Cervical cancer prevention in HIV-infected women within a high bacterial vaginosis setting in Kenya

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Abbreviations

AIS= Adenocarcinoma
AGC= atypical glandular cells
ASC-U= Atypical cells of undetermined significance
ASC-H= Atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion
BV= Bacterial vaginosis
CIN= Cervical intraepithelial neoplasia
FN= AGC favor neoplastic
FSW= Female sex workers
HAART= Highly active antiretroviral therapy
HSIL= High grade squamous intraepithelial lesion
HIV= Human immunodeficiency virus
ICC= Invasive Cervical Cancer
IMCI= Integrated Management of Childhood Illness
HPV= Human Papillomavirus
LEEP= Loop electrosurgical excision procedure
LLETZ: large loop excision of the transformation zone
LR HPV= low risk HPV
LSIL= Low grade squamous intraepithelial lesion
NOS= atypical glandular cells not otherwise specified
OR= Odds ratio
aOR= adjusted Odds ratio
pHR HPV= potential high risk
SCC= Squamous Cervical cancer
TV: Trichomonas vaginalis
VIA= Visual inspection with acetic acid

Preventie van baarmoederhalskanker bij HIV-geïnfecteerde vrouwen in een setting met hoge prevalentie van bacteriële vaginose in Kenia.

Abstract

Epidemiologische studies hebben vastgesteld dat een humaan papillomavirusinfectie (HPV) de hoofdoorzaak is van invasieve baarmoederhalskanker en voorloperletsels. Human immunodeficiency virus (hiv) is geassocieerd met een hogere prevalentie en persistentie van een breder scala van hoog risico HPV-genotypen, resulterend in een hoger risico tot ontwikkelen van baarmoederhalskanker. Er zijn epidemiologische onderzoeken die een verband aantonen tussen bacteriële vaginose (BV) en HPV. Voorts is bewezen dat BV en cervicitis onafhankelijke risicofactoren zijn voor cervicale intra-epitheliale neoplasie (CIN).

Wereldwijd staat invasieve baarmoederhalskanker op de vierde plaats van de meest voorkomende kankers bij vrouwen, en in ontwikkelingslanden op de tweede plaats. Vijf recente aanbevelingen van de Wereldgezondheidsorganisatie (WGO) betreffende de primaire en secundaire preventie van baarmoederhalskanker, zullen zeer waarschijnlijk gevolgen hebben voor de preventie van baarmoederhalskanker bij hiv-geïnfecteerde vrouwen in ontwikkelingslanden.

Deze WGO-aanbevelingen zijn:

1. Vaccineer meisjes in de leeftijd van 9-14 tegen de genotypen HPV 16 en 18, die verantwoordelijk zijn voor 70% van de gevallen van baarmoederhalskanker.

2. Hanteer een “screen en behandel”-benadering, waarbij visuele inspectie met 3-5 % azijnzuur (VIA) of, indien mogelijk, met een HPV-test, snel of direct gevolgd wordt door behandeling van gedetecteerde precancereuze letsels.

3. Indien een vrouw negatief bevonden werd met een pHR/HR HPV-test, dient zij niet meer na 5 jaar opnieuw getest worden, maar slechts na 10 jaar.

4. Hiv-geïnfecteerde vrouwen moeten eens per 3 jaar worden gescreend indien ze negatief zijn tijdens VIA- of cytologisch onderzoek.

5. Hiv-geïnfecteerde vrouwen moeten starten met antiretrovirale therapie (ART), ongeacht het klinische stadium van de ziekte of het aantal CD4-lymfocyten.

Baarmoederhalskanker is de meest prevalente kanker in Oost-Afrika, wat ook de regio is met de hoogste hiv-prevalentie. In Kenia is de geschatte volwassen hiv-prevalentie 5,6% en zijn er 1,2 miljoen mensen die leven met hiv. Een betere preventie van baarmoederhalskanker kan een significante impact hebben op hiv-geïnfecteerde vrouwen. Meer dan 75 % van de Keniaanse bevolking woont in plattelandsgebieden, en in de afgelopen jaren is de prevalentie van HIV in ruraal Kenia in gelijke mate gestegen als in de steden.

Kenia heeft een duidelijk beleid ontwikkeld om zowel hiv als baarmoederhalskanker, landelijk de tweede meest voorkomende kanker, te bestrijden. Er zijn echter weinig gegevens bekend over de verspreiding van HPV-genotypen, vooral onder de hiv-positieve bevolking met abnormale cervicale cytologie en baarmoederhalskanker. Dit ondanks de huidige invoering van het quadrivalente vaccin en de commercialisering van het nonavalente vaccin tegen de HPV-typen HPV 6, 11, 16, 18, 31, 33, 45, 52 en 58 antigenen. Kennis van de distributie van HPV-genotypen in kankergevallen is onontbeerlijk voor het voorspellen van de lokale impact van vaccinatie.

Hoewel er een succesvol vaccinatieprogramma is, zullen oudere vrouwen die niet in aanmerking komen voor vaccinatie blijvend behoefte hebben aan screening. Bij gevaccineerde vrouwen zal screenen ook vermijden dat andere (HR) HPV-genotypen met een hoog risico, die niet aanwezig zijn in huidige vaccins, alsnog baarmoederhalskanker veroorzaken.

Er is een gebrek aan studies over associaties. Kennis over specifieke combinaties van pHR/HR HPV-typen, die een risico zijn voor de persistentie en progressie van cervicale neoplasie, is nodig voor een betere behandeling van cervicale letsels en om de klinische evolutie van HPV-infecties preciezer te kunnen voorspellen. Momenteel zijn er slechts beperkte gegevens over het oncogene potentieel van potentiële hoog risico-(pHR HPV)-genotypen bij hiv-geïnfecteerde vrouwen. Daarnaast ontbreekt het aan gegevens over de synergistische effect van pHR/HR HPV-genotypen bij hiv-geïnfecteerde vrouwen met abnormale cytologie.

Daarenboven is er een gebrek aan studies uit Sub-Sahara-Afrika die de associatie tussen BV en HPV en/of cervicale intra-epitheliale neoplasie (CIN) onderzoeken. Gezien de prevalentie van BV wordt geschat op 20% tot 50%, is onderzoek naar het synergistische effect tussen hiv, HPV en BV van het grootste belang voor de volksgezondheid.

De screeningsprogramma's moeten worden aangepast aan de menselijke en financiële draagkracht en het specifieke gezondsheidsnoden van Kenia. Daarbij zal de groep van de meest kwetsbare hiv-geïnfecteerde vrouwen geïdentificeerd moeten worden. Zij kunnen baat hebben bij de aanbeveling van de Wereldgezondheidsorganisatie om meer preventieve screening te bieden, met één keer per drie jaar een controle.

Doel:

Deze thesis wil een bijdrage leveren tot het ontwikkelen van primaire en secundaire preventiestrategieën van baarmoederhalskanker binnen het kader van het hiv-preventie- en zorgbeleid in Kenia in setting van een hoge prevalentie van BV.

Methodologie:

In de artikelen die deel uitmaken van deze doctorale thesis werden transversale studies beschreven die de epidemiologie van de HPR-genotypen en CIN 2+ letsels bij hiv-geïnfecteerde

vrouwen in Kenia onderzochten. Daarnaast is er een systematische review en een meta-analyse uitgevoerd om het volgende te bestuderen: de prevalentie van pHR/HR HPV en van meerdere HPV-genotypen bij hiv-positieve vrouwen met normale of abnormale cytologie en baarmoederhalskanker in Kenia. Ook is er een systematische literatuurstudie gedaan om de epidemiologische associatie tussen ART en HPV, cervicale dysplasie en baarmoederhalskanker in kaart te brengen.

Resultaten:

De gepoolde prevalentie van de pHR/HR HPV-genotypen bij hiv-geïnfecteerde vrouwen was 64% (95% CI: 50% -77%). Meerdere pHR/HR HPV-genotypen waren zeer prominent, zowel bij vrouwen met normale cytologie als met HSIL (hogegraads squameuze intra-epitheliale laesies) en baarmoederhalskanker; 42% (95% CI: 35%; 49%) versus 35% (95% CI: 25%; 45%). Er was een borderline significant verschil in de prevalentie van de pHR/HR HPV-genotypen tussen vrouwelijke sekswerkers (FSW, *female sex workers*) in vergelijking met niet-FSW, bij vrouwen met zowel normale als abnormale cytologie. De meest voorkomende HR/HPV-genotypen bij vrouwen met abnormale cytologie waren HPV 16 (26%, 95% CI: 23%-30%) gevolgd door HPV 35 (21%; 95% CI: 18%-25%) en bij vrouwen met invasieve cervicale kanker, HPV 16 (37%; 95% CI: 28%-47%) en HPV 18 (24%; 95% CI: 16%-33%).

Risicofactoren voor pHR/HR HPV-genotypen:

Rekening houdend met de leeftijd, werd een associatie vastgesteld tussen het aantal CD4-lymfocyten < 200 cellen/µl en enerzijds het vertonen van meerdere HPV-co-infecties (OR=3,7; 95 CI: 1,2-12,1; p=0,03) en anderzijds met de aanwezigheid van HPV53 (OR=4,4, 95% CI: 1,4-13,6; p=0,01). Een associatie werd ook gevonden tussen een aantal CD4-lymfocyten ≥ 350 µl en HPV 16 (OR=2,9; 95% CI: 1,04-8,3; p=0,05). Geen associatie werd gevonden tussen BV en pHR/HR HPV genotypen.

Een multivariate analyse die rekening hield met leeftijd, aantal CD4-lymfocyten en HPV-co-infecties suggereerde de aanwezigheid van HPV 31 als een risiko factor voor CIN 2+ (OR: 4.9; p=0.05; CI: 1,0-22,6).

ART

De bevindingen suggereren dat HAART de HR HPV prevalentie verlaagt. HAART verlaagt de incidentie van CIN 2 en CIN 3, vooral in vrouwen met een laag aantal CD4 lymfocyten. De schaarse data betreffende HAART en baarmoederhalskanker zijn onovertuigend.

Conclusie:

Gezien de relatief hoge prevalentie van niet-HPV 16- en HPV 18-infecties bij hiv-geïnfecteerde vrouwen met abnormale cytologie en baarmoederhalskanker, is een regelmatige monitoring van deze bevolkingsgroep aan te raden. Het cervicale carcinoom-verwekkend potentieel van HPV 53 en zijn hoge prevalentie bij hiv-geïnfecteerde vrouwen, suggereert dat er behoefte is aan een betaalbare “point of care”-nucleïnezuur-amplificatiemethode om de pHR HPV-genotypen in Kenia te detecteren.

De WGO-richtlijn van 2014 om hiv-geïnfecteerde vrouwen om de drie jaar te screenen, kan effectiever zijn wanneer behandeling van BV en cervicitis een integraal onderdeel gaan vormen van primaire en secundaire preventie. Een screening triage voor een regelmatigere opvolging voor FSW, ongevaccineerde vrouwen en HIV + vrouwen ongeacht de CD4 lymphocyten concentratie is gewaarborgd. Gezien de clandestiene aard van het beroep van vrouwelijke sekswerkers, is het noodzakelijk om innovatieve manieren te hanteren om vertrouwelijkheid te waarborgen.

Het gebrek aan associatie tussen de duur en vroegtijdige start van ART versus een reductie van CIN 2+ bij vrouwen wijst op het belang van het voortzetten van cytologische en/of histologische screening, zelfs nadat ART de immuun functie hersteld heeft.

Ten slotte vragen de potentiële synergistische effect tussen cervicale dysplasie, BV, en de geassocieerde cervicitis om een integrale aanpak voor cervicale kankerpreventie. Door ART vroeger te starten onafhankelijk van het aantal CD4 cellen en door decentralisatie van de hiv-zorg, ontstaan er mogelijkheden voor een screeningprogramma's voor baarmoederhalskanker door eerstelijnsgezondheidsverleners en verpleegkundigen.

Cervical cancer prevention in HIV-infected women within a high bacterial vaginosis setting in Kenya

Epidemiological studies have established human papillomavirus (HPV) infection as the central cause of invasive cervical cancer (ICC) and its precursor lesions. HIV is associated with a higher prevalence and persistence of a broader range of high-risk HPV genotypes, which in turn results in a higher risk of cervical disease. Similarly, there are epidemiological studies suggesting an association between bacterial vaginosis (BV) and HPV and BV, cervicitis and Cervical intraepithelial neoplasia (CIN).

ICC represents the fourth most common malignancy, affecting women globally and is the second most common in resource poor settings. Five recent WHO global recommendations in the cervical cancer primary and secondary prevention landscape are likely to have an impact on cervical cancer prevention in HIV-infected women in low-income settings. These include:

- firstly, the WHO recommendation offering HPV vaccine against HPV 16 and 18 to girls at ages 9–14 naïve to the targeted types;
- secondly, a “screen and treat” approach, in which access to Visual Inspection with, 3-5% acetic acid (VIA) or if possible HPV testing followed soon or immediately by treatment of detected precancerous lesions;
- thirdly, that “once a woman has been screened negative for pHR/HR HPV testing, she should not be rescreened for at least 5 years, but should be rescreened within ten”,
- fourthly, that HIV-infected women be screened within 3 years if tested negative by VIA or cytology
- lastly, that HIV-infected women embark on Highly active antiretroviral therapy (HAART) regardless of WHO clinical stage or CD4 count.

Eastern Africa, where cervical cancer is the most frequent type of cancer is also the region burdened with the world's highest prevalence rates of HIV together. This overhaul in the global landscape of cervical cancer prevention is likely to have a significant impact on HIV-infected Kenyan women. With an estimated adult HIV prevalence of 5.6%, there are 1.2 million people living with HIV in Kenya. More than 75% of Kenyans live in rural areas, and, in recent years, HIV prevalence in rural areas has begun to reach levels estimated within urban setting.

Kenya has made a marked commitment to combat both HIV as well as the cervical cancer, which is the second most prevalent cancer in the country. However, there is a scarcity of data on the distribution of HPV genotypes, especially in the HIV positive population with abnormal cytology and ICC despite the current roll out of the quadrivalent vaccine and the commercialization of the nonavalent vaccine, containing additional HPV types HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 antigens. Knowledge of the HPV genotype distribution data in cancer cases are indispensable for predicting the local impact of vaccination.

Notwithstanding a successful vaccination program, older women ineligible for vaccines, along with vaccinated women will still require screening to detect those who will develop ICC from other High risk (HR) HPV genotypes not prevented by current vaccines. Currently, there is limited data on the oncogenic potential of pHR HPV genotypes HIV-infected women. In addition, there is a lack of data on synergistic potential HR/HR HPV genotypes coinfections in HIV-infected women with abnormal cytology. Knowledge of particular combinations of HPV types, which are risk factor for the persistence and progression of cervical neoplasia, could be helpful for management of cervical lesions and clinical prediction of the outcome of HPV infections.

Furthermore, there is a dearth of studies from sub Saharan Africa on the association between BV and HPV and/or CIN. In light of its prevalence estimates ranging from 20 % to 50 %, the elucidation

of HIV, HPV and BV interactions with one another is of utmost public health interest. Given the scarcity of resources, screening programs will need to be tailored to the human, financial resources and health profile of Kenya. This warrants the identification of most at risk HIV infected women who would benefit from a more regular follow up than once every three years as recommended by the WHO.

Aim:

This thesis purports to inform primary and secondary cervical cancer prevention programs within the framework of HIV preventive and care management in Kenya within a high BV setting.

Methodology:

The articles within this thesis employed cross sectional designs to explore the epidemiology of pHR/HR HPV genotypes and CIN 2 + in HIV-infected women in Kenya. A systematic review and meta-analysis was performed to report on the prevalence of pHR/HR HPV types and multiple pHR/HR HPV genotypes in Kenya among HIV positive women with normal, abnormal cytology and ICC. One systematic review was undertaken to examine the extent, range, and nature of research activities summarize research findings, and identify gaps in the existing literature on 1) the epidemiological association between HAART and HPV, cervical dysplasia and ICC.

Results:

Pooled prevalence of pHR/HR HPV genotype in Kenya

The overall prevalence of pHR/HR HPV genotypes among HIV-infected women was 64% (95%CI: 50%-77%). Multiple pHR/HR HPV genotypes were highly prominent in both normal cytology/HSIL and ICC, respectively (42% (95%CI: 35%-49%) versus 35% (95%CI: 25%-45%). There was a borderline significant difference in the prevalence of pHR/HR HPV genotypes between FSW

compared to non-FSW in women with both normal and abnormal cytology. The most prevalent HR HPV genotypes in women with abnormal cytology were HPV 16 with 26%, (95%CI: 23.0%-30.0%) followed by HPV 35, 21% (21 %; 95%CI: 18%-25%) and in women with ICC, HPV 16 (37%; 95%CI: 28%-47%) and HPV 18 (24%; 95%CI: 16%-33%).

Risk factors for pHR/HR HPV genotypes:

Statistically significant associations between CD4 counts <200 cells/µl and multiple HPV prevalence, adjusted for age were also noted (OR = 3.7; 95 CI: 1.2–12.1; p = 0.03) and HPV53 (OR = 4.4, 95 % CI: 1.4–13.6; p = 0.01). BV was not significantly associated with pHR/HR HPV genotypes. A multivariate analysis adjusting for age, CD4 count and HPV co-infections suggested the presence of HPV 31 as a predictor of CIN 2+ (adjusted OR:4.9; p=0.04; CI:1.0-22.6).

ART

Our findings suggest that HAART increases CD4 count, which in turn is associated with lower HR HPV prevalence. HAART decreases incidence of CIN 2 and CIN 3, particularly in women with low CD4 count. The scarce data on HAART and ICC is inconclusive.

Conclusion:

Given the relatively high prevalence of non HPV 16 and HPV 18 in HIV infected women with abnormal cytology and ICC, a regular follow up in this population may be warranted. The cervical carcinoma genesis potential of HPV 53 as a stand-alone genotype and its high prevalence in HIV infected women suggests the need for affordable point of care nucleic acid amplification methods to detect pHR HPV genotypes throughout Kenya.

The 2014 WHO guideline to screen HIV infected women within three years may be more effective if BV becomes an integral component of cervical cancer prevention. A screening triage for more

regular follow up for FSW, unvaccinated women, HIV + women irrespective of the CD4 count is warranted.

The lack of statistically significant associations observed in the systematic review between duration of ART intake and reduction of CIN 2+, in women initiating ART at an earlier CD4 count initiation suggests the importance of continued follow up with cervical cytological and/or histological screening, even after ART has been initiated and immune function restored.

The potential synergistic interactions between cervical disease, BV begs for an integrative cervical cancer prevention framework. In light of earlier ART initiation and decentralization of HIV care, there are opportunities for a more regular cervical screening program provided that front line nurses are better equipped to deal with challenges.

