the risk of HR HPV infection and development of pre-cancerous lesions of the cervix

Findings from this study confirmed previous observations that HIV positive women are at a greater risk (24.5%) of developing pre-cancerous cervical lesions compared to HIV negative women (15.9 %). The observed higher prevalence of HR HPV in HIV infected women has been noted in other regions of Africa, South America, and Europe, indicating that HIV-infected women are at increased risk of HR HPV infection irrespective of HIV type and strain (61, 63, 90,138,139). The higher prevalence observed has been attributed to HIV-related immune deficiency (90,94,145,146). In a meta-analysis of 20 publications, Clifford and colleagues showed that apart from viral types, immune-suppression of HIV infection significantly increased the risk of HPV infection (145). The higher multiple HPV infection rates reported in HIV-positive women in our study is consistent with previous reports (93,145-147) and may be attributable to the a) common mode of transmission of HPV and HIV, b) the persistence of HPV as a result of the inability to clear HPV infections, and C) the reactivation of latent HPV infections (148).

There was a significantly higher rate of SIL among HIV positive women in our study (14.3%) compared to those who were HIV negative (3.3%),

lending support to the reported association between HIV, HPV and cervical pre-cancerous lesions (136,137,142,145). The presence of HIV immunosuppression in a woman infected with HR HPV causes the persistence and eventual transformation of HPV to pre-cancerous lesions (90-93). The prevalence of SIL among HIV positive women in our study is within the range of 10.9% to 17.8% reported for HIV positive women in Nigeria (20, 149) and Thailand (150). However, it is lower than rate of 29% among HIV positive women in Jos, Nigeria (3). The reported lower prevalence of HPV among HIV positive women in this study is not surprising, as the average CD4 cell count of HIV positive women was significantly higher than that of women in Jos, confirming the findings of studies from southern Africa, North America, and Asia, where a higher prevalence of SIL was recorded among severely immune-compromised women (11-34,43-48). Sewande and colleagues (20) stated that the immunosuppressive effect of HIV infection, as measured by the decrease in CD4 cell count, is the greatest predictor of pre-cancerous cervical lesion development in HIV positive women. However, a review of the data supporting this statement revealed no rigorous control for other confounding variables. HIV infection and its associated immune deficiency are linked to a high prevalence of HPV infection, as confirmed by our study.

ART reduces the risk of HR HPV infection and the development of pre-cancerous lesions of the cervix

The potential of ARV drugs to reverse the chronic immunosuppression of HIV infection if correctly prescribed and taken has been confirmed by studies in both high- and low-income countries (151-153). Its use is also expected to reduce the burden of HPV infection as well as pre-cancerous and cancerous lesions of the cervix (18,90,95,139). Unfortunately, there is still no conclusive evidence to confirm this potential, especially in low income countries where the HIV and cervical burden is the highest (5-7, 11,13,14). While some studies have reported that the use of HAART restores immunity and decrease the persistence of HR HPV and its eventual transformation to pre-cancerous and cancerous cervical lesions

(5, 11,13,154), others found no such association (6,14). The contrasting findings have been attributed to the differences in virulence of the circulating HIV types and strains across different regions of the world (15). However, even within sub-Saharan Africa, findings reported from the south differed from those in the east (6,7,53). Findings may also be different in the West African sub-region, where the circulating HIV strain is different from that in other sub-Saharan countries (109,110,155).

Paper II and III provide evidence of the interaction between HAART, HPV and pre-cancerous lesion of the cervix. A statistically significant association was found between the use of HAART and a reduction in the prevalence of both HR HPV and pre-cancerous lesion of the cervix. HIV positive women on antiretroviral therapy were found to be at lower risk of acquiring high risk HPV infection than those not on ART (OR: 0.4; 95% CI: 0.3 – 0.5). The consistent use of HAART results in the restoration of immunity, which ultimately reduces the occurrence of HPV infection, prevents HPV persistence, and favours regression (6). HIV positive women not on HAART were found to be at a higher risk of developing SIL (aOR: 2.1; 95% CI: 1.4 – 3.5) compared to those on HAART. Even among HIV positive women, severely immune-compromised women were found to be at a higher risk of developing SIL (aOR: 1.9; 95% CI: 1.1 – 5.9) compared to those with CD4 cell counts above 200 cells/mm3. This suggests that in the presence of HIV infection and severe immune deficiency the body completely loses the ability to clear HPV from cervical cells, leading to persistence and transformation to pre-cancerous and cancerous lesion of the cervix.

VILI is inferior to VIA, and inadequate for detecting cervical cell abnormality in women living with HIV infection.