Contents

Preface

Cervical cancer prevention in HIV-infected women within a high bacterial vaginosis setting in Kenya

Cervical cancer is the fourth most common malignancy with 528000 new cases having been reported worldwide in 2015.[1] In low-income countries, where often diagnosis is late and cure impossible, cervical carcinoma is the second most common type of cancer. [1] HIV-infected women are particularly at risk for HPV infection and precancerous lesions, in whom lesions are more aggressive, persistent and more likely to recur following treatment. [2] [3] Eastern Africa, where cervical cancer is the most frequent type of cancer is also the region burdened with the world's highest prevalence rates of HIV together with Southern Africa, where according to UNAIDS in 2016, 19 million people were living with HIV, over 50 % of the total number of people living with HIV in the world.[4]

Whilst AIDS-defining cancers, such as Kaposi Sarcoma and Non-Hodgkin Lymphoma have been directly linked to the severity of immunosuppression, this association has been found to be much weaker and complex for ICC.[5] These observations underscore the need for understanding the natural history and epidemiology of HPV-induced cervical cancer in HIV-infected women. A number of factors have been identified that may influence acquisition of HR HPV and its progression to cervical disease, including concomitant BV [6] and cervicitis.[7]

In line with the poor elucidation of natural history and epidemiology of HPV induced cervical cancer in HIV-infected women, is the ill-defined interaction between HIV, HPV, HAART, and HPV infections, cervical dysplasia and the development of ICC.[8] In 2010, a meta-analysis of studies mostly carried out in industrialized nations on the impact of HAART on incidence, prevalence,

progression of HPV infection, yielded inconclusive results. [8] This contrasts with the decline of most opportunistic infections and certain malignancies in HIV-infected individuals on HAART. [9]

Women in rural areas of Kenya initiate sexual activity slightly earlier than their urban counterparts, with sexual activity beginning earliest in the Nyanza region (16.4 years) and latest in Nairobi (19.3 years).[10]The recent of a prophylactic HPV type vaccine for high risk HPV 16 and 18 constitutes a breakthrough for primary prevention. The WHO recommends the inclusion of HPV vaccination in national immunization programs provided HPV represents a public health priority and vaccine delivery is feasible and cost-effective.[11] Notwithstanding immunogenicity trials which have shown the effectiveness of a two-dose HPV vaccine to girls aged 9-14 naïve, [12] the WHO still recommends that immunocompromised/ HIV infected girls be administered a three-dose HPV vaccine. However, HPV vaccine uptake has been limited, with a recent longitudinal study in Eldoret, Kenya, reporting that only 31% (79/254) of those who entered the follow-up study have been vaccinated.[13]

However, vaccine development relies on knowledge of HPV genotypes characteristic in cases of ICC. Currently, there are limited data on pHR/HR-HPV genotypes among HIV-positive women in Africa. Knowledge of the prevalence and natural history of type specific HPV, either as single or multiple infections, in the development of cervical neoplasia will be important for an appropriate intervention in cervical cancer prevention programs in Kenya.

Prophylactic HPV type vaccines will not obviate the need for secondary prevention, which will remain pivotal for early identification of HIV negative and positive girls/women infected with HR/HPV genotypes not covered by bivalent HPV vaccine (Cervarix, GlaxoSmithKline) that protects against HPV genotypes 16 and 18 and the quadrivalent vaccine (Gardasil™ Merck) that protects against HPV genotypes 6, 11, 16 and 18[14], currently being rolled out in Kenya. With limited vaccine uptake in Kenya, in tandem with poor cervical cytology infrastructure, Kenyan

women with undiagnosed or untreated severe cervical lesions may be at high risk of developing ICC. [15,16]

Whereas early diagnosis and treatment of cervical pre-cancerous lesions prevents up to 80 % of cervical cancers in high resource countries where cervical cancer screening is routine,[17] in sub Saharan African, regular follow up of cervical cytology is not feasible. The WHO-approved, strategy for cervical cancer screening in low resource settings, like Kenya is visual inspection with acetic acid (VIA) or visual inspection with Lugol's iodine (VILI), although cytological screening or qualitative detection of HR-HPV genotypes is available in Kenyan urban settings.

The WHO recommends that women living with HIV be screened within 3 years in resource-constrained settings[18]. This recommendation is akin to the Belgian cervical cancer screening policy, which foresees one Pap smear or liquid-based cytology sample every three years for women of 25 to 64 years of age.[19] This recommendation contrasts with the CDC recommendation that HIV-infected women be followed up with cervical cytology alone or cytology and colposcopy together at 6-month intervals within the first year after initial HIV diagnosis and, if both tests are normal, annual screening can be resumed thereafter (CDC 2009). [20] Furthermore, in 2014 the WHO recommended that "once a woman has been screened negative for pHR/HR HPV genotypes, she should not be rescreened for at least 5 years, but should be rescreened within ten." However, there is current scant evidence to support the adequateness of this guideline in HIV-infected women.

Due to the paucity of resources, secondary prevention programs will need to be tailored to the human and financial resources of the region, and a triage for screening at a more regular interval be envisaged for the most vulnerable women in Kenya. This would require that the risk factors for pHR/ HR HPV infection and CIN 2+ in HIV-infected women on or not on HAART be better clarified.

Moreover, studies have shown a positive association between BV and HPV and HIV, where in Africa, BV was found to be significantly associated with vaginal inflammation. The high prevalence of HIV, HPV, and BV and its related cervicitis in the African continent makes elucidation of their interactions with one another of utmost public health interest.

By means of a meta- analysis in Kenya, two cross sectional studies carried out in Mombasa and Eldoret in Kenya, a systematic review for sub Saharan Africa, this dissertation aims to address the following research questions:

Aim 1: Risk factors for pHR/HRV genotypes to consider when designing an evidence based primary prevention

1st objective:

Systematic review & Meta-analysis: Distribution of pHR/HR HPV genotypes, in HIV-infected women with normal, abnormal cytology and ICC in Kenya.

What is the prevalence of pHR/HR HPV genotypes in the general HIV-infected female population versus the HIV-infected FSW population in Kenya? Which are the most prevalent pHR/HR genotypes in Kenya in HIV-infected women with normal, abnormal cytology and ICC? What is the percentage of HIV-infected women with normal, abnormal cytology and ICC harbouring multiple pHR HPV genotypes?

Hypothesis: There is a high prevalence of pHR HPV genotypes within this population in normal, abnormal and ICC subgroups.

2nd objective:

Epidemiology of pHR/HR HPV genotypes:

a) explore clinical and virological risk factors for pHR/HR HPV genotypes.

Hypothesis:

a) HPV 53 is associated with immunosuppression, while HPV 16 is not.

b) BV is associated with pHR/ HR HPV genotypes

Aim 2: Risk factors for abnormal cytology to consider when designing an evidence based secondary prevention program

3rd objective:

Epidemiology of CIN 2+/abnormal cytology:

a) Which pHR/HR HPV genotypes are independent predictors of CIN 2+ in a HIV-infected female population?

b) describe the most prevalent types of pairings in HIV infected women with abnormal cytology.

c) describe the most prevalent pHR/HR HPV genotypes in women with CIN 3 +

Hypotheses:

a) Single pHR/HR HPV genotypes in HIV-infected women are not independent predictors of abnormal cytology, but rather involve synergistic mechanisms

4th objective:

Explore the epidemiological associations between ART and pHR/HR HPV infection, cervical dysplasia and ICC in sub Saharan Africa

Hypothesis: Given the exploratory objective of this aim, no hypothesis was formed.

Original papers

The thesis is based on the following seven papers:

Published studies:

Menon S, Wusiman A, Boily MC, Kariisa M, Mabeya H, Luchters S, Forland F, Rossi R, Callens S, Vanden Broeck D. Epidemiology of HPV Genotypes among HIV Positive Women in Kenya: A Systematic Review and Meta-Analysis. PLoS One. 2016 Oct 20;11(10):e0163965. doi: 10.1371/journal.pone.0163965.

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Organization of the thesis:

The thesis is organized into six main sections:

I Background Section:

a) Chapter one describes the biological, clinical and epidemiological aspects of HIV
b) Chapter two describes the biological, clinical and epidemiological aspects of the HPV
c) Chapter three describes the biological, clinical and epidemiological aspects of BV
d) Chapter four describes risk factors for HPV infection
e) Chapter five describes risk factors for abnormal cytology

f) Chapter six reviews the literature around primary, secondary and tertiary cervical cancer prevention management in HIV-infected women in Sub Saharan Africa.

II Overview of Methods: This section describes the study area, HIV care in Kenya, the problem statement, study design, eligibility criteria for systematic reviews, and the justification for sample size. It also describes how confounders were adjusted for in cross sectional studies and how heterogeneity was dealt with in the meta analysis

*III Results sect*ions: this section includes five publications, and two which are currently being peer reviewed.

IV Discussion: this section includes a manuscript which presents an overview of the study findings to the larger research and global health issues at hand. the strengths and limitations of the findings are highlighted. Finally, specific recommendations are given for policy makers and research gaps.

I Background

1 Human immunodeficiency virus

1.1 Natural history of Human immunodeficiency virus

The human immunodeficiency viruses belong to the genus Lentivirus in the Retroviridae family and has genes, which are located in the central region of the proviral DNA and encode at least nine proteins. These are divided into three major structural proteins, Gag, Pol, and Env and two regulatory proteins, Tat and Rev and four accessory proteins, Vpu, Vpr, Vif, and Nef.

The primary cellular receptor for HIV entry is CD4. However, expression of CD4 on a target cell is insufficient for HIV entry and the help of chemokine receptors, including the major two co-receptors CCR5 and CXCR4 is required for HIV entry into cells.[21]

In genital tissues, likely candidates include dendritic cells (DC), macrophages and T cells, although many of these are located at the sub epithelial level and are therefore not directly exposed to the virus. Damage to the epithelium, albeit at the microscopic level, enables direct access to these cells. Alternatively, transmission across the intact genital epithelium is believed to involve Langerhans cells, a type of dendritic cell that extends its dendritic processes out to the surface of the epithelium in order to detect invading pathogens. From here, HIV can be spread to mucosal DCs macrophages and T cells in the underlying sub epithelial layers.

There are factors that may contribute to HIV evasion of the immune system. Even though the free virus circulating around the body may be cleared, concurrent virus production is sustained, which allows the viral load to be maintained. Secondly, the enzyme responsible for copying the viral genome, reverse transcriptase, is not equipped to proofread the newly synthesized DNA, resulting in a high error rate, which in turn contributes to the significant antigenic variation observed. Once

HIV has been integrated into the host genome, it is believed to remain integrated and in a non-replicating phase, thereby escaping immune surveillance, until the advent of all necessary transcription factors for activating the latent reservoirs.

1.2. Transmission

1.2.1 Sexual transmission

Transmission is via unprotected sexual intercourse, intravenous drug use, blood transfusion, infection with blood-derived products, or mother-to-foetal transmission.

1.2.1.1 Contribution of STIs to the sexual transmission of HIV

There is strong evidence that HIV transmission is enhanced by ulcerative sexually transmitted infection (STIs) [22] and by non-ulcerative STIs, such as gonorrhea and chlamydia [23] and trichomoniasis.[22] [23] These may increase HIV-replication and disease progression through immune activation of cellular mechanisms, rendering the CD4+T lymphocyte cells susceptible to increased HIV viral load, which in turn leads to higher transmission. [24] The presence of an untreated ulcerative or non-ulcerative disease may augment by a factor of up to ten the risk of becoming infected with HIV. [25] Moreover, HIV-infected individuals are also more likely to transmit the infection to their sexual partner if either of them already has a STI. [25]

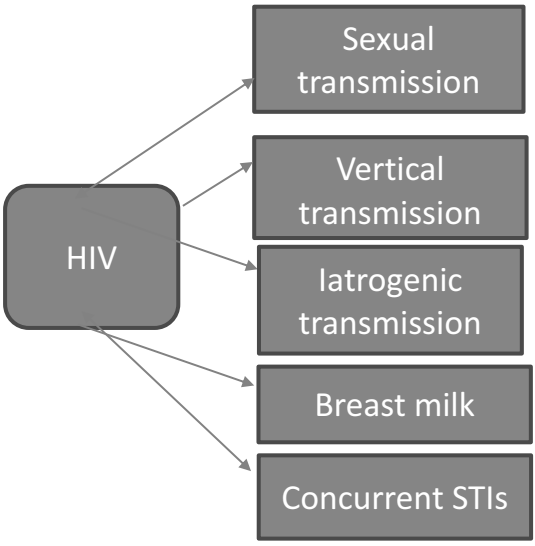
1.2.2 Vertical transmission of HIV

In the absence of any intervention, transmission rates range from 15% to 45%.[26]This rate can be reduced to below 5% with effective interventions, involving HAART for the mother and short course of HAART for the baby. Breastfeeding poses a substantial additional risk of acquisition of HIV, and if prolonged it more than doubles the overall rate of transmission.[27] Rates below 2% have

been reported from settings where HAART is applied during pregnancy and delivery, delivery is by elective caesarean section, and breastfeeding is avoided.[27]

1.2.3 Iatrogenic transmission of HIV

There is evidence that HIV transmission is fueled by parenteral exposures in health care settings, especially medical injections but also including transfusion of untested blood and others.[28,29] Injections are popular in sub Saharan patients and administered during medical visits, however they are also often unnecessary[30], and injection equipment is often reused without sterilization.[30]



1.3 Stages in HIV pathogenesis

1.3.1 Acute infection

Upon dissemination of the virus, a large-scale burst of viral replication characteristic of acute infection ensues. Virus replication and a loss of target cells continues throughout all stages of HIV infection, but is most pronounced during early stage of acute infection. During this phase of acute infection, complex changes take place within the immune system, including rapid depletion of CD4 cells. Notwithstanding the high number of anti-HIV antibodies produced, and HIV-infected cells destroyed by cytotoxic CD8+ lymphocytes, the response is imperfect and latent reservoirs of HIV infection become established throughout the body**.** Individuals who have symptomatic disease at the time of seroconversion appear to progress more rapidly than those who do not to the state of advanced immunosuppression. Following the acute burst of replication, the viral load decreases and remains at a relatively constant level, which heralds the start of the chronic phase of infection.

Chronic HIV infection begins after antibodies to the virus have fully developed and the initial immune response is complete. Active virus replication usually progresses during this asymptomatic period, and the rate of disease progression correlates with HIV RNA levels.

1.3.2 Symptomatic infection

Acquired immunodeficiency syndrome (AIDS) results from long-term HIV infection and is defined by an absolute CD4 cell count of less than 200 cells/μL and the occurrences of specific opportunistic infections or malignancies.[31] Exposure to HIV does not have a single common outcome in all individuals, with the interval between acute HIV infection and AIDS having a median time of approximately 10 years.[31] When the CD4 cell count falls to below approximately 200 cells/μL, the ensuing state of immunodeficiency puts the individual at significant risk for opportunistic infections and neoplasms.

As both CD4 cell count and viral load are expensive tests and often unavailable in most low-income settings, the WHO clinical staging system is used. This consists of using clinical manifestations of HIV disease to classify individuals as having asymptomatic, early, intermediate or late stage disease (WHO stages 1-4). To qualify as an AIDS-defining cancer, the malignancy should be at an increased risk in the HIV-positive population, and the risk should be inversely proportional to the degree of immunosuppression as expressed by the CD4+ T-cell count.[32] Cervical cancer before the age 35 years is considered an acquired AIDS-defining illness by the CDC.[33]

1.4 Antiretroviral for management of HIV infection

HIV management relies on thwarting viral replication. Sub-Saharan Africa remains most severely affected, with nearly 1 in every 25 adults (4.4%) living with HIV and accounting for nearly 70% of the people living with HIV worldwide,[34] the current standard recommendations for first-line adult ART include two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). [35] Persons not responding to first-line regimens are usually switched to a cocktail of two NRTIs plus a boosted protease inhibitors (PI).[36] As more persons are starting ART and the use of viral load monitoring is expanding, the need for second and third-line regimens is expected to increase.[37]

The WHO revised its recommendation in 2015 for all HIV-infected adults to start treatment, regardless of WHO clinical stage or CD4 cell count, following the results of a large, multi-national, randomized controlled clinical trial *(*START trial). [38]

2. Human papillomavirus

2.1 History

In 2008, the Nobel Prize for Medicine was awarded to Harald zur Hausen for the detection and isolation of HPV types 16 and 18 from cervical cancer cells. [39] Zur Hausen's specific field of research was the study of oncoviruses. In 1976, he published the hypothesis that human papillomavirus plays a central role in the cause of cervical cancer. Initially, his work was met with a great deal of scientific criticism but it was subsequently confirmed and the knowledge that he pioneered was also extended to other low- and high-risk papillomaviruses (HR HPVs).

2.1.1 Life cycle and carcinogenesis

Papillomaviruses, which are non-enveloped double stranded DNA viruses have a marked tropism for squamous epithelial cells. As the basal epithelial cells of squamous epithelium are the only dividing cells during keratinocyte differentiation, papillomaviruses must infect these cells to initiate an infection. HPV infects basal cells from mucosal epithelia through micro-abrasions, caused by micro-trauma during sexual intercourse.

The HPV encodes eight major proteins, six located in the “early” region and two in the “late” region. It is generally established that viral gene expression leads to the expression of six nonstructural viral regulatory proteins (E1, E2, E4, E5, E6 and E7) from the early region of the viral genome in undifferentiated or intermediately differentiated keratinocytes and two structural viral capsid proteins (L1 and L2) from the late region of the genome in keratinocytes undergoing terminal differentiation. E5, E6, and E7 are viral oncogenes and their expression induces cell immortalization and transformation. In particular, E6 and E7 are two viral oncoproteins that inactivate, respectively, p53 and pRb, two cellular tumor suppressor protein and do so more efficiently in high risk HPV genotypes compared to low risk HPV genotypes.[40]

Viral protein/ genomic element	Molecular weight/size	Function
Non-coding elements		
Long control region (LCR)	500–1000 bp	Origin of replication and regulation of HPV gene expression
Early proteins		
E1	68–85 kD	Helicase function; essential for viral replication and control of gene transcription; similar among types
E2	48 kD	Viral transcription factor; essential for viral replication and control of gene transcription; genome segregation and encapsidation
E3	Unknown	Function not known; only present in a few HPVs
E1^E4	10–44 kD	Binding to cytoskeletal protein
E5	14 kD	Interaction with EGF/PDGF-receptors
E6	16–18 kD	Interaction with several cellular proteins; degradation of p53 and activation of telomerase
E7	~ 10 kD	Interaction with several cellular proteins; interaction with pRB and transactivation of E2F-dependent promoters
E8^–E2C	20 kD	Long distance transcription and replication repressor protein
Late proteins		
L1	57 kD	Major capsid protein
L2	43–63 kD	Minor capsid protein

2.2 Natural history of HPV infection

2.2.1 Initial HPV infections

Women acquire HPV through sexual intercourse with an infected partner when HPV reaches the basal layer of the epithelium through a small tear in the cervical epithelium. Infections clear within 2 years in more than 90% of individuals.[41] The majority of HPV infections are self-limiting and spontaneously clear within several years as a result of cell-mediated immunity. However, many women who spontaneously clear one specific type of HPV may still become infected with another HPV type.

2.2.2 Subclinical HPV infection

A minority of HPV infections persist, which in turn puts an individual at a substantial risk of developing precancerous change dysplasia CIN1, 2, or 3 and these lesions are likely to progress to cervical cancer over a period of several years if left untreated.[42] Hence, the CIN3 lesions have become the targets of screening to prevent its progression to ICC within 10–20 years.

2.2.3 Cervical Cancer

The term 'cervical cancer' is generally used to denote squamous cell carcinoma (SCC) of the uterine cervix although there are some other known histological forms. SCC is however by far the most common form of cancer accounting for 80% of primary cervical cancers.[43] The progression from HPV infection to HPV persistence to the development of high-grade CIN and ultimately cervical cancer appears to take, on average, up to 15 years.[44]

2.3 Molecular interactions between HIV- HPV

Whilst most studies have suggested that the association between HIV infection and increased prevalence of HPV infection, there is also some evidence that mechanisms other than immunosuppression, such as direct molecular interactions between HIV and HPV viral genes, may influence the natural history of HPV.[45] [46] HIV may modify HPV-related carcinogenesis by altering the expression of cytokines in the cervix and reducing local cervical cellular immunity, resulting in an alteration of HPV regulation. [47]

2.4 TH1-TH2 shift

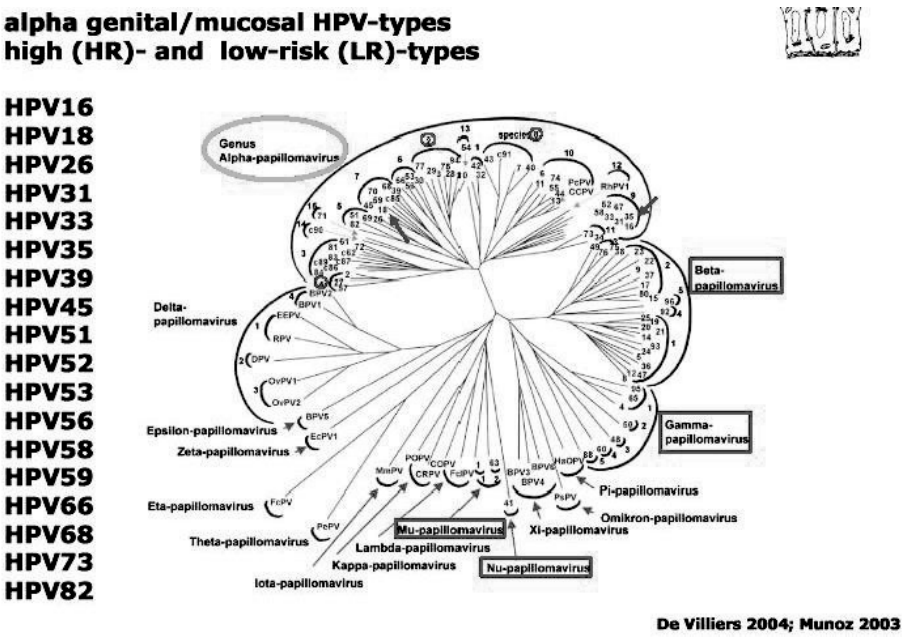
T helper cells can be classified as Th1 or Th2, a distinction based on the different cytokines that they secrete. Th1 cells secrete IL-2 and IFN-γ. Th2 cells secrete IL-4, IL-5, IL-10, and IL-13. HIV infection is characterized by a Th1-to-Th2 shift. IL-2 production has been shown to be decreased

and the IL-4 and IL-10 response increased with the persistence of viral infection and the development of neoplasms. [48]

Despite viral immune evasion, the host immune system is able to effectively combat most HPV infections. Persistent infections arise when the immune system fails to eliminate or control the HPV infection and is the greatest risk factor for development of SCC or, less commonly, adenocarcinoma of the cervix.[49] Persistence appears to be multi-factorial depending on viral, behavioural/environmental, and host factors.

2.5 Classification of HPV

HPV are viruses exhibiting a high degree of genetic diversity.[50] Two hundred and six HPV types, which are classified in different genus and species have been identified based on sequence analysis of the genomic sequence of the L1 gene which encodes the major capsid protein. [50] The clinically most important genus is referred as alpha-papillomaviruses are further subdivided into High risk (HR) and (Low risk) LR types according to the potential to cause cancer. [50]



Phylogenetic classification of HPV genotypes

2.5.1 High risk HPV

On the basis of molecular epidemiological evidence, HPV types can be classified into HR types, which are frequently associated with the development of premalignant and malignant epithelial lesions of the cervix.[51] Twelve of the 15 HPV types that are classified by a recent epidemiological meta-study as “HR” HPV types due to their association with cervical carcinogenesis are members of two species, HPV-species 7 (HPV-18, HPV-39, HPV-45, HPV-59, and HPV-68) and HPV-species 9 (HPV-16, HPV-31, HPV-33, HPV-35, HPV-52, HPV-58). In many epidemiologic studies these HPVs were found in more than 80% of HSIL and cervical cancers, with HPV 16 and 18,

being the two most common types that cause 70% of cervical cancer worldwide. [52,53] In one study, HR HPV genotypes were present in 81 % of ICC compared to 58 % of HSIL.[54]

2.5.2 Low risk HPV genotypes

The LR HPV genotypes include HPV 6, 11, 40, 42, 43, 44, 54. HPV 6 and 11 types causes 100% of genital warts and are frequently associated with LSIL.[55] Whilst HPV 6 and 11 have been identified in cancers of different organs or tissues, these HPVs are not considered oncogenic, as they have mostly been reported in low grade lesions or normal smears. [56] In addition, the p53 and pRB tumor suppressor proteins are not inactivated by the E6 and E7 genes of HPV 6 and 11.[57] [58]

2.5.3 Intermediate risk

There is currently only limited information about their relative prevalence in dysplastic or neoplastic lesions and specimens with normal cytology and the interaction of the E6 and E7 gene products of these HPVs with p53 and pRB proteins remains poorly elucidated.[59] The low frequency, lack of data on the active transcription and transforming potential in model systems of HPV 26, 53, 66, 67, 68, 70, 73,82, have led them to be classified as possible pHR HPV types.[60]

2.6 Classification of HPV induced cervical lesions

As the traditional methods of viral diagnosis such as electron microscopy, cell culture, and certain immunologic assays are unsuitable for HPV detection[61], the primary diagnostic tools have been cytology and histology. SIL and CIN are two terms, which describe precancerous lesions in the cervix. The Bethesda System 2001 classifies squamous cell abnormalities into four categories: (i) atypical squamous cells (ASC) which in turn contains two sub categories, the ASC-US

subcategory, which includes lesions that have cellular abnormalities suggestive of SIL and the "atypical squamous cells which cannot exclude HSIL" (ASC-H) category. (ii) LSIL, (iii) HSIL, and (iv) squamous cell carcinoma. [62]

Squamous cell carcinomas represent 75–85% of all cases, while adenocarcinomas occur in 11–25% and adeno squamous carcinomas in 2–3% of cases.[63] The natural history of the cervical adenocarcinoma is akin to that of the squamous cell carcinoma, particularly in terms of its existence of precursor lesions and their association with HR HPV infection.[64]Adenocarcinoma in situ (AIS) is well established to be a precursor of invasive adenocarcinoma and it is considered to be the glandular counterpart of cervical intraepithelial neoplasia (CIN) 3. Similar HPV types have been demonstrated in most invasive adenocarcinomas and AIS.[65] The changes made in the last Bethesda revision (2001) entailed classifying endocervical glandular cell abnormalities as less severe than AIS and breaking down invasive adenocarcinoma into two categories: atypical glandular cells (AGC) not otherwise specified (NOS) and AGC favor neoplastic (FN) because the risk of neoplasia associated with the latter is substantially higher.[66]

Classification of an abnormal papanicolaou smear

(According to the standard of the 2001 Bethesda classification of cytologic abnormalities)

squamous epithelial cell abnormalities	glandular epithelial cell abnormalities
↓Atypical squamous cells of undetermined significance (ASCUS) cannot exclude HSIL(ASC-H) ↓Low-grade squamous intraepithelial lesion(LSIL) encompassing:human papillomavirus/mild ↓High-grade squamous intraepithelial lesion(HSIL) encompassing:moderate and server dysplasia, carcinoma in situ; CIN2 and CIN3 ↓Squamous cell carcinoma	↓Atypical glandular cells(AGC) (specify endocervical, endometrial, or not otherwise specified) ↓Atypical glandular cells, favor neoplastic (specify endocervical, or not otherwise specified) ↓Endocervical adenocarcinoma in situ(AIS) ↓Adenocarcinoma

2.7 HPV transmission

2.7.1 Sexual transmission

In contrast to HIV, sexual transmission of HPV occurs primarily by skin-to-skin contact and/or mucosal contact, hence making it easier to contract HPV. Epidemiologic studies clearly indicate that genital HPV infection is primarily transmitted by genital contact, usually, however not exclusively through sexual intercourse. [67] Additional overwhelming evidence corroborating the hypothesis of sexual transmission is derived from studies reporting the consistent association between lifetime numbers of sexual partners and a positive trend of HPV prevalence with age in women. [62] Whilst condom usage may confer some protection to individuals from exposure to HPV,

HPV can still be transmitted by contact with infected labial, scrotal, oral or anal tissues that are not protected by a condom.[68]

2.7.1.1 Concurrent STI and sexual transmission of HPV

Like in HIV, a cofactor involved in increasing sexually transmission of HPV is co-infection with other STIs, either viral or bacterial in nature.[69] Such infections can cause inflammation, and in HPV-infected women, cervical inflammation is associated with higher HR-*HPV* transmission in women. [70]

2.7.2 Vertical transmission

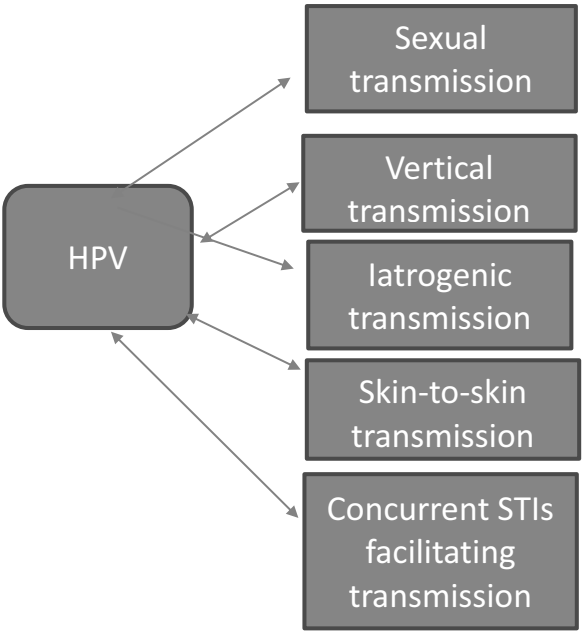
Vertical transmission from mother to infant is well documented. [71,72,73] Several studies have reported that the risk of vertical transmission is increased with vaginal delivery, suggesting that perinatal transmission occurs as the fetus passes through an infected birth canal. [74,75]Cason et al reported that, among infants who were positive for HPV-16 at birth, HPV-16 DNA could still be detected in 60% of infants at 6 months of age.[76] In contrast to HIV, HPV infection through maternal milk may occur, but its likelihood is low.[77]

2.7.3 Horizontal transmission

Horizontal transmission of HPV has been suggested to be an important contributor to infection. Non-sexual acquisition horizontal transmission of HPV can occur when HPV on skin surfaces or contaminated fomites comes in contact with microscopic injuries in the skin surface. [78, 79, 80]Auto inoculation occurs from the transmission of HPV from one site to another by scratching or bathing.[78]

2.7.4 Iatrogenic transmission

Iatrogenic viral transmission may occur with the reuse of nonsterilized vaginal specula could be a source of iatrogenic viral transmission.[81] Furthermore, HPV DNA has also been found on surgical gloves and biopsy forceps used in the care of patients with genital condylomata. Cryoprobe tips and biopsy forceps may still harbor HPV DNA, even after sterilization.[82]



Modes of HPV transmission

2.8 Diagnosing cervical dysplasia

2.8.1 Papanicoloau smear

Papanicoloau smear is a microscopic examination of cells scraped from the cervix and is used to detect cancerous or pre-cancerous conditions of the cervix. While in developed countries the introduction of large-scale cytological testing has resulted in a major decline in cervical cancer mortality, it has proven difficult to establish conventional cytology based screening programs in low-resource settings where the prevalence of HIV infection is greatest. This can be attributed to both, the high infrastructure requirements of cytology and subsequently the clinical expertise required to perform colposcopy when abnormal cytology has been found.[83] In these settings the high specificity of cytological testing is offset by its lack of sensitivity for detection of precursors of invasive cervical cancer, ranging from 30% to 90% and highly dependent on adequacy of sample collection, slide preparation and slide interpretation.[84]

In addition, pap smears are not point-of-care tests; they require the ability to notify women of abnormal results and to follow-up with further evaluation or treatment. In sub-Saharan Africa, a 60-80% default rate among those with cytologic abnormalities[85] at age 30, at ten year intervals was reported.[86,87]

2.8.1.1 Liquid based cytology

In well-resourced nations, ThinPrep and SurePath are 2 commonly used LBC systems, which are alternative methods of preparing cervical smears for microscopic examination, based on collecting cells from a vaginal, exocervical or endocervical specimen, which are transferred into a transport solution medium. Liquid-based cytology (LBC) is a relatively simple technique, which exhibits good nuclear and cytoplasmic details with the absence of obscuring background material.[88] Moreover, screening for other STIs can be performed on the LBC collection fluid, without the need

for collecting a separate specimen and HPV testing can be performed on the fluid in the vial, and HPV testing or cytology can be performed on the same sample.[89 90] Recent digital screening technology using thin prep medium uses digital images for telecytology, training, education, proficiency testing, and automated screening of Pap test slides.[91] However, due to the high cost of the LBC technology, conventional Pap smear remains the screening test of choice in Kenya and South Africa.[92] Furthermore, there is also a paucity of literature examining whether LBC can be successfully used to screen for cervical abnormalities in HIV-positive women.[93]

2.8.2 Visual Inspection Acetic acid

As part of an efficient cervical prevention programme, alternatives to cervical cytology have been sought, including VIA, which is a naked-eye screening tool for the detection of precancerous tissue after the application of dilute acetic acid to the uterus cervix.[94] VIA can be performed by midwives, nurses and other health care workers, which decreases barriers regarding staff shortages. [95]

Although VIA and cryotherapy are less complex than cytologic screening and colposcopy for screening and treatment of cervical cancer precursors, the simpler techniques still require significant infrastructure, training of providers, and quality assurance.

A meta-analysis in 2016 concluded that small differences in specificity resulted in fairly large absolute differences in overtreatment,[96] furthermore VIA has not been met with a high level of specificity, especially in a HIV-infected population.[97] A study of a HIV-infected population in Uganda reported that a VIA based see and treat strategy may have resulted in overtreatment by 72% (439 out of 625).[98]

In the general population, VIA has been shown to have a similar sensitivity (60-86%) to human papillomavirus (HPV) testing and cervical cytology.[99,100] Recent well-powered studies of VIA among HIV-positive women that use colposcopy or histology as the gold standard show a range of values for VIA sensitivity (63%-84%) and specificity (66%-89%), suggesting that HIV-related

and provider factors are important determinants of test performance.[101,102] Huschko et al 2015 in a study on 1432 HIV+ women undergoing VIA and colposcopy and using a histological reference standard reported that sensitivity and negative predictive value were higher among women with higher CD4+ counts and in non-HAART users in the >35 years age group. This finding of improved sensitivity of VIA among women with a healthier immune status is in agreement with previous data.

2.8.2.1 Visual Inspection with Lugol's iodine (VILI)

Results from a recent meta-analysis suggested that in the context of primary cervical cancer screening in sub-Saharan Africa, VILI to be the most accurate alternative to cytology and when performed by nurses, VILI was about 13% more sensitive and as specific as VIA in this region.[103] Color changes yielded by iodine impregnation of cervical mucosa have been reported to be more easily detectable than acetowhite switches observed after application of acetic acid.[104] It has also demonstrated to improve the performance of VIA on HIV-positive women.[105]

2.8.3 Alternative to screening: Preventive cryotherapy

A recent mathematical modeling study on the cost effectiveness of universal cryotherapy for women of screening age in low-resource settings indicated that preventative cryotherapy for women of screening age may yield greater health benefits than once-in-a-lifetime screening.[106] Cryotherapy has been shown to be effective and has relatively low risks.[107] Early complications including discharge, bleeding, abdominal pain, and infection and delayed complications including cervical stenosis are rare. Particularly in settings where there is limited access to screening or cancer treatment, preventative cryotherapy may be an effective and affordable alternative.[125]

The impact of cryotherapy on cervical shedding of HIV-1 among HIV-positive women remains unclear. Some studies have found that cervical treatments for CIN 2/3 disease, such as

cryotherapy, may inflame the cervix and cause ulcerations and watery discharge that increase HIV-1 shedding.[108] [109] In turn, high levels of HIV-1 cervical shedding may in turn increase an HIV-1-positive woman's infectivity and risk of HIV-1 transmission to HIV-uninfected sexual partners.[110] However, two prospective cohort studies, suggested that there was no significant increase in detectable cervical HIV-1RNA after cryotherapy in women on HAART. [111,112]

2.9 Molecular techniques of detecting HPV

Unlike the conventional pap smear test, HPV testing lacks interobserver variability of cervical cytology, and is reported more sensitive than cytology and VIA for detecting precancerous lesions and invasive carcinoma of the cervix. [113]Testing for HPV is typically done using automated molecular amplification or hybridisation techniques. The most reliable tests are consensus primer polymerase chain reaction (PCR) assay or specific primer PCR and the Hybrid Capture 2 microtiter assay (HC2; Digene, Gaithers-burg, MD). [114,115]

Hybrid Capture II version of the assay is now widely used in clinical diagnostic laboratories. It is less sensitive than PCR assays with a detection limit of approximately 5000 genome equivalents[116] and several recent studies have shown a significant analytical inaccuracy of the HC2 test near the cutoff, mainly due to the cross-reactivity of its high-risk probe cocktail[117,118,], which would reduce the clinical importance of positive results.

In most epidemiological studies PCR is the most widely used technology to test for HPV DNA, which uses target amplification methods that allow for the multiplication of unique regions of the DNA so they can be detected. The majority of studies using PCR to date have used consensus primers to amplify a broad spectrum of HPV types in a single PCR amplification. These primers target conserved regions of the HPV genome such as the L1 capsid gene. As PCR assays are highly sensitive and can detect 10 to 1000 DNA molecules in a specimen[119], their high analytic

sensitivity detects low levels of HPV that is not predictive of disease requiring treatment, these PCR tests are not useful clinically.[120] A large number of comparative studies have presented HC2 as the HPV detection method with the lowest analytical sensitivities , PCR with GP5+/6+ and PGMY09/11 with intermediate analytical sensitivity, and PCR with SPF10 with the highest sensitivity.[121]

Emerging epidemiologic studies suggest that screen-and-treat strategies using HPV genotyping might be most suitable in resource poor settings, targeted after the peak age of new and typically self-limiting infections. A cost-effectiveness modelling comparing screening strategies in five resource poor countries predicted that for 35-year-old women screened only once in their lives, a single-visit or two-visit approach with the VIA method could reduce the lifetime risk of cervical cancer by 25%, and HPV DNA testing could reduce it by 36%.[122] However, HPV testing, along with its requirement for skilled laboratory personnel renders it unaffordable for most resource poor settings.

However, a recent breakthrough may result in a change in the diagnostic landscape in low-income settings. The Cepheid Xpert HPV assay, the point-of care HR HPV testing, a qualitative, real time polymerase chain reaction assay detecting 14 HR-HPV DNA demonstrated good clinical performance in identifying women with CIN2/3 and it was found to have similar performance as other FDA approved HR-HPV tests among HIV-uninfected women.[123] Similar results have been observed when using the Hybrid Capture 2 DNA assay (Qiagen, Germantown, MD, USA).[124]

2.9.1 Cost effectiveness of different cervical cancer screening in HIV treatment clinics

In 2017, a cost-effectiveness study using a SIL-based model comparing cryotherapy without screening, VIA, Panicolalau smear and HPV testing in a HIV treatment clinic in Kenya found that the costs of cryotherapy, VIA, Pap, and HPV for women with CD4 200–500 cells/mL were $19,

$94, $124, and $113 from a clinic perspective, with 17.3, 17.1, 17.1, and 17.1 years of life expectancy, respectively. [125] Women at higher CD4 counts (>500 cells/mL) given cryotherapy VIA, Pap, and HPV resulted in better life expectancies (19.9+ years) and lower clinical cost ($13, $51, $71, and $56 respectively).