The World Health Organization and several national programmes, including that of Nigeria, recommend the integration of CC prevention services into existing HIV programmes as the optimal strategy for the prevention of CC among HIV-infected women in low-income high HIV

burden countries. However, access to such screening by HIV positive women is limited by inadequate human resources, infrastructure and financial capacity (16,17,156,157). The morbidity and mortality associated with CC among HIV positive women in resource-limited settings can be reduced if technically appropriate detection methods are introduced and implemented. Chung and colleagues (158) from Kenya compared the ability of Pap smear, VIA and HPV DNA testing to detect HSIL in HIV-infected women. Some earlier studies evaluated the test characteristics of VILI and VIA and found both useful screening tests for CC (122,156,158-160), but to the best of our knowledge only one study compared the test characteristics of VIA and VILI in the presence of HIV infection (121). However, rather than a direct comparison, it compared screening with VIA alone and screen after application of VIA followed by VILI. Even those who compared the two screening tests in a population of unknown HIV status primarily utilized non-randomized study designs (119,159,160). In addition, few studies have evaluated the effect of HIV immune deficiency on the characteristics of the two tests (156). This information is necessary in order to determine whether HIV infection and its associated immune deficiency impacts on the accuracy of the tests.

Using cytology-diagnosed SIL as the benchmark, the test performance of VIA was found to be superior to VILI. In addition, VILI was found to be inferior to VIA sensitivity and specificity for detecting SIL diagnosed by cytology in HIV positive women. The performance of VILI deteriorated further as the CD4 cell counts of the HIV positive women dropped below 200 cellsmm3 (the NPV of VILI went down to 50.0%). This finding is similar to what has previously been reported in the cases of tuberculosis (TB), where TB diagnostic tools were found to be less sensitive in the presence of HIV infection and severe immune deficiency (123). While the reason for this was attributed to the inability of the body to mount sufficient tissue response in presence of HIV infection and disseminated TB disease (123), the explanation in our study is not immediately obvious. It may be related to the poor expression of some cervical tissue proteins that interact with Lugol's iodine to generate colour change observed during screening. Thus,

in settings of high HIV burden VILI should not be used unless the HIV status of the woman is known to be negative or not significantly immune deficient. Also found in our study is the extremely good NPV of VIA which ranged from 88.2% to 99.5%. Similar results have been reported previously (19,158), confirming that if the VIA test result is negative, women, irrespective of their HIV status, can be given assurance and safely sent home. Although the NPV of VILI was generally above 70% under most circumstances in our study, the 50% NPV score in HIV positive women with CD4 cell counts below 200 cells makes it an unreliable test in high HIV burden countries. With an NPV as low as 50%, health workers cannot confidently reassure HIV positive women, and those of unknown HIV status that the result is truly negative for pre-cancerous lesions of the cervix. The PPV of VIA ranged from 83.3% to 93.4%, making it a very good test for screening in our region. The use of VIA in low resource settings like ours can result in a significant reduction of the cost associated with over-treatment of false positive cases when VILI is used for screening. It will also reduce the danger of morbidity as a result of unnecessary treatment due to a false positive test. Most importantly, health care workers can accurately inform women who screen positive after VIA testing of the likelihood that they have a pre-cancerous lesion of the cervix and thereby counsel them to seek confirmation and treatment**.**

Access to CC screening is a key determinant of service uptake

A major challenge to most public health initiatives in resource-limited settings is the poor awareness of these initiatives, resulting in the inability of the populace to seek or accept health care services when offered. Evidence has shown that when the public is informed about the benefits of public health initiatives, their willingness to make use of those services increases (161). Studies IV and V showed a low awareness and poor knowledge of CC or CC screening among women studied. It also shows no differences between HIV positive and negative women. In addition, we found very little experience of past CC screening in the case of both HIV positive and HIV negative women. Nevertheless, more than 75% of the

women interviewed were willing to undergo CC screening, suggesting that access to such services is a key determinant to their uptake in our environment. We also found that women who were aware of CC were almost two times more likely to accept screening than those who lacked such knowledge. Several studies have also shown that educated women are more likely to accept CC screening than those with no formal education (162-165). Similarly, we found that respondents with tertiary education were more likely to accept testing than their peers with lower educational attainment.