CD4 (200–500 cells/mL)	US $ 19	US $ 94	US $124	US $113
CD4 (>500 cells/mL)	US $ 13	US $ 51	US $71	US $56

Clinical cost in $ US 2014

3. Bacterial vaginosis

3.1 Natural history

BV is attributed to a disturbance in the vaginal flora, with fewer lactobacilli and increasing numbers of anaerobic Gram-negative rods. [126] Clinically, BV is characterized by vaginal discharge, odor, and an increased vaginal pH (> 4.5); however, symptoms are nonspecific and as many as 50% of women remain asymptomatic. [127] In turn, this alteration may result in an increased risk or reactivation of HPV infection, as well as other many STIs, including infection with *Trichomonas vaginalis* (TV), *Chlamydia trachomatis*, *Neisseria* according to the geographical region. [128] Despite its prevalence and adverse sequelae, the etiology and natural history of BV are poorly understood. [129]

3.1.1 Epidemiology of bacterial vaginosis

A meta-analysis found the epidemiological data to be consistent with BV having a sexual mode of transmission, [130] but additional factors may potentially contribute to its aetiology. Intra vaginal practices, including insertion or application of substances like herbs, pulverized rock, or commercial products to prepare the vagina for sexual intercourse can cause physical or chemical abrasions [131] that could be exacerbated during intercourse are believed to alter the dominant flora of the vagina, thereby increasing susceptibility to BV. [132,133] BV prevalence in Sub-Saharan African

women being among the highest worldwide. Estimates in BV range from 20 % to 50 % in African populations. [134]

3.2 Diagnosing bacterial vaginosis

The gold standard for diagnosing BV include Nugent's scoring system, which is a gram stain method that determines the relative amounts of gram-positive *Lactobacilli* and gram-negative rods (low score of 0–3 indicating mainly *Lactobacilli*/normal vaginal "flora"; high score of 7–10 indicating BV). The clinical criteria for diagnosing BV is d iagnosed by three of the four physiological Amsel criteria which include 1) pH≥4.5; 2) positive whiff test for diagnosing a fishy amine odor when 10 percent potassium hydroxide solution is added, 3) at least 20 percent clue cells consisting of vaginal epithelial cells with border obscured by adherent coccobacilli on wet-mount preparation or Gram strain, and 4) thin, homogenous vaginal discharge [135,136] In Kenya, like in sub-Saharan Africa, diagnostic testing for BV is often not available and most women are managed using syndromic algorithms,[137] where only the first three Amstel criteria are adhered to. A study in Kenya, reported poor detection of BV using syndromic diagnostic algorithms.[138]

3.3 Treatment of bacterial vaginosis

A 7-day metronidazole oral regimen of 500 mg twice a day has been shown to provide an initial cure in 80–90% of BV cases. However, treatment trials report cure rates of 80–90%, but recurrence rates of 43% within 3 months. [139] Little research has been undertaken on the occurrence and recurrence of BV in key populations for HIV infection. In a recent study on the epidemiology of BV in a cohort of women at high risk for STI and HIV infection in Kampala, Uganda, of the HIV-infected women who were treated for BV, 72% had a second episode by 3 months.[140]

A review of randomized controlled studies has reported the safety and benefits of probiotics in the prevention or treatment of BV and recommends daily consumption of probiotic products to improve public health among women.[141]

3.4 Bacterial vaginosis related cervicitis

There is growing evidence from other studies that BV is an independent predictor for cervicitis. [142] A direct link between the two disease states is demonstrated in findings by Schwebke et al,[143] who reported that cervicitis did not respond to recommended treatment unless coexisting BV was treated. BV could lead to cervicitis through a loss of bactericidal H202-producing lactobacilli, reduced levels of protective vaginal mucins, and increased pro-inflammatory enzymes and cytokines, which in turn may decrease the cervical mucus barrier .[142] A study has shown that HIV infection is associated with elevations in genital inflammatory cytokines, as a marker of genital inflammation, which would explain the high risk of HIV acquisition in African women. [144]

4. Risk factors for HPV infection

There are a number of epidemiological risk factors for the acquisition of HPV infection.

4.1 Behavioral determinants of HPV infection

Epidemiological studies investigating risk factors for HPV infection have clearly and consistently shown that the key determinants of infection in women are the age at which sexual intercourse was initiated and the number of sexual partners.[145] In populations where female monogamy is predominant, female sex workers play an important role in the maintenance and transmission of HPV infections.[145]

Vertical transmission of HPV from mother to fetus is known to occur, although much controversy still exists about maternal-to-fetal transmission of HPV, specifically about the magnitude of the risk

and the route and timing of such vertical transmission.[146] Cason et al reported that, among infants who were positive for HPV-16 at birth, HPV-16 DNA could still be detected in 60% of infants at 6 months of age.[147] The precise route of vertical transmission as well as the clinical significance of HPV DNA detection in neonates needs to be further elucidated. [146]

4.2 Age

The detection of HPV infection in women has been found to start consistently with a peak just after the onset of sexual relations, usually from 15 years of age,[148] reaching prevalences up to 80% in some populations mostly at the expense of transient infections that clear rapidly.[149] Coinfection with multiple HPV types has been observed more frequently among younger women and among those with cytologic abnormalities or impaired immune response.[150]

4.3 Immunosuppression

There is evidence that the high rate of HPV in HIV-infected women is associated with reactivation in HIV-infected women is a CD4 count less than 200 cells/mm3,[151] suggests that functional immune systems keep HPV infections in a sub-clinical state and that they may be reactivated by immunosuppressive conditions.[152]

Differences in the capacity to evade the immune system have been observed among different HPV types. HPV 16 has been shown not to depend as much on immune status as other less prevalent types would.[153] It is hypothesized that HPV 16 enrichment in SCC may be related to its greater ability to escape immune surveillance compared to other types. [154]

4.4 Bacterial vaginosis

The association between BV and HPV infection has been inconsistent among studies. A recent meta-analysis of available literature also suggested a positive association between BV and HPV infection, of OR, 1.43; 95% CI: 1.11-1.84), although no study in Africa was eligible to be included.[6]

As most included studies had a cross-sectional design, where data on prevalence of BV and HPV infection were gathered simultaneously, instead of over time, a causal relationship cannot be established.

5. Risk factors for abnormal cytology

However, only a fraction of precancerous lesions progress to ICC and a strong candidate factor for differential progression is HPV type.[155] Apart from HR-HPV being the central etiological agent in the development of cervical cancer and include HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68,[156] there are viral and immunological risk factors which play a role.

5.1 Multiple HPV genotypes as risk factor

The clinical importance of co-infection with multiple HPV types remains a controversial area of investigation. A recent study reported that women infected with multiple HPV infections were at a lower risk of high risk cervical lesions compared to their single genotype infection counterparts[157], suggesting a possible cross protection triggered by multiple infections. [158] However, a recent large study on multiple HPV infections in Costa Rica, young non-HIV positive women with multiple infections were observed to be at significantly increased risk of CIN 2+, when compared with those with single infections. [159]There is also conflicting evidence on whether genetically similar HPV types reduce the likelihood of progression to high-grade lesions in HIV positive women .[160]

5.2 HIV as a risk factor for CIN 2+

HIV significantly impacts the natural history of HPV infection. Among HIV-infected women, rates of persistent HR- HPV infection have been shown to increase multifold. [161] Furthermore, among HIV-infected women the progression from persistent infection to CIN2/3 occurs within a shorter period of time and occurs more frequently than in HIV-negative women.[162, 163,164] When ICC does

develop in the setting of HIV, it tends to occur at 20-25 years younger and with less immunosuppression as compared with HIV-positive women with other AIDS-indicator conditions. [165] HIV-infected women with ICC may metastasize to unusual locations, have poorer responses to standard therapy, and have higher recurrences and death rates, compared with HIV-negative women of similar stage.[166]

Whilst it is speculated that ART may reduce ICC incidence and progression of cervical dysplasia as a result of improved immunologic function, the consequent prolonged survival, along with only partial restoration of immune function, could also result in an higher incidence of ICC due to increased longevity allowing time for disease progression.[167]The association between ART and HPV infection and cervical cancer is still controversial.[162] A recent systematic review evaluating the impact of ART on incidence of cancers suggests that the use of ART does not appear to reduce the risk of cervical cancer genesis.[168]

5.3 Bacterial vaginosis

A meta-analysis suggested a positive association between BV and cervical pre-cancerous lesions, with a significant overall estimated odds ratio of 1.51.[169] Alterations in inflammatory cytokine profile present in a disturbed vaginal environment could promote development of cervical lesions.[170] In a prospective study, Tavares-Murta et al (2008) reported that patients with BV and CIN presented a similar local cervical immune profile, as assessed by cytokine (IL-6, IL-8 and IL-10) and nitric oxide concentrations. [171] However, it has been reported that cervical inflammation is associated with CIN, and may be a confounder for high-grade cervical lesions in HPV-infected women [7] and as BV frequently coexists with cervicitis, [172] a disturbed vaginal microflora might therefore indirect predispose to cervical dysplasia.

Nevertheless, the majority of included studies had cross-sectional designs, which precludes imputing a causal role in cervical carcinogenesis. Whereas BV may influence onset and progression to cervical pre-cancerous lesions, it is also plausible that cervical dysplasia favors conditions for disruption of the normal vaginal environment and promotes an abundant growth of anaerobes.[169] Only one study was included in this meta-analysis was conducted in South Africa.[173]

Furthermore, in a cohort of HIV uninfected women in Uganda and Zimbabwe, it was estimated that 17% of HIV infections were attributable to BV and 12% to intermediate microbiota. Studies have shown that by causing vaginal inflammation, BV or with low levels of lactobacilli shed more HIV viral particles in their vaginal secretions,[174,175] which in term can increase cervical dysplasia progression.[176,177]

5.4 Cervicitis

Two studies have found a significant association between cervicitis and abnormal cytology.[178] [179] Marrazzo et al 2006 found a trend of increasing cervical inflammation associated with high-grade lesions in oncogenic HPV-infected women, (P_{trend} = 0.05) and overt cervicitis was associated with a 1.9-fold increase in risk of high-grade lesions (95% confidence interval, 0.90–4.1). The results of this study suggest that cervical inflammation may be associated with high-grade lesions and may be a confounder for high-grade cervical lesions in women infected with oncogenic HPV.

5.5 Allogeneic stem cell

Other immunocompromised populations are at increased risk of HPV-related SIL and cancers. A study suggested a high incidence of SIL in long term allogeneic stem cell survivors, which underscores the importance of cytological screening after transplantation. [180] The survival of an increased number of women undergoing allogeneic stem cell transplantations may portend an increased risk of genital or other HPV-related malignancies.[180]

5.6 Behavioral determinants for CIN 2+ progression

Pooled analyses of case-control data have established several risk factors for cervical cancer, including OC use, parity, smoking and lack of cytology-based screening.[181,182,183] However, a recent study on how these factors act at different stages of cervical carcinogenesis have suggested that CIN 2 and CIN3 are heterogeneous with respect to known cervical cancer risk factors and whereas hormonal factors are important for the progression from HPV infection to pre-cancer, smoking promotes progression from HPV infection to pre-cancer.[184]

Early age at first sexual intercourse is an important risk factor for cervical cancer.[185] Plummer et al 2012 reported an OR for ICC to be approximately proportional to the square of time since first intercourse (exponent 1.95, 95% CI: 1.76–2.15) up to age 45. [186] Plummer's model suggested that delaying first exposure to HPV by vaccination would have the same lifelong effect as delaying age at first sexual intercourse and that a delay of first HPV infection of 12 years from age 18 to 30 years would prevent almost all cervical cancers below age 40 and reduce the risk more than 3-fold above age 45.[186]

6. Cervical cancer prevention programs in sub Saharan Africa

6.1 Primary prevention

6.1.1 Prophylactic vaccines

Prophylactic vaccines for HPV are likely to greatly reduce the future burden of cervical cancer, particularly where screening is scarce, like in Sub Saharan Africa. The immunogenicity of both the bivalent (Cervarix, GlaxoSmithKline) and quadrivalent (Gardasil, Merck) vaccines that protects against HPV genotypes 6, 11, 16 and 18 **[187]** has also been recently observed in HIV-infected women. A recent study suggests that the quadrivalent HPV vaccination of HIV positive individuals

with the A5240 trial of 319 HIV-infected women in US, Brazil and South Africa are more immunogenic in women HIV viral load <10,000 copies/mL and/or CD4 counts >200 cells/mm³.[188]

A new vaccine in the cervical cancer prevention landscape is now being commercialized. A nonavalent vaccine containing additional HPV types HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 will have direct implications for cervical cancer incidence and prevention in all regions of the world with almost the potential to prevent 90% of ICC cases worldwide. In a recent estimation of the epidemiological burden of HPV-related anogenital cancer, precancerous lesions, and genital warts in women and men in Europe, Hartwig et al (2015) demonstrated how the large public health impact that was achieved by the first-generation HPV vaccines could be further increased by second generation vaccines.[189]

6.1.2 Target group and vaccination schedule

The primary target group in most of the countries recommending HPV vaccination is young adolescent girls, aged 9-14 and as of 2017, the CDC does not recommend the HPV vaccine over age of 26, regardless of HIV status.[190]

A study combining data from two large trials found similar protection against HPV 16 and 18 infections from a two-dose vaccination as from the current two and three dose schedules.[191]For both HPV vaccines, the vaccination schedule depends on the age of the vaccine recipient.[192] Females <15 years at the time of first dose: a 2-dose schedule (0, 6 months) is recommended. If the interval between doses is shorter than 5 months, then a third dose should be given at least 6 months after the first dose.[192] For females above 15 years at the time of first dose and immunocompromised and/or HIV-infected, a 3-dose schedule (0, 1 or 2, 6 months) is recommended.[192]

The latest recommendation of the advisory Committee for Immunization Practices of the CDC recommends 2 doses for the 9valent vaccine, based on a recent study which found that immunogenicity was non-inferior with 2 doses in persons aged 9 through 14 years compared with 3 doses in a group in which clinical efficacy was demonstrated (GRADE evidence type 3).[193]

6.1.2.1 One-dose schedule

Despite the lack of cross-protection, a single dose may be sufficient to vastly reduce the number of cervical cancers worldwide.[194] Apart from helping to overcome programmatic barriers in low-income settings, if HPV vaccines could be delivered as one dose, while retaining their efficacy against the most oncogenic HPV types 16 and 18, it may open a great opportunity to extend the reach of protection using HPV vaccines to more people.[195]

However, protection against phylogenetically related HPV types, which is probably attributable to cross-neutralizing antibodies, is likely to be lower with alternate vaccine schedule as compared with the standard three-dose schedule.[196]

6.1.3 HPV vaccination for boys

In 2011, the CDC began recommending HPV vaccination for boys, not only prevent transmission to girls, but also to protect themselves and women from anogenital and oropharyngeal cancer. [197] Consequently, worldwide, 15 high-income countries have adopted a gender neutral HPV vaccination policy.[198] In an middle-income country like Brazil, where coverage of girls is high, a cost-effectiveness of including boys *versus* girls alone in a pre-adolescent vaccination programme against HPV types 16 and 18, suggested that the added value of including boys will be relatively small compared with settings in which coverage of girls is low.[199] Current WHO recommendations to prioritize vaccine access for girls for the purpose of cervical cancer prevention is based on modelling data showing that, for the prevention of cervical cancer, reaching high coverage in girls

is more cost-effective than attaining lower coverage in both boys and girls.[200] By June 2016, 6% of low-income countries had introduced the HPV vaccine,[201] which underscores why consideration for adopting a gender-neutral immunisation policy should be a country-level decision based on factors such as disease burden, local sexual behaviour patterns, equity concerns, programmatic implications, cost-effectiveness, and affordability.[202]

Assuming affordable vaccine cost, the addition of a catch-up round is, worth considering in medium/low-income countries to extend vaccine benefits to less young adolescent girls whose future access to cervical screening is uncertain.[203]

6.1.4 Delaying sexual debut

Other primary prevention strategies to reduce HPV infection include delaying sexual debut, reducing the number of lifetime sexual partners, and increasing the use of condom through educational interventions.[204] Kahn et al (2012) found that a subset of adolescents believed they were at less risk for STIs other than HPV after HPV vaccination, which underscores the importance of strengthening educational interventions to prevent misperceptions and promote healthy behaviors after vaccination.[205]

6.2 Secondary prevention

Albeit the current HPV vaccines is expected to reduce the prevalence of the most prevalent oncogenic genotypes, recent data presented at the 31st HPV 2017 conference suggested that there may be emerging genotypes, not included in the current nonavalent vaccine, including HPV 51, 53 and 66.[206] This potential post-vaccination shift, in tandem with lack of elucidation of any potential cross-protection mechanisms conferred to these genotypes by the nonavalent vaccine suggests that secondary prevention may still be warranted.

Whilst HPV-16 and HPV-18 pose much higher cancer risks than any other HPV type and replacement by a nononcogenic type or an oncogenic type is not expected to have any major consequences in a general population[207], it is unclear what this shift in a HIV-infected population may entail. Moreover, due to the broader range of pHR/ HR HPV genotypes in HIV women and HIV-infected women ineligible for the HPV vaccine due to prior exposure to the HPV genotypes contained within the vaccines, secondary prevention consisting of screening and treatment of precancerous lesions will still be needed. In HIV-infected women in sub-Saharan Africa, the prevalence of the non-vaccine targeted types could increase, reflecting the distribution of regional HPV types and the co-infecting genotype patterns. [208]

In many low-income countries, there are no screening programs, and women present late for care and treatment. The WHO recommends that planners should take into account that cervical cancer is rare before the age of 30 years and screening younger women will detect many lesions that will never develop into cancer, will lead to considerable overtreatment, and is not cost-effective. [37] However, global change in sexual behavior, including earlier exposure and multiple sexual partners is likely to change this threshold.

New guidance from WHO in December 2014 advocates for a "screen and treat" approach in resource poor settings, in which access to Visual Inspection with Acetic acid or HPV testing for screening, is followed soon or immediately by treatment of detected precancerous lesions.[209] This approach contrasts with conventional screening approaches by forgoing confirmation of a diagnosis prior to treatment by colposcopy and cervical biopsy, which are expensive, labor-intensive and reliant on well-functioning clinical, laboratory and referral systems.

In 2014, the WHO recommended, that "once a woman has been screened negative by means of HPV tests, she should not be rescreened for at least 5 years, nevertheless she should be rescreened within ten".[210] The WHO recommends cytological/VIA screening within 3 years for

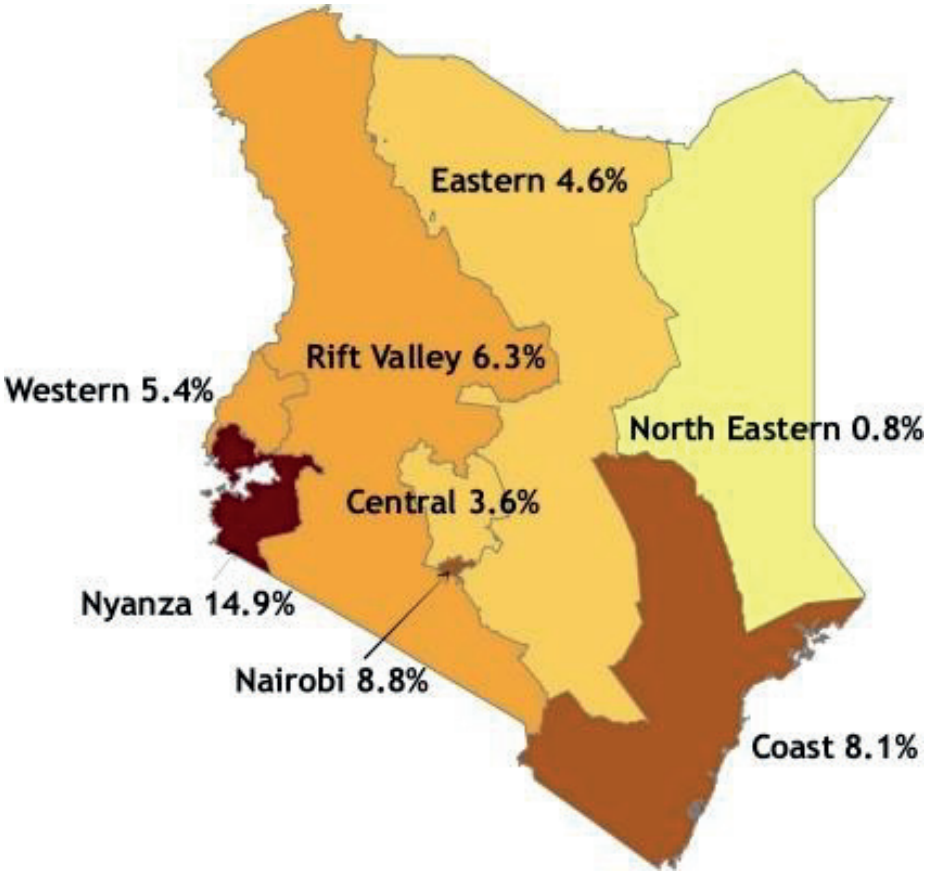
women living with HIV,[211] however there has been no evidence to support that this guideline is appropriate in all settings, especially for HIV-infected women living in resource-constrained conditions. This contrasts with the CDC 2009 recommendation for higher income countries that women with HIV infection should have more frequent screening with cervical cytology: twice in the first year after diagnosis of HIV and, if normal, annually thereafter.[212]

6.3 Tertiary prevention

Tertiary prevention consists of cryotherapy, which involves the freezing of cervical lesion by application of liquid nitric oxide or carbon dioxide [213] performed by mid-level providers in low-income settings has been used for the management of up to 85%-90% of abnormalities detected by screening. [214] Women with cryotherapy-ineligible VIA-positive pre-cancerous lesions are referred for further evaluation and therapy excision methods[215], including, cold knife conisation, and thermal excision called either large loop excision of the transformation zone (LLETZ) or loop electrosurgical excision procedure (LEEP). In HIV-infected women, premalignant lesions tend to be larger and involve the endocervical canal, which makes it more likely that LEEP is needed. [216] Estimates for the incidence of disease recurrence after treatment vary widely from 25% to 55% at 12 months in HIV-infected women [217] [218] compared with 5%–16% in HIV-negative women.[219] [220]

Women with severe cervical dysplasia that remains undiagnosed or untreated can develop ICC [221] Albeit, early diagnosis and treatment of cervical pre-cancerous lesions prevents up to 80 % of cervical cancers in high resource countries where cervical cancer screening is routine,[222] in sub Saharan Africa, studies have found that a significant percentage of women (56–80.6 %) in Kenya, Tanzania, and Nigeria are only identified once their cervical cancer is at an advanced stage. [223] Women with severe cervical dysplasia that remains undiagnosed or untreated can develop ICC.[224] Furthermore, a study in Tanzania reported that women co-infected with HIV are less likely to be treated. [225]

Staging the severity of ICC requires a combination of clinical and endoscopic procedures to determine the stage of progression.[226] Inadequate laboratory facilities and personnel shortages may result in treatment decisions being made without proper diagnoses or adequate information. Furthermore, the lack of surgical facilities and external and intracavitary radiotherapy infrastructure required for performing hysterectomy and radiotherapy[227] limit treatment options typically experienced outside of African capital cities.[228]



Map of Kenya, with provincial HIV prevalence among all adults aged 15-64 years.

II Methodology

7 Overview of methods

7.1 Justification for the studies

7.1.1 Study area

Kenya is one of the countries in the world most severely affected by the HIV epidemic. With an estimated adult HIV prevalence of 5.6%, in 2012, there was an estimated 1.2 million people living with HIV in Kenya.[229] According to the 2012 Kenya AIDS Indicator Survey, fewer than half of all HIV-infected adults in Kenya know their HIV status and less than two-thirds of individuals eligible for therapy are on HAART.[229] In Kenya, as in many parts of sub-Saharan Africa, FSW bear the greatest burden of HIV infection, and as early as 1985, a study reported that HIV prevalence was as high as 61% among a group of FSW in Nairobi.[230]

Kenya has also recently witnessed a geographic spread of the HIV epidemic. More than 75% of Kenyans live in rural areas, and, in recent years, estimated HIV prevalence in rural areas has begun to reach levels estimated within urban settings—suggesting that the population in need of HIV care in Kenya is largely, and increasingly, rural.[231] Rural women living with HIV often face oppression in their relationships with male partners and within the wider community because of their gender, HIV status, economic and social marginalization.[232] As a result, their vulnerability makes them more at risk for sexual and other gender-based violence, which in turn makes them more likely to contract other STIs.

From the onset, HIV care and treatment throughout Kenya was mainly provided in provincial and district hospitals in urban settings. However, with a view to improving patient access to services and reducing staff burden in large facilities, HIV care was decentralized, resulting in an increase in services from 15 health facilities in 2003 to over 700 facilities by December 2008.[233] A pivotal aspect of this process has been the establishment of HIV clinics at health centers and

dispensaries, close to those in need as well as the integration of HIV programs with other services provided at the health facilities.[233] A recent study reported that death rates were comparable between primary and secondary health facilities, whereas loss to follow up among pre-ART patients was lower at primary health facilities, suggesting that decentralization was successful at expanding HIV care.[234]

7.2 Problem statement

Amidst the roll out of the quadrivalent vaccine, there is a scarcity of data on the distribution of pHR/HR HPV genotypes among HIV-infected women with abnormal cytology and ICC in Kenya. Yet, with the advent of preventive HPV vaccines that target HPV 16 and 18, and the nonavalent vaccine targeting 90% of all ICC cases, such HPV genotype distribution data are indispensable for predicting the impact of vaccination and HPV screening on prevention.

Nevertheless, a successful vaccination program will not obviate the need for women to undergo screening to detect other oncogenic HPV genotypes. The WHO now recommends that “once a woman has been screened negative, she should not be rescreened for at least 5 years, but should be rescreened within ten”.[235] Whereas some advocate treatment with cryotherapy after a positive VIA test ('see and treat'), others advocate for cryotherapy after VIA and positive colposcopy ('see-see and treat').

There is current scant evidence to support the adequateness of this guideline in HIV-infected women in high BV settings. In Sub-Saharan Africa, the region with the highest HIV/AIDS burden, substantial international funding along with evidence-based clinical practice have resulted in an unparalleled scale-up of access to antiretroviral treatment at a higher CD4 count. In addition, the role and timing of HAART in mediating cervical disease remains unclear, which is all the more critical since the most recent revision of the policy recommending treatment initiation for all all HIV

infected adults, regardless of WHO clinical stage or CD4 cell count, following the results of the START trial. [209]

Given the scarcity of resources, screening programs will need to be tailored to the human and financial resources of the region. Evidence-based locally relevant primary and secondary cervical cancer prevention protocols within the new HIV and cervical cancer prevention landscape in Kenya must be developed.

7.3 Study objectives

The overall aims of this thesis are: firstly, to assess the pooled prevalence of pHR/HR HPV genotypes and overall burden in HIV-infected women (FSW and general population) in Kenya in order to inform primary and secondary cervical cancer prevention; secondly, to explore risk factors for pHR/HR HPV genotypes; thirdly, explore HPV-related risk factors for CIN 2+; fourthly, systematically synthesize literature on HAART and the epidemiological association between HR HPV genotypes, cervical dysplasia and ICC.

7.4 Ethical considerations

Staff obtained written informed consent from patients, collected demographic and behavioural data using structured questionnaires. All human subject protocols were approved by the Ethics Committee at the Kenyatta National Hospital and the Institutional Research and Ethics Committee at the MOI University, (Ref: KNH-ERC/01/3618) and (No 000187) respectively. For the two systematic reviews, no ethical approval was required as it consisted of an analysis of secondary data.

7.5 Inclusion and exclusion criteria

Meta-analysis on the prevalence of pHR/HR HPV genotypes in the HIV-infected female population in Kenya

Studies were eligible if they reported overall pHR/HR HPV frequency as well as specific pHR/HR HPV genotype frequency in both a general HIV-infected study population or in a HIV-infected FSW study population. Studies conducted on males, studies that did not provide pHR/HR HPV infection data by HIV status or if it was not possible to calculate the above information from data given. Another inclusion criterion was that HPV testing had to be performed through validated and commercial or PCR testing.

HPV-related risk factors for CIN 2+; Epidemiology of pHR/HR HPV genotypes

In the cohort study which took place in Mombasa, Kenya, only HIV-infected women aged with abnormal cytology between 18 and 50 years, between November 2005 and April 2006 were eligible. In the study population of FSW in Western Kenya, recruited between 2010 and 2016, the inclusion criteria for the study were being HIV-negative or HIV-infected females >18 years of age and engaging in transactional sex in exchange for money, goods, services, or drugs in the last 3 months. In these three studies, a history of hysterectomy, and pregnancy were the only exclusion criteria.

Systematic review on the association of ART on HPV infection, cervical dysplasia and ICC in sub Saharan African women

Article selection criteria included any clinic-based randomized-controlled trials, meta-analysis/systematic reviews, observational or population-based linkage studies documenting both ART status and HPV/ICC rates in Sub-Saharan Africa.

7.6 Sample size

For objective 2 and 3, a sample size was calculated to allow for a prevalence of at least 15 % for abnormal cytology,[236] resulting in 74 HIV-infected women with abnormal cytology being recruited from the initial cohort and 87 FSW with abnormal cytology.

7.7 Data management and analysis

Data manipulation for cross sectional studies:

Age was dichotomized into ≥30 years and <30 years; this categorization was used to reflect the WHO 2014 guideline concerning cervical screening. The number of pHR/HR HPV co-infections was also dichotomized as a categorical variable with 1 and ≥2 genotypes. Condom use was dichotomized as always or irregular, according to self-report. CD4 cell count was analyzed both as a continuous variable and as two categorical variables, using a cut off of CD4 count <200 cells/µl (vs ≥ 200 cells/µl) and CD4 count <350 cells/µl (vs ≥350 cells/µl), when HIV-infected women are severely immunosuppressed and moderately immunosuppressed, respectively. The number of sexual partners was also dichotomized as per other studies (up to 5 versus 6 or more) to make the studies more comparable to others and to explore the risk from a public health point of view. After cleaning the data, it was entered into Excel and exported and analyzed in STATA 12 and 13.

Assessment of heterogeneity in the meta-analysis

Due to the anticipated heterogeneity between study populations, the Der Simonian and Laird random-effects model to pool overall HPV prevalence. The Q test was performed to assess the presence of heterogeneity and the I2 index to quantify the extent of heterogeneity. A leave-one-out sensitivity analysis was performed by iteratively removing one study at a time while

recalculating the co-infection prevalence rate to assess the robustness of pHR/HR HPV prevalence. Forest plots were produced to depict the pooled estimate of the distribution of genotypes among the FSW and non FSW population.

7.8 Confounding, heterogeneity and interactions

Confounding may provide an alternative explanation for results obtained and need to be considered within observational studies. A confounder has three key characteristics: it is a risk factor for the outcome, associated with the exposure and it is not an intermediate factor in the causal pathway. To control for confounding, multivariate regression models were fitted.

Epidemiology of HPV Genotypes among HIV-infected women in Kenya: A Systematic Review and Meta-Analysis: Due to lack of individual information for clinical parameters, including age, CD4 cell count, HIV viral load or antiretroviral treatment a meta-regression could not be performed. To explore heterogeneity, a sub-analysis was performed. A pre-2004 period versus 2004-2008 dichotomization was elected to reflect modifications in PCR sensitivities, screening protocols, and the 2003 review of the epidemiological classification of oncogenic HPV genotypes.[51] The sub analysis did not reveal any statistically significant differences between pre 2004 and post 2004 suggesting that heterogeneity may not have significantly caused by an increase of PCR sensitivity, a wider range of pHR/ HR HPV genotypes testing over time, and a review of classification of HPV genotypes. However, sensitivity and specificity within PCR-based methods vary largely, aside from changes due to the development of techniques over time and may also depend on how the specimen in processed.[237] Also of consideration is that some tests are performed at analytical sensitivity, while others have been validated clinically and use a clinical threshold, hence contributing to the variability of PCR outcome.

Epidemiology of pHR/HR HPV genotypes: A final multivariable logistic regression model was derived using a forward stepwise modeling procedure where covariates, concomitant STIs, age, and the number of sexual partners in the past week were added to the model in an iterative manner. The same method was also used to explore the simultaneous effect of covariates. To assess for a potential interaction effect due to age, logistic regression models were fitted with and without the interaction term.

Epidemiology of CIN 2+ in HIV-infected women: A multivariable logistic regression analysis was performed to simultaneously control for age, presence of pHR/HR HPV co-infections, CD4 count and/or concurrent BV, TV, and Candida to assess the adjusted association for various risk factors. To assess for a potential interaction effect due to age, logistic regression models were fitted with and without the interaction term.

Overview of papers and their respective objectives, study population and data analysis employed.

Paper	Study Design and Objectives	Study population Sample size	Data analysis method
Objective 1/ Paper 1: "Epidemiology of HPV genotypes among HIV positive women in Kenya: A systematic review and meta-analysis" (PLos ONE)	Meta-analysis of pooled prevalence of pHR/HR HPV genotypes in HIV-infected women in Kenya	1244 HIV-pHR/HR HPV co-infected women (FSW and non FSW)	The random-effects model was used to pool overall HPV prevalence. The Q test was used to assess the presence of heterogeneity and the I^2 index to quantify the extent of heterogeneity
Objective 2/ Paper 2: "Distribution of human papillomaviruses and bacterial vaginosis in HIV positive women with abnormal cytology in Mombasa, Kenya" (Infectious agents and Cancer)	Cross sectional design; Risk factors for pHR/HR HPV genotypes in Kenya	74 HIV-infected women with abnormal cytology from Mombasa	A multivariate analysis was fitted adjusting for CD4 count, age and pHR/HRHPV co-infections
Objective 2/Paper 3: "Associations between Vaginal Infections and pHR/HR HPV genotypes in FSW in Western Kenya" (Clinical Therapeutics**)**	Cross sectional design; Risk factors for abnormal cytology	616 HIV-uninfected and infected FSW from Western Kenya	A multivariate analysis was fitted, adjusting for age, STIs and multiple pHR/HR HPV genotypes
Objective 3/ Paper 4 "Multiple HPV infections in female sex workers in Western Kenya: implications for prophylactic vaccines within this sub population (Infectious agents and Cancer)	Cross sectional design; Risk factors for abnormal cytology	616 HIV-uninfected and infected FSW from Western Kenya	A multivariate analysis was fitted, adjusting for concomitant STIs, age, and the number of sexual partners in the past week
Objective 3/ Paper 5; "HPV correlates of moderate to severe cervical dysplasia in HIV-infected women in Mombasa, Kenya: a	Cross sectional design; Risk factors for CIN 2+	74 HIV-infected women with abnormal cytology from Mombasa	A multivariate analysis was fitted, adjusting for age, the number of sexual partners and CD4 count < 200 µl and CD4 count ≥350 cells/µl

cross-sectional analysis" (in press: BMC Virology)			
Objective 4/ Paper 6; "Associations between HAART on the presence of HPV, pre-malignant and malignant cervical lesions in SSA, a systematic review: Current Evidence and Directions for Future Research" BMJ Open Access	Systematic review on the impact of HAART on HPV infection, cervical dysplasia and ICC	19,345 HIV infected women	Narrative synthesis of the 22 studies included, of which 7 prospective studies literature. Adherence to the PRISMA methodology
Conclusion/ Paper 7 ("Public Health Approach to Preventing Cervical Cancer in HIV-infected women in Kenya, Issues to Consider in the Design of Prevention Programs") Gynecology Oncology reports	Narrative review article which summarizes the risk factors to be considered when designing a primary and secondary cervical prevention program in a post-vaccination era for HIV-infected women in Kenya	HIV-infected women in Kenya and in sub Saharan Africa	A literature search of PUBMED, EMBASE, SCOPUS, and PROQUEST and a synthesis of all eight papers.

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III Results

This sections includes paper I to VI

EPIDEMIOLOGY OF HPV GENOTYPES AMONG HIV POSITIVE WOMEN IN KENYA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Epidemiology of HPV genotypes among HIV positive women in Kenya: A systematic review and meta-analysis

Abstract

Background

There is a scarcity of data on the distribution of human papillomavirus (HPV) genotypes in the HIV positive population and in invasive cervical cancer (ICC) in Kenya. This may be different from genotypes found in abnormal cytology. Yet, with the advent of preventive HPV vaccines that target HPV 16 and 18, and the nonavalent vaccine targeting 90% of all ICC cases, such HPV genotype distribution data are indispensable for predicting the impact of vaccination and HPV screening on prevention. Even with a successful vaccination program, vaccinated women will still require screening to detect those who will develop ICC from other High risk (HR) HPV genotypes not prevented by current vaccines. The aim of this review is to report on the prevalence of HR HPV types and multiple HR HPV genotypes in Kenya among HIV positive women with normal, abnormal cytology and ICC.

Methods:

PUBMED, EMBASE, SCOPUS, and PROQUEST were searched for articles on HPV infection up to August 2nd 2016. Search terms were HIV, HPV, Cervical Cancer, Incidence or Prevalence, and Kenya.

Results:

The 13 studies included yielded a total of 2116 HIV-infected women, of which 89 had ICC. The overall prevalence of HPV genotypes among HIV-infected women was 64% (95%CI: 50%-77%). There was a borderline significant difference in the prevalence of pHR/HPV genotypes between Female Sex workers (FSW) compared to non-FSW in women with both normal and abnormal cytology. Multiple pHR/HPV genotypes were highly prominent in both normal cytology/HSIL and ICC. The most prevalent HR HPV genotypes in women with abnormal cytology were HPV 16 with 26 %, (95%CI: 23.0%-30.0%) followed by HPV 35 and 52, with 21% (95%CI: 18%-25%) and 18% (95%CI: 15%-21%), respectively. In women with ICC, the most prevalent HPV genotypes were HPV 16 (37%; 95%CI: 28%-47%) and HPV 18 (24%; 95 %CI: 16%-33%).

Conclusion:

HPV 16/18 gains prominence as the severity of cervical disease increases, with HPV 16/18 accounting for 61% (95%CI: 50.0%-70.0%) of all ICC cases. A secondary prevention program will be necessary as this population harbors multiple pHR HPV co-infections, which may not be covered by current vaccines. A triage based on FSW as an indicator may be warranted.

Background:

Human papillomavirus (HPV) is a sexually transmitted infection, and high-risk (HR) HPV DNA has been shown to be present in 99.7% of cervical cancers worldwide [1]. Over 200 HPV genotypes have been identified and are divided into high-risk (HR) and low-risk (LR) carcinogens depending on their potential to cause cancer. HR types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68 and 59. Others are classified as potential High risk (pHR) (types 53, 66, 70, 73, 82). HPV 16 and HPV 18 are the most virulent HR-HPV genotypes, causing about 70% of all invasive cervical cancer (ICC) in the world [2].

Apart from a higher prevalence and broader range of HR HPV, HIV immunosuppression has been linked to multiple HPV infections [1, 3-5]. The inability to clear HPV infections and the reactivation of latent HPV infections, a result of immune suppression, have been attributed to multiple HPV genotype co-infections [6, 7].

Moreover, HIV positive women are more at risk for progression to cervical intraepithelial neoplasia grade 3 (CIN3). In 2007, the World Health Organisation (WHO) included ICC to the stage "4" of the AIDS classification of its clinical staging and case definition of HIV for resource-constrained settings [8].

Several countries, including Kenya, have licensed and adopted the bivalent HPV vaccine (Cervarix™) that protects against HPV genotypes 16 and 18 and the quadrivalent vaccine (Gardasil™) that protects against HPV genotypes 6, 11, 16 and 18 [9]. However, HPV vaccine uptake has been limited, with a recent longitudinal study in Eldoret, Kenya, reporting that only 31 % (79/254) of those who entered the follow-up study to have been vaccinated [10].