Education has consistently been shown to impact positively on the acceptance and uptake of reproductive services. Moreover, it is also linked to self-perception, empowerment to make decisions, and the ability to pay for services without recourse to one's partner (162,163). Our findings further showed that the fear of disrupting HIV treatment services if CC screening is integrated into them, is unfounded, as the majority of HIV positive women who participated in our study were willing to screen. What remains, is for the government to ensure that all public HIV treatment facilities implement the recommendations set out in the Nigerian National HIV Treatment Guidelines and make screening a standard of care for HIV positive women (78). Such screening should be integrated into existing HIV services, rather than establishing a parallel programme outside HIV treatment facilities. Unfortunately, this important recommendation is not yet operational in most HIV clinics in Nigeria (18,100), despite a previous study showing that a large majority of the HIV positive women in south east Nigeria (96.0%) were willing to be screened for CC (19). The argument made at the time was that the study was conducted among women who were not yet in HIV care because it was conducted among HIV positive women undergoing post-test counselling. This limitation made the information difficult to extrapolate to women who were already in HIV care. The present study overcame such a limitation as it was conducted among women already in a care, treatment and support programme. Hopefully the finding of the present study will

assist policy makers advocate for compliance with the recommendations of the national guidelines.

The CC screening refusal rate of 20%, although low in comparison to previous studies in Nigeria and other HIV high burden countries (166-170), is still unacceptable for a disease that is entirely preventable and a significant contributor to avoidable deaths in Nigeria. In addition, the extremely low previous CC screening experience found in our study has most likely contributed to the high CC burden in the country. Nigeria, with less than 1% of the world's population, contributes 10% to the global CC burden – a figure that is alarming and unacceptable. For Nigeria to reduce the morbidity and mortality due to its CC burden, it is urgent to implement the plan outlined in the national CC control policy. It is comprehensive and provides for the use of low cost tools by nurses and midwives at primary and secondary level facilities (16).

Currently in Nigeria and most sub-Saharan African countries, the majority of women with CC present during the later stages of the disease, and their treatment consequently pose major challenges to the health care system due to a lack of surgical facilities, skilled providers, and radiotherapy services (107). In addition, the lack of a well-organized health care system makes it difficult to provide the effective multidisciplinary approach that is required for management of advanced stage CC. These challenges make the outcome of late stage cervical cancers predictably bad (107). The option that seems to be the most viable in Nigeria is to scale up public health education and increase awareness of CC so that women will be encouraged to undertake lifesaving CC screening and accept treatment if the result is positive.

Opportunistic outreach CC screening is associated with high default rates.

CC screening by means of DVI has been operational in Nigeria since 2010, although not in a well-organized fashion (16). While some studies have

compared the test outcomes of various CC screening tools, none have evaluated the outcome of DVI after a positive test (17,156,158,160). In our study we tracked 108 women who tested positive for CC during the outreach programme screening and were referred to the clinic for follow-up care. Almost half of the women (47.2%) did not keep their appointments. The most common reason(s) given for default were a) cost, b) time constraints, and c) disbelief that anything was wrong with them. These findings suggest that the current practice of CC screening using the outreach strategy only inefficient, as well as a waste of manpower and resources. In fact, we found the default rate to be much higher than rates reported from other low-income countries. This may be attributable to sociocultural, environmental, and health system factors (160,171 -174). Nigerian society expects women to be subservient to their spouse and only make decisions – even those related to their health and wellbeing – after spousal permission. The result is a challenge to the uptake of reproductive health services by women. Our study was mainly conducted in rural communities where women were dependent on their spouses for daily sustenance and even their bus fare. Such women must obtain their spouse's approval before they can visit a clinic. A poor public transportation system, the distance of health facilities from rural communities, and a weak, poorly organized healthcare system add to these other challenges confronting women. Where transportation even exists, its cost is usually beyond the reach of poor peasants in rural communities (174,175). Challenges like these make it difficult for most women to keep their appointment. Moreover, the vast majority of Nigerian women prefer to seek care in private health facilities because of the long waiting time and perceived poor quality of services in public facilities (176). Eventually women will have to choose between honouring an appointment for a disease they are not aware off to obeying their spouses and avoiding consequences that may include violence (175-177). Women are also mindful of the fact that in a country with a weak health system and long waiting times, keeping an appointment do not equate to finding a solution to their "health challenge". In addition, the encroachment of faith-based institutions into health care field without

basic health infrastructure and human resources is another possible contributor to the high default rate (178). These faith-based institutions promise a total cure without drugs or surgery for all diseases, including cancer. Moreover, they often do not charge fees directly and thereby prey on the vulnerability of the poor and uneducated. The women in our study who defaulted from follow-up care were mainly poorly educated and resided lived than 10 km from the health centre.