In 2014, a nonavalent vaccine, not yet commercialized in Kenya, containing additional HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 antigens will have direct implications for cervical cancer

incidence and prevention in all regions of the world with the potential to prevent almost 90% of ICC cases worldwide.

Whilst the immunogenicity of both the bivalent and quadrivalent vaccines has also been recently documented in HIV positive women, the effectiveness of these vaccines in curbing the incidence of ICC is contingent upon the prevalence of oncogenic vaccine genotypes in HIV positive women [11, 12].

Considering the very high incidence of HIV and ICC observed in sub-Saharan Africa, the HPV type distribution is still not well characterised among HIV-infected populations in the region [13]. Whilst in some European populations a positive association of HIV infection and ICC has been reported, the picture lacks clarity in Africa [14-16]. Additionally, with the establishment of HAART programmes and consequent increase in life expectancy in HIV-positive women, a change in the pattern of the burden of HIV-related cancers can be expected for these patients.

Preceded only by breast cancer, ICC is the second most prevalent cancer among women in Kenya, and its incidence is increasing [17]. Despite high ICC prevalence, cervical screening uptake is low, with a 2014 cross sectional study in the Kisumu East District of Nyanza Province reporting a 17.5% screening uptake [18]. A successful vaccination program will not obviate the need for women to undergo screening to detect other oncogenic HPV genotypes. The WHO now recommends that “once a woman has been screened negative, she should not be rescreened for at least 5 years, but should be rescreened within ten” [19]. However, there is current scant evidence to support the adequateness of this guideline in HIV-infected women.

Given the scarcity of resources, screening programs will need to be tailored to the human and financial resources of the region, and a triage for HPV screening among vulnerable women envisaged. In Kenya, as in many parts of sub-Saharan Africa, female sexual workers (FSW) bear

the greatest burden of HIV infection, and as early as 1985, a study reported that HIV prevalence was as high as 61% among a group of FSW in Nairobi [20]. A recent study estimated that 5 percent of the urban female population of reproductive age could be sex workers, making this population particularly at risk for HIV-HPV co-infection. Some published studies have suggested differential prevalence of pHR/HR HPV infection among FSW, however there are no pooled estimates for this population [21-23].

The overarching aim of this review is to guide primary and secondary prevention of cervical cancer programs in Kenya and has as objectives: 1) determine the prevalence of pHR/HR HPV genotypes among HIV-infected women with normal cytology to ICC; 2) explore the differential prevalence of pHR/HR HPV infections among FSW and non FSW women; 3) establish the pooled estimates for different pHR/HR HPV genotypes in these populations; 4) determine the pHR/HR HPV genotypes and multiple HPV genotypes in Kenya among HIV positive women with normal cytology/abnormal cytology to ICC.

Method

Search Strategy and Selection Criteria

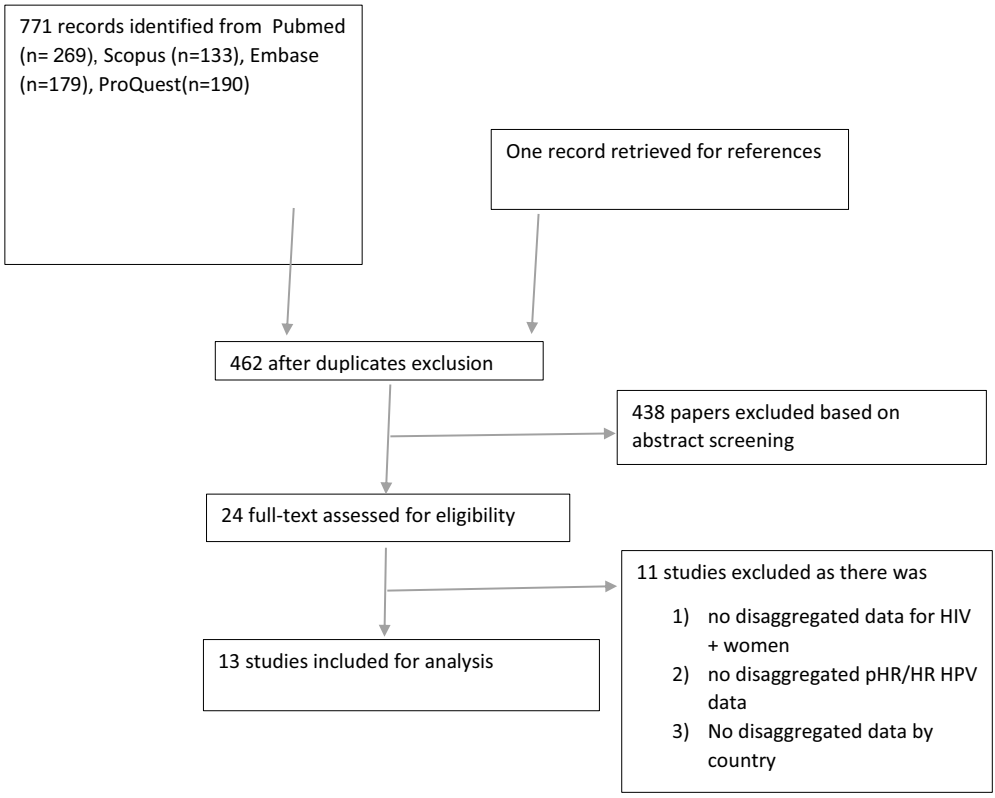
The search strategy was designed by a medical librarian to identify studies reporting HPV genotypes associated with normal, abnormal cytology and cervical cancer in HIV infected women living in Kenya. S1_Search Strategy. We conducted this systematic review and meta-analysis based on a pre-defined search protocol that conformed to the criteria set out by the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) group and was in accordance with the PRISMA statement [24, 25]. (S2_ PRISMA Checklist)

We systematically searched PubMed, EMBASE, PROQUEST, and SCOPUS without any language restrictions. Reference lists of all retrieved articles and previous systematic reviews were checked for further eligible publications up to August 2nd 2016.

The domains of the search terms were HIV, HPV, cervical Cancer, incidence or prevalence, and Kenya. We combined HPV and cervical cancer with the Boolean operator "OR", and the result was combined with the other terms with "AND". Full search strategy for the databases is given in S1 Literature search strategy.

Studies were eligible if they reported our main outcomes of interest, overall pHR/HR HPV frequency as well as specific pHR/HR HPV genotype frequency in both a general HIV study population or in HIV infected FSW study population. If the HPV prevalence was not disaggregated into pHR/HR HPV genotypes, or no frequency of pHR/HR HPV genotype, multiple pHR/HR HPV genotypes or/and no disaggregated frequency of pHR/HR HPV genotype provided for Kenya, the authors of the retrieved articles were contacted. Another inclusion criterion was that HPV testing had to be performed through validated and commercial or WHO CE labelled PCR testing**.** Studies were included if their design was a cohort, cross-sectional, case-control or case-case study. Potential duplicate data were searched for.

We excluded studies conducted on males, studies that did not provide pHR/HR HPV infection data by HIV status or if it was not possible to calculate the above information from data given. For flow diagram, see (Fig 2)



For the first research question, data were extracted on the total number of HIV infected people with any pHR/HR HPV genotype. The prevalence among HIV infected women was calculated using the total number of women with pHR/HR HPV genotypes divided by the total number of HIV-infected women. We did not disaggregate by cytological status as we were interested in obtaining a general picture of the burden among HIV-infected women in Kenya, which would include women with ICC.

For the second research question, the pooled prevalence of pHR/HR HPV genotypes was compared between FSW and non FSW. As no cases of ICC have been observed among FSWs and ICC cases having a very high pHR/HR HPV genotype, the denominator included only HIV infected women with normal and abnormal cytology.

For the third research question, due to the small number of studies and study population, the frequency of pHR/HR HPV genotypes were only broken down into three groups: normal, abnormal cytology and ICC. Indeterminate cytological results were excluded, and inflammation was categorized as normal cytology.

For the fourth research question, the number of multiple infection pHR/HR HPV genotypes in HIV-infected women with normal to abnormal cytology was compared to the number of multiple infections in women with ICC. Their respective population was used as a denominator.

Subgroup analyses:

A sub-analysis was performed to assess differential prevalences during the total time of sample collection 1994-2008, by categorizing observations as pre-2004 or 2004-2008. This dichotomization was chosen to reflect changes in PCR sensitivities, changes of screening protocols, and the 2003 review of the epidemiological classification of oncogenic HPV genotypes [2].

Data Abstraction

All studies were independently reviewed and critically evaluated for inclusion by two authors (SM and WA). All data was extracted independently and in duplicate manner by two investigators (SM and WA). The following items were recorded: first author, study period, publication year, study type, type of sampling, sample collection method, study population, total sample size, HIV and pHR/HR HPV prevalence of infected women, mean and standard deviation /median age with IQR

number of women with specific pHR/HR HPV types if available, normal/abnormal cytology, invasive cervical cancer (ICC), HPV detection method, and data on FSW.

Statistical Analysis

The HPV prevalence, type specific prevalence, 95% CI of prevalence of HPV infection, and specific HPV genotype were calculated according to Wald method. We used the DerSimonian and Laird random-effects model to pool overall HPV prevalence, as we expected the level of heterogeneity to be significant. The Q test was used to assess the presence of heterogeneity and the I^2 index to quantify the extent of heterogeneity; $p<0.10$ was considered indicative of significant heterogeneity. To assess the robustness of HR HPV prevalence in HIV infected women, we performed a leave-one-out sensitivity analysis by iteratively removing one study at a time while recalculating the co-infection prevalence rate. Forest plots were produced to show the pooled estimate of the distribution of genotypes. Analysis was undertaken using STATA version 13 (Corporation, College Station, TX, USA). The STATA command Metaprop was used as it provides appropriate methods for dealing with proportions close to 0 [26].

Ethical approval:

No ethical approval was required as this was a meta-analysis (analysis of secondary data).

Results

Search results and study characteristics

On August 2nd, 2016, we retrieved 771 studies from PUBMED, EMBASE, SCOPUS, and PROQUEST of which 310 were duplicates. We title/abstract-screened 461 articles, of which 29 studies were eligible for full text screening. Finally, 13 studies were included for this review (Fig. 2). For pooling the pHR/HR HPV prevalence, all 13 studies reported this prevalence and hence were eligible. For pooling the different pHR/HR HPV genotypes, only 10 studies provided data on different HPV genotypes. Of these 10 studies, one could not be used due to lack of disaggregated

pHR/HR HPV genotype data. Another study did not break down the HPV genotypes according to cytological results, and another study did not provide geographically disaggregated data, and so were also excluded from our analysis.

From the 7 eligible studies, 5 studies reported on pHR/HR HPV genotypes in HIV infected women with normal cytology and 6 reported on abnormal cytology. Three studies reported on frequency of pHR/HR HPV genotypes in HIV infected women with ICC.

From the 13 eligible studies, data on multiple pHR/HR HPV genotypes were only reported in 7 studies. Data were broken into two groups, normal/abnormal cytology versus ICC to juxtapose the difference in multiple pHR/HR HPV genotypes prevalence.

The 13 studies included in this meta-analysis yielded a total number of 2116 HIV positive women with individual sample sizes ranging from 46 to 498 (median = 74 women). Seven studies were based on convenient samples from gynaecology, HIV and family planning clinics in Kenya, (Rahman et al 2011, Yamada et al 2008, Vuyst et al 2003 & 2012a & 2012b, Temmerman et al 1999 and Menon et al 2016); four studies included random samples from HIV clinics (Chung et al 2013, Maranga et al 2013, Vuyst et al 2012, Luque et al 2010); one study used peer leader recruitment (Patel et al 2010), and another used snowball sampling of FSW (Luchters et al 2010). Different protocols and techniques were used to determine HPV infection throughout Kenya.

Meta-analysis results

Among the total 2116 HIV positive women, 1244 women were diagnosed with pHR/HR HPV infections. See S3_ Results. Pooled prevalence of pHR/ HR HPV genotypes among HIV infected women was 64% (95%CI: 50%-77%) and displayed significant heterogeneity, I^2 = 98.1 %, p<0.000. Pooled HPV prevalence was not substantially sensitive to the exclusion of any single study test. (Fig 3)

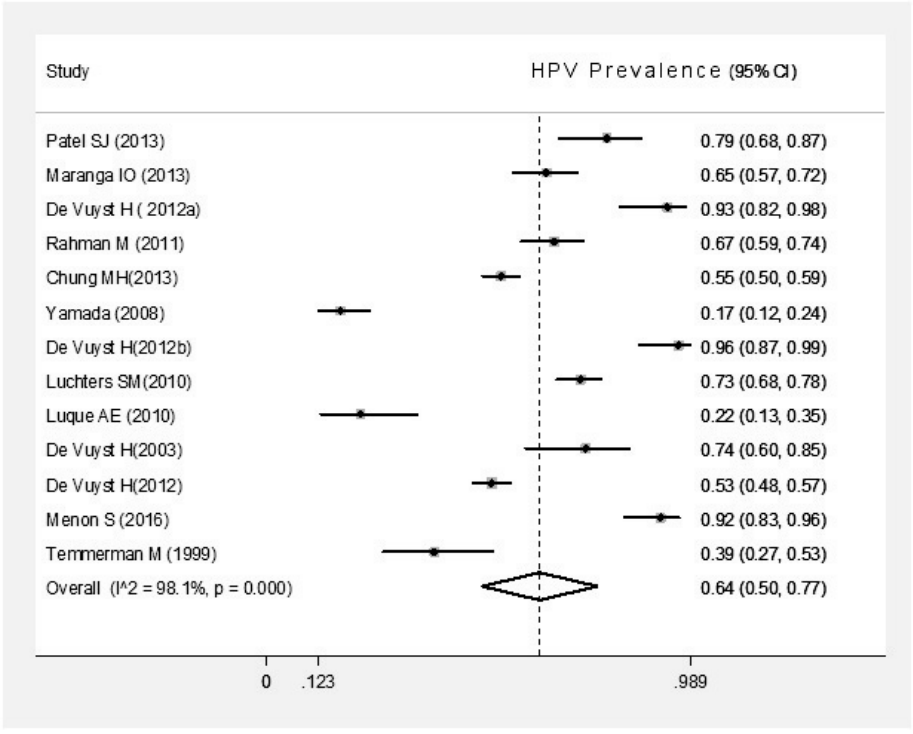


Fig 3 Pooled pHR /HR HPV prevalence among HIV-positive women

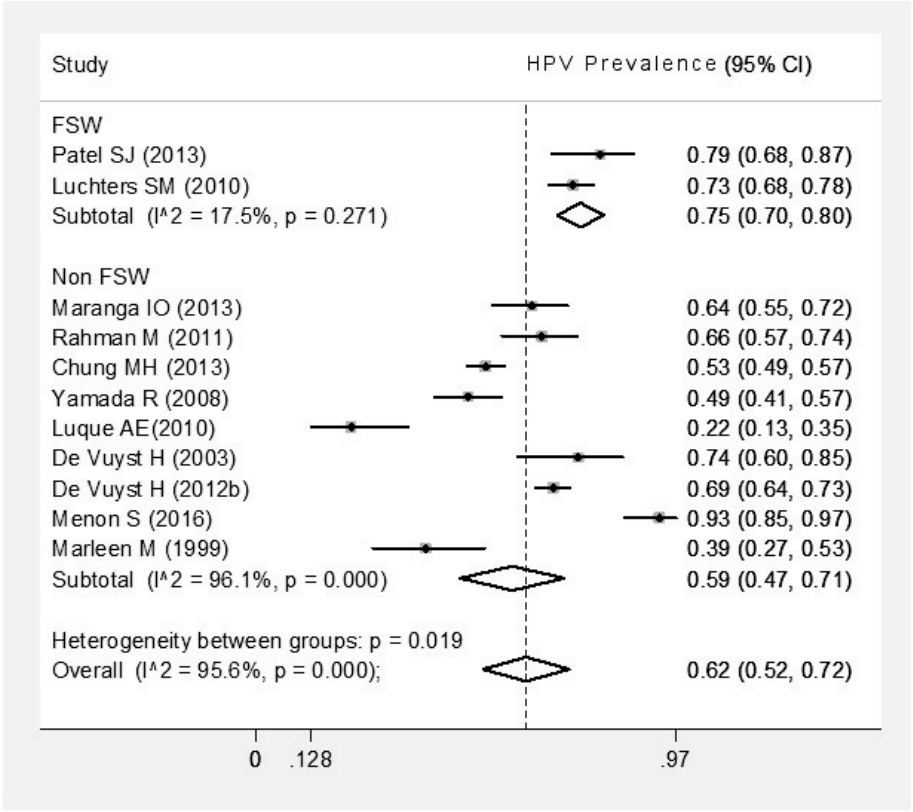


Fig 4: pHR/HR HPV prevalence in FSW versus non FSW

There was a borderline statistically significant pooled HR HPV prevalence among FSW. Pooled HR HPV prevalence among FSW was 75% (95%CI: 70%-79%) compared to 57 % (95%CI: 45%-70%) among non FSWs. (Fig 4)

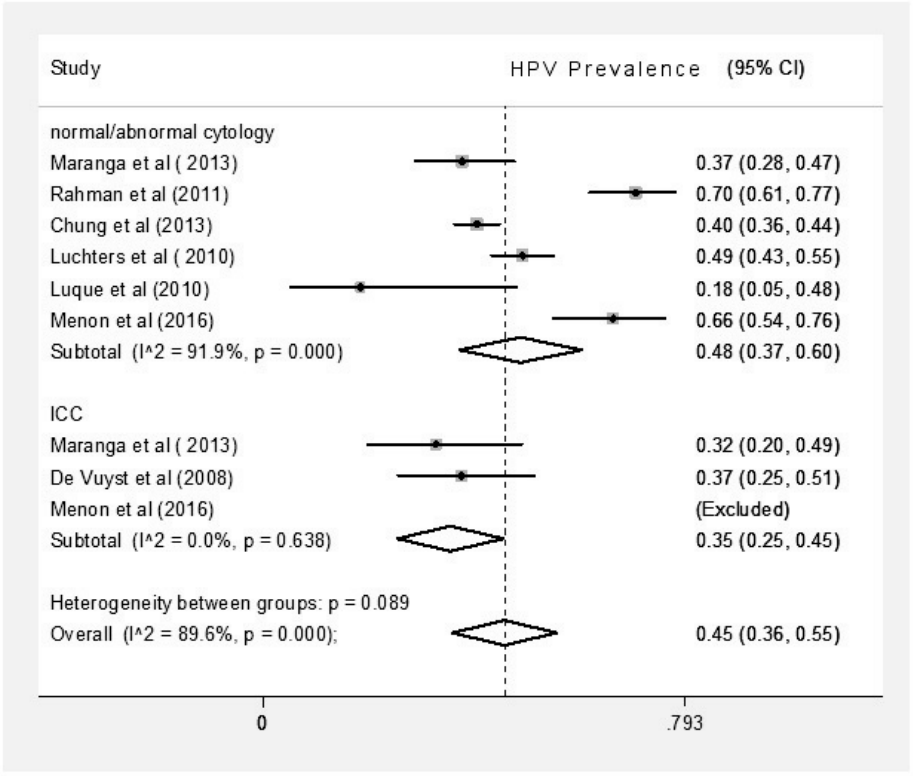


Fig 5 Prevalence of multiple pHR/HR HPV infection among HIV positive women, separated for women with normal cytology to HSIL and for women with ICC

Both HIV infected women with normal cytology to HSIL and ICC had a high prevalence of multiple pHR HPV genotypes, 42% (95%CI: 35%; 49%) versus 35% (95%CI: 25%; 45%). (Fig 5)