CC screening in most low-income countries, including Nigeria, is mainly opportunistic and characterized by low coverage and an absence of quality control procedures. Such services rarely reach women at greatest risk for CC – poorly educated women residing in rural areas and urban slums (106,178). The CC screening system that requires women to make multiple return visits after screening positive to pre-cancerous cervical lesions is associated with a high default rate (99,179). The near total absence of effective mechanisms for the recall of women with abnormal smears makes the multiple visit strategy ineffective (99,106,178-184). The Johns Hopkins Program for International Education in Gynecology and Obstetrics (Jhpiego) pioneered and championed the “single visit approach” or “see and treat strategy”, which effectively addressed the challenge of the high default rates associated with a multiple visit approach (185). The “see and treat strategy” involves visual inspection of the cervix after application of dilute acetic acid or Lugol’s iodine i.e., VIA or VILI, followed by immediate cryotherapy of any pre-cancerous lesions seen during the same visit. Several studies have evaluated the “see and treat” strategy and reported very good outcomes with a significant reduction in the default rate that was attributed to the reduction in the number of visits, reduced service and transportation costs, as well as a reduction in man hours of work (181,182,184-187).

The “see and treat” approach is now the preferred strategy for low-resource settings since it results in high treatment completion rates and reduced loss to follow-up. The opportunity to get screened, obtain results, and receive treatment during a single visit makes it attractive to both

patient and health care provider (181, 182,184-187). The same strategy is also ideal for HIV treatment clinics, as women receive both services at a single visit. This increases the chance that women who screen HIV positive will receive in one visit the potentially lifesaving care they need if found to have pre-cancerous lesion (183,184).

However, this strategy can only achieve its full potential if non-physicians are trained to carry out screening and treat pre-cancerous lesions that may be detected during screening, since in Nigeria over 65% of the population lives in rural communities where less than 5% of the country's doctors practice. The facilities in these communities are mainly staffed by nurse/midwives and community health extension workers. Training this cadre of health workers (especially the midwives, because of the technical quality of their pre-service training) will not only reduce the pressure on the doctors and referral facilities, but will bring CC screening and treatment services close to women who live in rural settings. It may also serve as motivation for the non-physician health workers to do more (80).

Methodological consideration

Strengths and limitations

External validity

In epidemiological studies, external validity or generalization refers to the degree to which the findings of a study can be applied to other settings or groups (188). The participants in this study were drawn from a public health facility, rural, and urban communities in the cosmopolitan Nigerian states of Lagos and Ogun. With the high rural to urban migration rate into Lagos, the commercial capital of Nigeria, religious and ethnic boundaries have dissolved, creating a Lagos-Ogun continuum that has become a mini-Nigeria. This is illustrated in the socio-demographic characteristics of the women in the study, who came from all ethnic, religious, educational, and socioeconomic groups in Nigeria. Therefore, the findings of this study can

be said to be a fair representation of Nigeria women in general. On the other hand, there is the possibility that migration itself implies a selection process, i.e., migrants differ from non-migrants in their socio-demographic or psychological and behavioural characteristics.

Studies II, III and IV applied a cross-sectional design so that disease and exposure statuses in a given population may be measured simultaneously. It provides a "snapshot" of the frequency and characteristics of a condition in a population at a given point in time. Although it is widely believed that a cross-sectional study can only be used to assess the prevalence of conditions in a population but not to determine the direction of the association (since it is not known if the exposure preceded the disease (189)), in some situations it can provide a good indication of the causal direction. This is because exposures like age, ethnicity and education generally precede the outcome, even if measured at the same time. The utilization of a cross-sectional design for studies II, III, and V had the advantage of ensuring that they were concluded in a short time (190).

In Study I, randomised control trial design was used as it is known that statistical techniques may not always control confounding adequately, especially if a confounding factor is not measured very well (189). To ensure objectivity and avoid bias, Cook advised that one conceal future allocations since that prevents manipulation (whether conscious or not) of group allocation and preserves the merit of randomizing balanced groups at baseline (191). In addition, whatever the mechanism of randomization, third party control is highly desirable and that procedure should be applied soon after randomization. These recommendations were implemented in Study I: computer-generated numbers were held by the lead investigator and only released as the screening was about to begin. Interpretation of the outcome was based on clearly defined criteria, and testing and reporting quality was maintained through training and supportive supervision of all research assistants by the lead investigator.

In Study V, we utilized a prospective design to evaluate the community outreach CC screening programme. This permitted adequate control over the selection of participants and control for the most obvious confounders by means of multivariable regression analysis. In addition, it allowed for follow-up of participants who tested positive for pre-cancerous lesions of the cervix.

Internal validity

The internal validity of a study is defined as the absence of systemic error (selection bias, misclassification, and confounding) as an explanatory factor of the results presented in the study (192).

Selection bias

According to Aschengrau and Seage, selection bias is an error due to systematic differences in characteristics of participants and non-participants in a study (190). It results from procedures used in choosing the participants and leads to outcomes that are different from the results that would have been obtained from other eligible individuals who were not included. Although it is more likely to be encountered in prospective and case control studies, it may also occur in other study designs. Since the risk of selection bias was anticipated, efforts were made to reduce it to the bare minimum. In Studies I – III and V, although we recruited participants from both rural and urban communities, the recruitment of individuals from only a few local government areas and communities may have introduced selection bias, as the latter may not have been representative of women in the two states. However, the distribution of the socio-demographic characteristics of the participants in the studies suggests very minimal selection bias, if any.

In Study IV, recruitment was hospital-based and restricted to only one site. This may have introduced selection bias because often patients in low-resources setting who present in hospital are already self-selected due to their educational background and social status. However, as the HIV

prevalence in Nigeria is low, utilising the largest HIV treatment clinic in southwestern Nigeria for recruitment of participants seemed a rationale strategy for achieving the desired sample size in a reasonable amount of time. Selection bias not only poses a challenge for the generalisation of study findings, but it may also create other challenges such as refusal to participate, dropping out, and loss to follow-up. If these challenges are related to socio-demographic characteristics like age and education, it may reduce the ability of the study to show the causal relationship between the exposure and the outcome variables. We believe that since this anticipated and effort was made to control it, it was not a major problem in these studies

Misclassification

Misclassification or response bias for variables measured in these studies may have resulted from the nature of the questions or observations reported. Misclassification is particularly a problem if it is differential, i.e. if the assessment of outcome status is biased by exposure status, or vice-versa. Exposure variables like age, socioeconomic class, sexual and reproductive behaviours, number of sexual partners, age at sexual debut, history of sexually transmitted diseases, induced abortion history, outcome variables of willingness and acceptance of CC screening, cervical cell abnormality, and outcome of DVI may all have been affected by recall bias, personal prejudice or inter-observer error. While recall bias may have led to errors in documenting age, number of previous sexual partners and age at sexual debut, prejudices or attempts to prevent appearing promiscuous may have caused some of the respondents to give false information. To prevent this type of bias, only female research assistants fluent in the local languages and trained to obtain sensitive information were used in the study. Misclassification may have occurred in Studies I and II during the reading of Pap smear slides or while assessing cervical changes after painting with Lugol's iodine or acetic acid. If so, these would have over or under estimated the outcomes measured. However the quality controls built into the study sought to reduce these to a minimum.

Confounding

As in most epidemiological studies, confounding (i.e., mixing the effect of two exposures) is present and must be minimized. In order to do this, we used a randomized control design in Study I and in Studies II, III, IV, and V adjusted for the effects of possible confounders in the analysis. Multivariate logistic regression was used to control for the effect of potential confounders in different models. We discussed how to optimize the selection of exposure variables in the models in order not under or over fit, deciding whether the selected variables were confounders or mediators in the same causal chain. For example, in Study IV we did not adjust for confounders associated with sexual behaviour, as they are more of a proxy marker for the acquisition of HPV. Controlling for them may have led to over fitting the model.

Non-systematic errors

Assessment errors that are not systematic in nature do not represent bias although they could be problematic since they will "blur" the picture by reducing the precision of the data, which may require using a larger sample size to demonstrate causal associations with sufficient statistical power. Inter-observer error might occur from visual inspection and cytology reporting. All of our research assistants received skill/competency-based training on the how to read, interpret, and report study outcome measures in an attempt to prevent inter-observer error. However, differences in an individual's comprehensions of the procedure may be an obstacle to the complete elimination of this type of error. Visual charts and diagnostic criteria were displayed in each screening room as a reminder that this specific error must be reduced to a minimum. Inter-observer error may also have occurred during the reading of cytology slides, hence the choice of a team from a university teaching hospital with vast experience in this type of work. Moreover, a quality assurance system was put in place to reduce inter-observer error to almost nothing. A senior pathologist read all slides originally classified as abnormal and 15% of those classified as normal. In the event of a conflict between the reports of the cytopathologist and the senior pathologist, the slides in question were

sent to another pathologist for independent review. In such cases, that review constituted the final diagnosis.

The use of cytology-diagnosed lesions as the standard comparator test rather than the histology-diagnosed lesions in Study I is also a possible source of error. Our study compared two approved tools for CC screening, so was not evaluating entirely new tools. Consequently, the effect on the outcome of the screening test is likely to be minimal and may have no significant impact on the test characteristics observed. Although we initially planned to use histology as the standard comparator test, the ethics committee declined its approval, stating that: *"The proposed plan to use histology diagnosis as the gold standard instead of a less invasive standard like cytology for a study that aimed to compare the test performance of tools that are already approved and in use, involving HIV positives and partly conducted in community outreach programme, may not add any extra value. Rather it may pose a risk to participants and health workers. The risk of HIV transmission using invasive procedures like cervical biopsy outweighs the advantage, if any. We recommend that histology should be replaced with cytology as a standard comparator".*

Great care was taken to improve the precision of the measurements used in this study, but since we cannot exclude such errors completely, there may be cases of "type two errors" in certain instances, i.e., increased CIs due to reduced precision might have rendered some estimates non-significant from a statistical point of view.