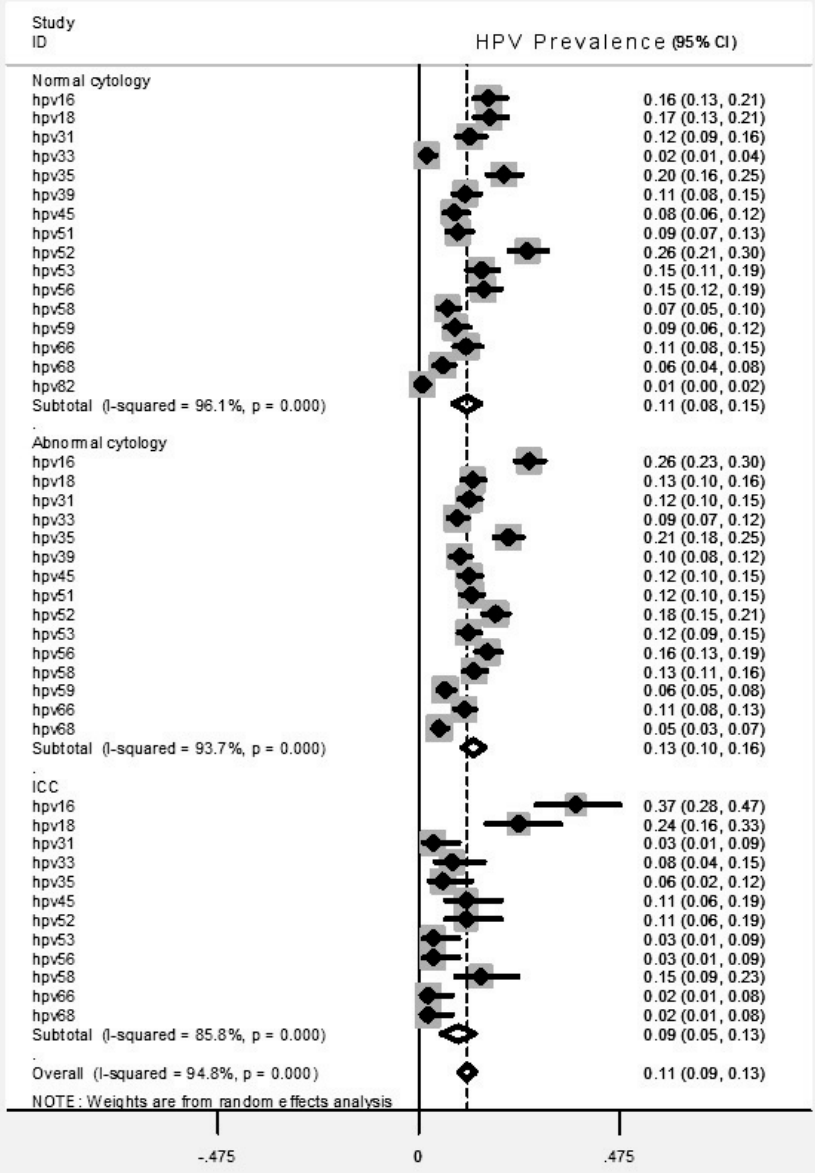

Study
ID
HPV Prevalence (95% CI)
Normal cytology
hpv16 0.16 (0.13, 0.21)
hpv18 0.17 (0.13, 0.21)
hpv31 0.12 (0.09, 0.16)
hpv33 0.02 (0.01, 0.04)
hpv35 0.20 (0.16, 0.25)
hpv39 0.11 (0.08, 0.15)
hpv45 0.08 (0.06, 0.12)
hpv51 0.09 (0.07, 0.13)
hpv52 0.26 (0.21, 0.30)
hpv53 0.15 (0.11, 0.19)
hpv56 0.15 (0.12, 0.19)
hpv58 0.07 (0.05, 0.10)
hpv59 0.09 (0.06, 0.12)
hpv66 0.11 (0.08, 0.15)
hpv68 0.06 (0.04, 0.08)
hpv82 0.01 (0.00, 0.02)
Subtotal (I-squared = 96.1%, p = 0.000) 0.11 (0.08, 0.15)
Abnormal cytology
hpv16 0.26 (0.23, 0.30)
hpv18 0.13 (0.10, 0.16)
hpv31 0.12 (0.10, 0.15)
hpv33 0.09 (0.07, 0.12)
hpv35 0.21 (0.18, 0.25)
hpv39 0.10 (0.08, 0.12)
hpv45 0.12 (0.10, 0.15)
hpv51 0.12 (0.10, 0.15)
hpv52 0.18 (0.15, 0.21)
hpv53 0.12 (0.09, 0.15)
hpv56 0.16 (0.13, 0.19)
hpv58 0.13 (0.11, 0.16)
hpv59 0.06 (0.05, 0.08)
hpv66 0.11 (0.08, 0.13)
hpv68 0.05 (0.03, 0.07)
Subtotal (I-squared = 93.7%, p = 0.000) 0.13 (0.10, 0.16)
ICC
hpv16 0.37 (0.28, 0.47)
hpv18 0.24 (0.16, 0.33)
hpv31 0.03 (0.01, 0.09)
hpv33 0.08 (0.04, 0.15)
hpv35 0.06 (0.02, 0.12)
hpv45 0.11 (0.06, 0.19)
hpv52 0.11 (0.06, 0.19)
hpv53 0.03 (0.01, 0.09)
hpv56 0.03 (0.01, 0.09)
hpv58 0.15 (0.09, 0.23)
hpv66 0.02 (0.01, 0.08)
hpv68 0.02 (0.01, 0.08)
Subtotal (I-squared = 85.8%, p = 0.000) 0.09 (0.05, 0.13)
Overall (I-squared = 94.8%, p = 0.000) 0.11 (0.09, 0.13)
NOTE: Weights are from random effects analysis
-.475
0
.475

Fig 6 Pooled estimates of genotype specific pHR/HR HPV prevalence, separated for women with normal/abnormal cytology and women with ICC

Out of the total number of HIV-positive women, the most prevalent HR HPV genotypes in women with normal cytology were HPV 52 with a pooled estimate of 26% (95%CI: 21%-30%-38.4; n=92), followed by HPV 35, 20% (95%CI: 16 % - 25%; n=72). The most prevalent pHR/HR HPV genotypes in women with abnormal cytology were HPV 16 with 26 %, (95%CI: 23%-30%; n= 156), followed by HPV 35 and 52, with 21% (95%CI: 18%-25%; n= 126) and 18% (95%CI: 15%-21%; n=108), respectively. (Fig 6)

In HIV infected women with ICC, the most prevalent HR HPV genotype in women were HPV 16 (pooled prevalence 37%; 95%CI: 28 % - 47 %; n=33), followed by HPV18 (24 %; 95%CI: 16%-33%; n= 21), followed by a 15% prevalence of HPV 58 (95%CI: 9%-23%; n=11). HPV 31, 33, 35, 53 and 56 were also detected. (Fig 6)

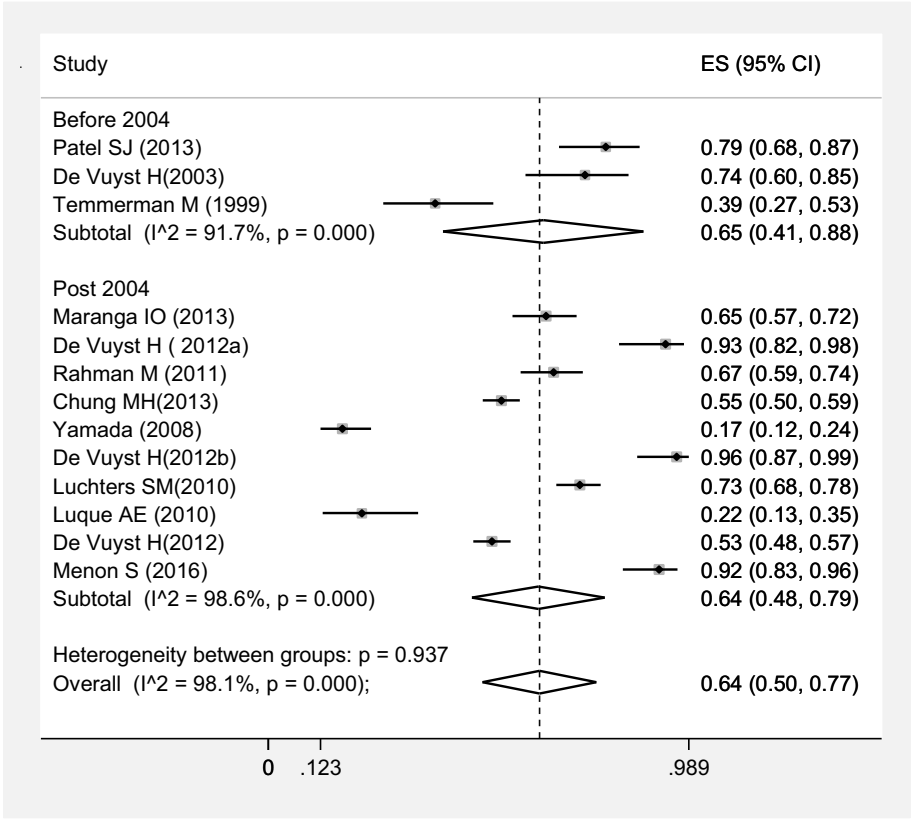


Fig 7 pHR/HR HPV prevalence among HIV-positive women stratified by pre 2004 and post 2004 time period.

In our subgroup analysis, time period did not appear to impact the pHR/HR HPV prevalence, with pooled estimates overlapping, pre 2004 65% (95%CI: 44%-88%) and post 2004, 64% (95%CI: 50%-77%). (Fig 7)

Discussion

Our meta-analysis demonstrated a high burden of HR-HPV genotypes in a general HIV population, significantly higher than the recent meta-analysis on pooled HPV prevalence in the general female population in eastern Africa (42.2%) [39].

Prevalence of specific PHR/ HR HPV genotypes in HIV-infected women with abnormal cytology:

It was also found that, whilst HPV 52, followed by HPV 35, 53 and 56 were the most prevalent pHR/HR HPV genotypes detected in HIV positive women with normal cytology, in women with abnormal cytology, HPV 16 shifted to first place and was followed by HPV 35 and 52. Nevertheless, in women with ICC, HPV 18 gained prominence, ranking second after HPV 16.

Data on HPV type distribution in invasive and pre-invasive cervical cancer is required to predict the future impact of HPV16/18 vaccines, the future nonavalent vaccine and HPV-based screening test. Our systematic review suggests that HPV 16 regains it prominence in HIV women with abnormal cytology, though this difference was not statistically significant.

Our high pooled estimates suggest a prominent place for non 16/18 HPV types, including HPV 35, 52, 56, 58, 53, and 31 in HIV infected women with abnormal cytology. Similar observations have also been made from previous studies carried out in Africa and in the world where detection of non-16/18 HPV type patterns in HIV infected individuals is commonplace [40, 41].

Our pooled estimates are also in line with those of a 2015 meta-analysis on the general female population with LSIL from sub Saharan Africa for which HPV 16, 35, 52, 18, 53, 56, 58, 51, 45 and 66 were the top ten most common genotypes. Albeit, we do not have pooled estimates for HSIL, the ranking of HPV 16, followed by HPV 35 and 52 observed in our study is in line with that

observed in eastern and southern regions of Africa where HPV 16 was estimated at 28%, followed by HPV 52 (16.5%) and HPV 35 (15.1%).

The relatively high pooled prevalence of HPV 53 in our meta-analysis suggests that HPV 53 plays a more prominent role in HIV infected women. Our pooled estimate in abnormal cytology of 12% is about twice as high as the pooled 6.8% estimate in HIV infected women with LSIL and the 2.1% estimate observed in women with HSIL by Clifford et al (2006) [42].

Prevalence of specific PHR/ HR HPV genotypes in HIV-infected women with ICC

The preponderance of both HPV 16 and HPV 18 in ICC cases in our meta-analysis is in agreement with the findings of the systematic review undertaken in Uganda and with the recent meta-analysis of HPV prevalence in women with normal cervical cytology to neoplasia in Africa [3, 39]. However, our pooled HPV 16/18 estimate of 61% (95%CI: 50.0%-70.0%) is lower than the 75.4% observed in Eastern African women with ICC.

Compared to the pooled estimates of 4.1% reported in the meta-analysis of HPV prevalence in eastern African women with ICC, we found a HPV 52 prevalence of 11% (95%CI 6.0%-19.0%) which was also present in single-type infections, However, our study found a similar prevalence of HPV 45, 11% (95%CI:6.0%-19.0%) compared to 9.1%.

In this review, we found that the prevalence of multiple HR HPV co-infections was analogous in women with normal cytology/HSIL to that of women with ICC, which may suggest that a synergistic interaction between pHR/HR HPV genotypes remains high in HIV women.

In our analysis comparing FSW who are at higher risk for STIs, our meta-analysis suggested that the prevalence of having pHR/HR HPV genotypes in FSW was statistically significantly higher

than in non FSW. However, our subgroup analysis of pre-2004 compared to post 2004, revealed no statistically significant differences in pooled pHR/HR HPV prevalence.

Strengths and limitations:

To our knowledge, this systematic review represents the first attempt to evaluate the prevalence of HPV infections in HIV infected women, prevalence of different HR HPV genotypes in HIV infected women with normal, abnormal cytology and ICC in Kenya, and the second one on the sub Saharan continent attempting to measure the burden of HPV infection and ICC [43]. Another strength of this study is that it includes studies from different settings, including a family planning clinic, HIV clinics, and a community-based setting, which enabled us to capture a more representative HIV positive female population in Kenya. In addition to the diversity of settings and median age of the study population, the catchment of clinics may have differed in terms of socioeconomic status, which as a corollary, may have had an impact on the levels of immunosuppression.

The data should be interpreted in light of a number of limitations, including the small number of studies examining genotypes specifically in HIV infected women, especially with ICC as an endpoint. Our sub analysis did not reveal any statistically significant differences between pre 2004 and post 2004 suggesting that heterogeneity may not have significantly caused by an increase of PCR sensitivity, a wider range of pHR/ HR HPV genotypes testing over time, and a review of classification of HPV genotypes. However, of consideration is that some of the tests described are performed at analytical sensitivity while others have been validated clinically and use a clinical threshold, rendering a high variability of PCR outcome.

Another cause of heterogeneity may be that HIV-infected women with low CD4 counts are at risk for reactivation of HPV. However, a meta regression for age, CD4 cell count, HIV viral load or

antiretroviral treatment could not be performed, as no individual information was available for these clinical parameters, which would allow adjustment or stratification of type-specific HPV prevalence for these variables. The high heterogeneity may be attributed to the different settings from which the study population was derived, including family planning facilities, HIV clinics and community-based settings. The high I2 that we encountered is in line with that observed in the 2015 meta-analysis from sub-Saharan Africa, where heterogeneity was found to be substantial, ranging from ICC: I^2 = 88.8% to 99.1% in women with normal cytology [39].

In addition, another limitation relates to the cross-sectional analysis of studies with cross sectional study designs as well as baseline data on HPV and HIV derived from cohort studies. The temporal criterion of causality is not fulfilled, therefore reversal causality cannot be excluded.

Potential reduction of ICC following an effective vaccine:

Given that very few studies looked at type distribution in ICC in HIV positive women in Kenya, it is difficult to estimate the potential effectiveness of the bivalent or nonavalent vaccines. The still poorly characterised association between HIV and ICC causes a public health concern in Kenya, which currently has 1.6 million HIV-positive women who may well have an increased risk of developing ICC [44].

Data from the present meta-analysis indicate that current HPV 16/18 genotypes can be found in 61% of women with ICC. However, pooled estimates suggest that a 90% reduction of cervical cancer afforded by the nonavalent vaccine may be attained.

Whether Gardasil and Cervarix can attain a 70% reduction of cervical cancer may be contingent upon the natural history of the imputed pHR/HPV genotype in cancer genesis. However, with the high presence of other pHR/HR HPV genotypes in HIV infected women with ICC in Kenya, it is

currently not possible to attribute a direct association between a specific genotype in cancer genesis as its relevance may be inflated.

In order to assess the potential reduction of ICC cases by 70%, an important research gap to address is the vaccines' cross protection against HPV 45. Studies have shown that both current vaccines Gardasil and Cervarix afford cross protection against type 45, with Cervarix offering a higher degree of effectiveness[45]. However, this cross protection still has to be determined in HIV infected women.

Apart from limited information on cross protection, it should be established whether any concurrent HPV types may augment or decrease the efficacy of HPV vaccines, as a result of competition among the non-vaccinated HPV types[46]. These caveats and the pooled prevalence of above 90% of all the genotypes incorporated in the nonavalent vaccine outline the benefits of the nonavalent vaccine within this population.

Secondary prevention:

Secondary prevention, using cancer prevention tools such as visual inspection with acetic acid and/or Lugol's iodine, or detection of high risk HPV genotypes will remain indispensable for various reasons, including the necessity of early identification of patients infected with HR/HPV genotypes not covered by current vaccines, as well as for post bivalent, quadrivalent and nonavalent vaccine surveillance and for unvaccinated young and older women. It is noteworthy that the impact of HPV vaccination will not be observed for years, especially with respect to types associated with pre-invasive and invasive cancers, as those tend to occur in older women.

Previous studies have shown a lack of association between HPV 16 and immunosuppression [7]. Moreover, HAART use is associated with increased clearance of some high risk HPV types, with the exception of HPV16. Hence, a pooled estimate of 26 %, of older HIV infected women with

abnormal cytology infected with HPV 16 or 37 % of women with ICC in Kenya may still need to be monitored regularly. Although most HPV infections may resolve without treatment, the immuno-epidemiology of some genotypes is still poorly characterized, especially in regards to resolution of infections and regression of non-HPV 16 infections.

Because of the potential to alter their behavior in the post vaccine area and so resulting in case type replacement, the relatively high prevalence of HPV 53 and other undiagnosed pHR/HR HPV genotypes not covered by the nonavalent vaccine underscores the need for it to be considered in a screening protocol. This is hampered by the lack of affordable nucleoacid amplification methods, such as Papillomacheck, which requires specific apparatus [47, 48].

Conclusion:

Our pooled estimates of HPV 16 and 18 of 61% in HIV-infected women with ICC suggest the need for a wider protection that the nonavalent vaccine would confer.

Whilst HPV 52 is most prominent in HIV-infected women with normal cytology, in women with abnormal cytology, HPV 16 regains its preponderance and is followed by HPV 35, only to be replaced by HPV 18 in women with ICC.

To assess the potential vaccine efficacy in Kenya, the synergistic interactions between the multiple genotypes harboured by HIV positive women with premalignant and malignant diseases in the post vaccine era need to be elucidated.

Borderline statistically significant differences between pooled estimates of HR HPV genotypes in FSW and non FSW suggest that cervical cancer prevention may warrant a triage based on this indicator. An effective secondary prevention programme in HAART era, will require that the immuno- epidemiology of specific HPV types in HIV-infected women be explored

List of abbreviations:

HIV: human immunodeficiency virus

HPV: Human papillomavirus

LSIL: Low-grade squamous intraepithelial lesions

HSIL: High-grade squamous intraepithelial lesions

ICC: Invasive cervical cancer

WHO: World Health organization

Supporting Information

S1_Search strategy

S2_ PRISMA checklist

S3_ Results

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DISTRIBUTION OF HUMAN PAPILLOMAVIRUSES AND BACTERIAL VAGINOSIS IN HIV POSITIVE WOMEN WITH ABNORMAL CYTOLOGY IN MOMBASA, KENYA

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Abstract

Background: HPV is the major etiological factor in the causal pathway for cervical cancer, which is the leading cancer among women in sub-Saharan Africa. HIV is associated with a higher prevalence and a broader range of high-risk HPV genotypes. Studies have shown a positive association between Bacterial vaginosis (BV) and HPV and HIV. Also, in African women, BV was found to be significantly associated with vaginal inflammation. The high prevalence of BV, HIV and HPV infections in the African continent makes elucidation of the interactions with one another of utmost public health interest. The aims of the current study are to examine the frequency of HPV genotypes and BV as well as their respective risk factors within an HIV infected population with abnormal cytology in the resource-constrained setting of Mombasa, Kenya and, secondly, highlight issues to consider for triple co-infection clinical management.