Statistical power

In conducting the study, an effort was made to ensure that any difference in the rate of a certain outcome existing between exposed and unexposed individuals could be clearly demonstrated in our analysis. To do this required the enrolment of an adequate number of participants. Minimum sample size was calculated for each of the five studies, with a 10% to 25% safety margin to ensure that the number of participants at the conclusion

of the study was not below the minimum sample size. In doing this we took into consideration the expected differences in outcomes between groups (Studies I – III and V), the background rate of the outcome, and the probability of alpha and beta errors (Studies I to V).

In Study I, a sample size of 530 participants in each arm was calculated to be sufficient to achieve 80% power at a 5% significance level (i.e., one-sided) in order to demonstrate the difference between VILI and VIA. The sample size formula $N = Z\alpha^2 P\ (1 - P)/d^2$ where Zα is the Z statistic for a 95% confidence level, N is the sample size, p is the prevalence, and d is the precision (126), was used to determine the minimum number of participants for Studies II and V, since the prevalence of outcomes of interest were known. In Study IV, the prevalence of outcome of interest was not known but the population of women of interest was known. Based on the most conservative response distribution of 50% (allowing a 2.5% margin of error at 95% CI), the required sample size for Study IV was calculated. Non-response was anticipated and the sample size was determined based on a 15% non-response in a previous study at the same HIV treatment centre.

Statistical tests

We used statistical tests to assess whether our findings might be due to chance. *P* values, ORs, and 95% CI were used in the analysis of the five papers in this thesis to determine the role of chance. Results and findings in which the 95% CI included 1.0 or p-value was greater than 0.05 were considered to have occurred by chance and was thus reported as statistically insignificant. Although many researchers use *p*-values for evaluating the role of non-systematic error**,** many epidemiologists prefers confidence interval to p-value for evaluation. Unlike *p*-values, CIs provide an indication of precision, which is related to the actual magnitude of the association between exposure and outcome (190). In this study we utilized both measures to provide an opportunity for all to evaluate our study using their preferred tool.

Implication for future research

The distribution of HR HPV infection in our study differs from that of other regions of the world, supporting the findings of three previous studies in Nigeria (48-50). In addition, a recent multi-country study involving two centres in Nigeria showed a disparity between the distribution of HR HPV in women with a normal cervix and those with CC (53). However, the multi-country study (53) and a previous study from Ibadan (52) suggest that the vaccine currently in use could prevent most cases of CC in Nigeria. Both studies are hospital-based and conflict with the findings of previous community or population-based studies (48-50). Resolving these conflicts require a nationwide study involving women in all the 36 states of Nigeria and including samples from women with CC as well as those with a healthy cervix. This is very important as most of previous studies are from the southern Nigeria (48-50, 52, 53).

Also, the nearly 50% default rate from follow-up after community CC outreach screening programme in study , needs to be investigated further in order to understand the reason for the failure of this public health initiative. An implementation research that will incorporate task shifting and a single visit approach as alternative strategy is urgently needed to inform future policy. Finally, there is also a need to confirm the results of Study I that VILI lacks the specificity to be used as a CC screening tool in HIV positive women.

Conclusions and Recommendations

1. VILI was found not only inferior to VIA but inadequate in detecting cervical cell abnormalities in HIV positive women. With grossly inadequate PPV and NPV, it is recommended that VILI should not be used as a CC screening tool in a setting of high HIV burden like that of Nigeria because it has been associated with high false positive and negative test results.

2. The vaccine-preventable HPV types 16 and 18 were found in less than one-quarter of the women in our study. Other common high risk HPV types that we found (HPV 35, 58, and 31), may play a more important role in CC pathogenesis in Nigeria than previously. It is recommended that until the role of other common high risk HPV types is fully elucidated, the current vaccine should considered insufficient to prevent HPV-related CC in Nigerian women. In addition, equal emphasis should be placed on CC prevention strategies such as public health education, cancer screening, and treatment of precancerous lesions, even among women vaccinated with the current HPV vaccine.

3. Women living with HIV infection were found to be at a greater risk of high risk HPV and SIL. However, those on ARV drugs were found to be at a reduced risk for HR HPV and SIL. Whenever resources permit, it is recommended that all HIV positive women should be started on ARV drugs. This should have the twin advantage of reducing HIV- associated deaths and reducing CC burden and death as well.

4. The awareness of CC screening tests was found to be low, with higher acceptance of screening among those who were aware. Most women, including those who were HIV positive agreed to take the test when it was offered to them. It is recommended that

the government intensify the current effort to educate the public on the morbidity and mortality associated with CC and the importance of CC screening and other prevention methods. Government officials and health facility managers should enforce the introduction of CC screening into all facilities where women receive any type of health care services. In addition, the various reasons given by those who refused screening (i.e., the cost of the test, long waiting times, and need for spousal permission) should be addressed through public health education and improved access to CC screening and treatment.

5. Opportunistic CC outreach screening programme are associated with unacceptable high default rates as women who test positive rarely receive the follow-up care required. Such screening is not only inefficient, but economically wasteful. An effective strategy should be adopted by primary health care facilities with a single visit strategy in which screening and treatment are provided on the same day.

Summary in Swedish

Denna avhandling avser fylla kunskapsluckor om effekten av HIV-infektion, nedsatt immunfunktion och medicinering vid HIV-infektion (ARV) på cellförändringar i livmoderhalsen som är förstadier till cancer. Vidare kunskapsluckor om förekomsten av olika genetiska typer av Humant Papillomvirus (HPV) samt om egenskaper hos två av de diagnostiska tester som idag används rutinmässigt för att hitta förstadier till cancer i livmoderhalsen, i ett geografiskt område (Lagos i Nigeria) där HIV och cervixcancer är vanligt förekommande och har sina specifika genetiska varianter av HIV-viruset.

Cervixcancer rankas som nummer två då det gäller den globala förekomsten av cancer och är den vanligaste cancerformen bland kvinnor i låginkomstländer. Cirka 80 % av alla globala fall av cervixcancer rapporteras från dessa länder. Samtidigt har HIV/AIDS-pandemin överväldigat hälsosystemen och blivit en mycket vanlig orsak till ohälsa bland kvinnorna. Nigeria är rankat som nummer två bland världens länder vad gäller antalet HIV-infekterade personer och rapporterar ca 10 % av alla fall av cervixcancer i världen.

Tills nyligen överlevde inte de flesta HIV-infekterade kvinnorna i låginkomstländerna länge nog för att cellförändringar i livmoderhalsen skulle hinna utvecklas till en etablerad cancer. Ökad tillgång till ARV-mediciner har drastiskt förändrat denna bild och idag överlever långt fler kvinnor så länge att sådana cellförändringar hinner utvecklas till cancer.

Nyvunnen kunskap från afrikanska länder söder om Sahara visar att HIV-infekterade kvinnor, jämfört med kvinnor som inte bär på detta virus, har en ökad risk för att utveckla cervixcancer men det verkar samtidigt som om ARV-mediciner kan minska denna risk. Tyvärr använder sig inte dagens cancerförebyggande verksamhet i denna del av världen av denna viktiga kunskap.

Den forskning som redovisas i denna avhandling har tagit sig an detta problem genom belysa fem viktiga aspekter på kunskap som behövs för att komma ur det beskrivna dilemmat.

Den första studien som ingår i avhandlingen var en så kallad öppen randomiserad jämförande studie (med namnet CANHIV) där 1160 kvinnor hade genomgått undersökning för livmoderhalscancer/cellförändringar. I denna studie utvärderades tillförlitligheten hos såväl HIV-positiva som HIV-negativa kvinnor avseende två olika metoder som idag används rutinmässigt för att upptäcka cellförändringar med blotta ögat vid en gynekologisk undersökning. En metod använder ättiksyra för att pensla livmoderhalsen vid undersökningen (VIA) och en annan metod använder Lugols jodlösning (VILI) för detta syfte. Resultatet av dessa två metoder jämfördes sedan med en mer sofistikerad metod (som kräver tillgång till ett specialiserat laboratorium) där cellprover från livmoderhalsen undersöks i mikroskop, så att graden av cancerutveckling kunde fastställas, dvs. låggradig respektive höggradig Squamous Intraepithelial Lesions .
I denna studie kunde man visa att den metod som använder Lugols jodlösning (VILI) för den förberedande penslingen av livmoderhalsen, inte är tillräckligt känslig och specifik för att säkert hitta tidiga former av livmoderhalscancer hos HIV-positiva kvinnor. Utifrån dessa observationer, dras slutsatsen att VILI inte bör används som metod för att hitta tidiga former av cancer i livmoderhalsen bland HIV-positiva kvinnor.

I avhandlingens andra studie *v*ar syftet att kartlägga förekomsten av HPV-infektion och några tänkbara vanliga riskfaktorer för detta bland 515 HIV-positiva kvinnor från lokalsamhällen, såväl på landsbygden som från stadsområden i sydvästra Nigeria.

De cellprov som togs på dessa kvinnor analyserades avseende förekomsten av 13 olika genetiska typer av HPV som anses innebära en särskilt hög risk för utveckling av cancer i livmoderhalsen, med hjälp av så kallad PCR-metodik (Polymerase Chain Reaction-based assay). Förekomsten av HPV-typer med hög cancerrisk var 19.6 % i hela den

studerade gruppen, högre för HIV-positiva kvinnor (25.4 %) jämfört med kvinnor som inte samtidigt var infekterade med HIV (15,9 %).

HPV-16 (3,9 %), HPV-35 (3,5 %), HPV-58 (3.3 %) och HPV-31 (3.3 %) var bland de vanligaste högrisktyperna av HPV som dessa kvinnor var bärare av. Kvinnor som behandlades med ARV-mediciner hade en minskad risk med 60 % för HPV-infektion medan kvinnor med allvarlig immundepression hade en ökad risk med 140 % för HPV-infektion.

I den tredje delstudien samlades information in från 1140 kvinnor som uppvisade tecken på cellförändringar i livmoderhalsen som var synliga för blotta ögat vid en gynekologisk undersökning. Med hjälp av statistiska analysmetoder (multivariata regressionsanalyser) beräknades sambandet mellan HIV-infektion, nedsatt immunfunktion, användandet av ARV-medicin å ena sidan, och förekomsten av begynnande cervixcancer, å andra.

Den totala förekomsten av cellförändringar i hela den undersökta gruppen var 8,5 %. Cellförändringar var betydligt vanligare bland HIV-positiva kvinnor (13,4 %), jämfört med HIV-negativa (3,3 %). Denna studie är den första från ett västafrikanskt land, med sina särskilda typer/stammar av HIV, som kunde visa att intag av ARV-mediciner verkar kunna minska risken för HIV-positiva kvinnor att utveckla cellförändringar i samband med samtidig infektion med HPV. Kvinnor som inte behandlades med ARV-mediciner visade sig nämligen ha en mer än dubbelt så hög risk för att utveckla sådana cellförändringar.

I avhandlingens fjärde studie studerades kunskapen om och villigheten att delta i gynekologiska hälsoundersökningar för att upptäcka livmoderhalscancer, bland 1517 kvinnor som besökte en stor HIV-klinik i Lagos, Nigeria.

Flertalet av dessa kvinnor (56.2 %) var medvetna om och hade viss kunskap om cervixcancer och 79.8 % accepterade att delta i sådana hälsoundersökningar. De vanligaste orsakerna till att avböja erbjudandet om hälsoundersökning var en förväntad hög kostnad för provet (35.2 %)

samt religiösa invändningar (14.0 %). Kvinnor med en hög utbildningsnivå, kvinnor som inte hade några barn, kvinnor som nyligen diagnosticerats som HIV-positiva och kvinnor som hade större kunskap om cervixcancer, var mer benägna att acceptera gynekologiska hälsoundersökningar.

I avhandlingens femte studie undersöktes hur kvinnor som hade genomgått en gynekologisk hälsoundersökning för tidig upptäckt av livmoderhalscancer, följde upp denna med det planerade återbesöket och vilka faktorer som påverkade deras val att komma på detta återbesök.

Totalt valde 47.2 % (51 av 108 kvinnor) att inte komma till uppföljningsbesöket och de skäl som angavs var oftast tidsbrist och dyra transportkostnader. Kvinnor med låg utbildning, kvinnor som bodde längre än 10 km från kliniken och kvinnor som aldrig tidigare genomgått en sådan hälsoundersökning, var mer benägna att utebli från uppföljningsbesöket. På grundval av dessa observationer föreslås en ändring av den nuvarande strategin som innebär flera återbesök, till en ”See and Treat”-strategi, vilket innebär undersökning och behandling på en och samma gång.

Studierna i avhandlingen har bidragit med viktiga nya kunskaper om förekomsten av olika genetiska typer av HPV i Nigeria, och styrkt antagandet om en positiv effekt av ARV-mediciner både för att minska risken av för HPV-infektion och för att en sådan infektion utvecklar förstadier till livmoderhalscancer. Vidare så har den idag rutinmässigt använda VILI-metoden visats vara otillräckligt tillförlitlig för att tidigt upptäcka livmoderhalscancer bland HIV-positiva kvinnor.

Avhandlingen visar också att program för gynekologiska hälsoundersökningar för att hitta livmoderhalscancer som inkluderar flera besök, upplevs som ett hinder för kvinnorna men att det trots detta samtidigt finns en hög villighet att delta i sådana undersökningar bland kvinnor som bor i urbana områden, eller på den omgivande landsbygden i sydvästra Nigeria.

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