Method: cross-sectional analysis with a sample drawn from an ongoing cohort study. All consenting, non-pregnant HIV infected women, between 18 and 50 years of age, without a history of cervical cancer or hysterectomy, between November 2005 and April 2006 were screened for HR HPV DNA in Mombasa, Kenya. 1 out of 4 HIV positive women fulfilled the criteria by having SIL (24.9%). 600 HIV infected women were tested to reach a cohort of 74 HIV women with abnormal cytology. To assess which factors were associated with HR HPV, crude statistical analysis was performed through logistic regression

Results: Bacterial vaginosis (BV) was found in 46 women out of 74 **(**62.2 %). Cervicitis was diagnosed in 15% of women (n=11), of which 8 had BV. The most prevalent HPV genotypes were HPV 16 (33.8%) and HPV 53 (24.3%), while 65% of the participants had multiple genotype infection.

Statistically significant associations between CD4 counts <200 cells/µl and multiple HPV prevalence, adjusted for age were also noted (OR: 3.7; p=0.03; 95%CI: 1.2- 12.1) and HPV53 (OR=4.4, 95% CI: 1.4-13.6; p= 0.01). A statistically significant association was found between CD4 count ≥ 350 µl and HPV 16 adjusted for age (OR: 2.9; p=0.05; 95%CI: 1.0- 8.3).

A borderline statistically significant association was observed between BV and HPV58 (crude OR=4.1, 95%CI: 0.8-21.0; p=0.07).

Conclusion: The most prevalent HPV genotypes observed were HPV 16, HPV 53, and HPV 18, which have a combined prevalence of 76%. Our results show that a triage based on CD4 count should start at CD4 count ≥ 350 µl as our study suggests that HPV 16 are more prevalent when women are moderately immunosuppressed. Given the high prevalence of HPV 53 in a HIV infected population with abnormal cytology, its cervical carcinoma genesis potential as a stand alone genotype and as well as its synergism with multiple infections should be investigated. The new WHO guideline in resource-poor settings to rescreen women for HPV within ten years may be more effective if BV and cervicitis management become a major component for HIV-HPV management.

Key words: HIV, HPV, CD4 count, BV, cervicitis

Introduction

Cervical carcinoma is the fourth most prevalent cancer in the world and the most common female cancer in sub-Saharan Africa.[1] It is the second most prevalent cancer among women in Kenya, after breast cancer, and its incidence is increasing.[2]

Infection with a high risk (HR) Human Papillomavirus (HPV), a sexually transmitted DNA virus, is the central etiological agent in the development of cervical cancer and include HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.[3] Others HPV types 26, 53, 66, 67, 70, 73, and 82 are classified as "possible/probable" (pHR) carcinogens according to the recent review of International Agency for Research on Cancer (IARC) assessing carcinogenicity of biological agents.[4]

Cervical cancer is the result of a change in the cell cycle control caused by HP. Cervical intraepithelial neoplasia (CIN) can be histologically graded into mild dysplasia (CIN 1), moderate dysplasia (CIN 2), and both severe dysplasia and carcinoma in situ belonging to CIN 3.[5]

Apart from a higher prevalence and broader range of HR HPV, HIV immunosuppression has been linked to multiple HPV infection,[6] [7] particularly in those with CD4 count <200 cells/µl,[8] meaning when immunosuppression becomes severe. Multiple HPV genotypes co-infections has been attributed to the inability to clear HPV infections as well as to the reactivation of latent HPV infections, both occurring as a result of immune suppression.[8] [9] [10] [6] In a recent large study on multiple HPV infections in Costa Rica, young non-HIV positive women with multiple infections were observed to be at significantly increased risk of CIN 2+, when compared with those with single infections.[11] [12]

Moreover, concurrent genital infections are common in HIV-1–infected women. The prevalence of Bacterial Vaginosis (BV), characterized by an overgrowth of vaginal anaerobic flora and

reduction of H_20_2-producing lactobacilli in African women is among the highest worldwide,[13] which is of particular concern, as there is evidence that BV is a risk factor for acquisition and transmission of many STI's, including HIV and HPV.[14] [15] A recent meta-analysis of available literature also suggested a positive association between BV and HPV infection, though no study in Africa was eligible to be included. [16] A recent HIV Epidemiology Research study in Tanzania showed that BV is also associated with increased odds for incident HPV as well as delayed clearance among women.[17] These findings are in line with biologic plausibility since, unlike most cervical HPV infections, BV causes major changes in the local vaginal environment leading to degradation of innate defenses.

In previous studies, BV was associated with many sexually transmitted infections (STIs), including infection with *Chlamydia trachomatis*, *Neisseria gonorrhoea*, HSV-1 and 2.[18] [19] [20]There is growing evidence from other studies that BV is an independent risk factor for cervicitis.[21] BV could lead to cervicitis through a loss of bactericidal H_20_2-producing lactobacilli, reduced levels of protective vaginal mucins, and increased pro-inflammatory enzymes and cytokines, which in turn may decrease the cervical mucus barrier.[22]

New guidelines from WHO in December 2014 advocates for a "screen and treat" approach in resource poor settings, in which treatment of detected precancerous lesions ensues Visual Inspection with Acetic acid (VIAC) or HPV testing for screening soon or immediately.[23] The use of HPV tests for cervical cancer prevention is expected to reduce the frequency of screening and "once a woman has been screened negative, she should not be rescreened for at least 5 years, but should be rescreened within ten."[23] However, there is current scant evidence to support the adequateness of this guideline in HIV-infected women living in low resource settings. Screening programs need to be tailored to the resources and capacity in each area. This secondary preventive strategy would accompany the comprehensive strategy to prevent

cervical cancer in Kenya, which includes plans for vaccinating girls with the quadrivalent vaccine (Gardasil™) by 2015.[24 25]

This analysis of HIV-positive women with abnormal cytology purports to 1) describe the distribution of HPV genotypes and multiple HPV infections, cervicitis, BV and STIs within our population 2) explore risk factors for the most prevalent HPV genotypes; 3) discuss the potential effectiveness of a screening protocol triaged by CD4 count and age and the public heath management of HIV-HPV-BV.

Methods

This cross-sectional analysis reports the findings of a sample of women enrolled in a prospective cohort study, whose aim was to define the best way to manage low grade squamous intraepithelial lesions in HIV-1 infected women, aged between 18 and 50 years in Mombasa, Kenya, between November 2005 and April 2006. As all women were to receive the same intervention depending on their squamous intraepithelial lesion, no formal sample size calculation was needed. 600 women were screened within this period. Non-pregnant women of reproductive age and attending the Comprehensive Care Centre for management of HIV infection at Coast Province General Hospital in Mombasa, Kenya, were invited to participate. Within the 600 HIV positive women undergoing cytological examination, we expected to find 10 % prevalence of Squamous Intraepithelial Lesions (SIL) for the HIV positive patients not on HAART and 15 % for those under HAART period, resulting in 74 HIV women with abnormal cytology being recruited from the initial cohort.

Cytological abnormalities, a prerequisite for participating in the study, were all histologically confirmed. Exclusion criteria included pregnancy, less than 6 weeks post-partum, history of cervical cancer or hysterectomy. Self-reported behavioral risk factor assessment included the

presence of an STI, number of sexual partners, the age of first sexual intercourse and regular use of a condom. Blood plasma samples for measuring CD4 count were taken, and a gynaecological examination was performed with speculum insertion, and collection of endocervical and high vaginal swabs.

Biologic specimens

Cervical samples were collected using a cervix brush (Cervex-brush®, Rovers®, Oss, The Netherlands), and cervical cytology was assessed with conventional Papanicolaou (Pap) smears. Slides were read by a cytologist with master level training, supervised by a pathologist. An external cytopathologist provided quality control. The Bethesda Reporting System was used for cytological classification.[26]

The cervix brush tips were preserved in a liquid-based cytology collection medium (SurePath®, Tripath Imaging Inc., Burlington, North Carolina, USA) and stored at 4°C until further processing.

HPV DNA Extraction, Detection and Typing:

HPV testing was done as described by Depuydt et al (2006) Micalessi IM et al (2012) in an accredited laboratory (ISO certification: ISO15189).[27] [28] Briefly, HPV DNA was extracted from exfoliated cervical cells using the standard proteinase K-based digestion protocol, following the manufacturer's instructions. Cells were incubated with proteinase K solution (100µg/ml) for 3 hours at 55°C. DNA was then further purified by spin column chromatography. HPV types were determined using a series of real-time PCR reactions with specific primers and TaqMan® (Invitrogen, La Jolla, USA) probes for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 IARC (2006). Low risk (LR) HPV types 6 and 67 were also detected. HPV

DNA was tested according to de Meijers' et al (2009) guidelines for HPV DNA test requirements.[29]

The national recommendation was followed to diagnose HIV and a parallel testing algorithm was used for HIV diagnosis using rapid immunoassays: Uni-Gold™ Recombigen® HIV (Trinity Biotech plc, Bray, Ireland) and Determine® HIV-1/2 (Abbott Japan co Ltd, Minato-Ku, Tokyo, Japan). In the case of indeterminate results, an enzyme-linked immunosorbent assay was used to confirm HIV status.

Diagnosis of STIs

A gynecological examination was performed and blood, urine, PAP smear, and high vaginal and endocervical swabs collected. PAP smears were read using conventional cytology and classified using the Bethesda classification. Infection with *Trichomonas vaginalis* was determined by wet mount and/or PAP smear slides. To test for *Neisseria gonorrhoeae* infection, culture plates (blood agar; International Diagnostic Group, Lancashire United Kingdom) were inoculated with endocervical swabs Presence of HIV was confirmed in the patient's serum using a parallel algorithm comprising Uni-Gold® HIV (Trinity Biotech plc, Bray, Ireland) and Determine® HIV-1/2 (Abbott Japan co Ltd, Minato-Ku, Tokyo, Japan). Elisa was used when parallel test results were discordant.

Cervicitis:

Cervicitis was diagnosed from inflammatory cells by means of a microscopic test.

Statistical analysis

Analysis was done using STATA version 12 (Stata-Corp LP, College Station, TX). In order to evaluate whether a median or mean should be given for continuous variable, including age,

number of sexual partners, CD4 count, number of HPV co-infections, a Shapiro-Wilk test was performed done to assess whether the variable was parametric; continuous variables were then converted into categorical.

Age was dichotomized into > 30 years and ≤ 30 years; this categorization was used to reflect the WHO 2014 guideline concerning cervical screening. Condom use was dichotomized as always or irregular, according to self-report. CD4 cell count was analyzed both as a continuous variable and as two categorical variable, using a cut off of CD4 count <200 cells/µl (vs ≥ 200 cells/µl) and CD4 count <350 cells/µl (vs ≥350 cells/µl), when HIV infected women are severely immunosuppressed and moderately immunosuppressed, respectively. The number of sexual partners was also dichotomized as per other studies (up to 5 versus 6 or more), this dichotomization was used to make our studies more comparable to others and to explore the risk from a public health point of view. The risk factors for the most common PHR/ HR HPV genotypes in this study are explored. This outcome variable was dichotomized into low-risk HPV (LR-HPV) and absence of HPV genotypes versus HR-HPV genotypes (see above).

The method employed for the first objective was to calculate the proportion of the specific HPV genotype divided by 74 (the sample size), the number of co-infected women with abnormal cytology, as well as the proportion of STI, BV and cervicitis, along with a 95%CI. For the second objective, logistic regression univariate analysis was performed to identify risk factors for HR-HPV. The resulting odds ratio was used to measure the strength of the association between HPV infection and each risk factor in turn. A multivariable logistic regression analysis was performed to simultaneously control for potential confounders and to assess the adjusted association for various risk factors. To assess the impact of CD4 count on the effects of the parameters, interaction parameters were assessed in the models by doing a Wald test. As no interaction terms were significant, no model reflecting the differential impact for that particular outcome was included.

The same method was also used to explore the simultaneous effect of age and number of sexual partners on the HPV genotype and to explore whether there was an association between the two or more HR HPV co-infections and CD4 count, while adjusting for age and the number of sexual partners. Given the public health impact of our high prevalence of BV in this population, we also explored associations between BV and related types from the alpha nine family, HPV 16 - 31 - 33 - 35 - 52 – for us to compare results with available literature.

Statistical tests were considered significant when the p-value, derived from the Wald Test, is 0.05 or less.

Ethical considerations

Staff obtained written informed consent from patients, collected demographic and behavioural data using structured questionnaires. All human subject protocols were approved by the Ethics Committee at the Kenyatta National Hospital, which also gave overall ethical approval for this study (Ref: KNH-ERC/01/3618).

Results

Characteristics of the population

The study included 74 non-pregnant HIV-infected women with an abnormal cytology. The mean age was 34.2 years of age (SD=6.5). The median age at first sexual intercourse was 18.0 (IQR: 15.5-20.0). The median number of sex partners was 2 (IQR: 1-4). The median CD4 count was 236 cells µl / (IQR: 158- 374). The mean number of HR HPV genotypes per participant was 2.3 (SD=1.6). The majority of patients (82%) were under treatment with highly active antiretroviral therapy (HAART) (table 1).

Type of infection	n	Percentage (95%CI)
HR HPV:		
HPV 16	25	33.8% (22.8-44.8)
HPV 18	13	17.6% (8.7-26.4)
HPV 31	11	14.9% (6.6- 23.2)
HPV 33	12	16.2% (7.6-24.8)
HPV 39	8	10.8% (3.6- 18.1)
HPV 51	11	14.9% (6.6- 23.2)
HPV 53	18	24.3% (14.3-34.4)
HPV 56	15	20.3% (10.9-29.6)
HPV 58	13	17.6%(8.7-26.4)
HPV 66	11	14.9% (6.6- 23.2)
HPV 68	4	5.4% (0.1-10.7)
Multiple HR HPV	48	64.9% (53.7-76.0)
Women on HAART	60	82.0(80.0-90.2)
LR HPV:		
HPV 6	4	5.4% (4.4-6.4)
HPV 67	0	0
No HPV infection:	6	8.1% (3.0- 16.8)
STIs:		
BV	46	62.2 %(50.9-
Genital ulcer	12	73.5)
Genital warts	8	16.2% (7.6- 24.8)
Trichomonas	1	10.8% (3.6-18.1)
vaginalis	11	1.4%(0.1-4.0)
Cervicitis	51	14.9% (6.6-23.2)
STI prevalence	8	68.9% (58.1-79.7)
More than one STI		10.7% (3.5-17.8)

Table 1: Prevalence of High and Low Risk (HR, LR) HPV infection and other Sexually Transmitted Infections

Variable	n	Percentage (95%CI)
Age group:		
>30 years	54	73.0% (61.4-82.6)
≤30 years	20	27.0% (17.4-38.6)
Sexual behaviour:		
First sexual encounter <15 years	29	60.4% (52.7-74.2)
old	19	39.6% (25.8- 54.7)
First sexual encounter >=15 years old		
>6 sexual partners	7	12.1% (5.0-23.3)
≤5 sexual partners	51	87.9% (76.7-95.0)
Regular use of condom	10	18.2% (9.1-30.9)
No regular use of condom	45	81.8% (71.3-92.3)
CD4 count:		
CD4 count <200 cells/ **µl**	26	35.1% (24.0-46.3)

CD4 count ≥200 cells/ **µl**	48	64.9% (69.1-91.0)
CD4 count <350 cells/ **µl**	52	70.3% (58.5-80.3)
CD4 count ≥350 cells/ **µl**	22	29.7 % (19.1- 40.4)

Table 2: Distribution of various categorical variables: age, sexual behaviour and CD4 count

Concerning sexual behavior, 81.8% of women reported irregular use of condoms, and 87.9% had up to 5 sexual partners (table 3).

Variable	n	Percentage (95%CI)
Age group:		
>30 years	54	73.0% (61.4-82.6)
≤30 years	20	27.0% (17.4-38.6)
Sexual behaviour:		
First sexual encounter <15 years old	29	60.4% (52.7-74.2)
First sexual encounter >=15 years old	19	39.6% (25.8- 54.7)
>6 sexual partners	7	12.1% (5.0-23.3)
≤5 sexual partners	51	87.9% (76.7-95.0)
Regular use of condom	10	18.2% (9.1-30.9)
No regular use of condom	45	81.8% (71.3-92.3)
CD4 count:		
CD4 count <200 cells/ **µl**	26	35.1% (24.0-46.3)
CD4 count ≥200 cells/ **µl**	48	64.9% (69.1-91.0)
CD4 count <350 cells/ **µl**	52	70.3% (58.5-80.3)
CD4 count ≥350 cells/ **µl**	22	29.7 % (19.1- 40.4)

Table 3: Distribution of various categorical variables: age, sexual behaviour and CD4 count

Prevalence of cervical abnormalities

Atypical squamous cells with possible high significance (ASC-H) were detected in 4.0% and atypical squamous cells of undetermined significance (ASC-US) in 16.2%, LSIL 58.1% and HSIL in 20.3%.

Histology results found the following abnormalities: Cervical intraepithelial neoplasia (CIN I) in 58.3%, CIN 2+ 43.2%, of which 1 had ICC. Cervicitis was recorded in 15% and normal biopsy in

4.0%. In 5 women with CIN 1, HPV was absent and in the two normal biopsies, HPV was present.

Prevalence of HPV genotypes

The most prevalent HPV genotypes found in this HIV-infected cohort of women are displayed in Table 1. HPV 16 and 53 were the most prevalent, with 34.8% and 24.3%, respectively. A large proportion, 64.9%, of the women in this cohort has multiple HPV genotypes (table 1). Most multiple infections were dual infections, (24%), but two women had up to 7 co-infections (table 2).

Number of co-infections	n.	%
2 co-infections	18	24%
3 co-infections	12	16%
4 co-infections	12	16%
5 co-infections	3	4%
6 co-infections	1	1%
7 co-infections	2	3%
Total	48	64%

Table 2: prevalence of multiple HPV genotypes among the total sample size (N=74)

At least one of the HR HPV genotypes was observed in 86.5% of the women, 5.4 % with LR-HPV types and 8.1 % without any contemporaneous HPV infection.

Prevalence of concurrent STIs

STIs were observed in 68.9% of all women, and 15.7% had more than 1 STI co-infection (Table 1). 16.2% were diagnosed with genital ulcer disease, 1.4% *trichomonas vaginalis* and 10.8% had genital warts.

Sexually enhanced disease:

Out of the 11 women with cervicitis, 8 had BV.

Risk factors for specific HR-HPV infection: results of uni- and multivariable analysis

No significant association was observed between HPV 16 and age (OR:1.4; p:0.5; 95%CI 0.5-3.6), adjusted for ≥6 sexual partners (aOR: 1.1 p=0.8; 95%CI: 0.3-4.1); HPV 53: (OR:05; p:3;95%CI: 0.2-1.6) adjusted for ≥6 partners (aOR: 0.9 p=0.8; 95%CI: 0.2-3.2) and HPV 18 (OR: 0.4; p:0.2; 95%CI 0.1-1.6): adjusted for ≥6 partners (OR: 2.3; p=0.3; 95%CI: 0.5- 11.5). No significant association was found for multiple HPV genotypes and age (OR: 1.8; p=0.3; 95%CI: 0.6-5.1) and adjusted for sexual partners (OR: 2.4; p=0.2; 95%CI: 0.7- 8.9). (table 4)

HPV Genotype	Odds ratio	95% CI	P value*
CD4 count < 200 cells/ µl			
HPV 16	0.7	0.2-1.9	0.4
HPV 18	1.3	0.4- 4.6	0.7
HPV 31	1.1	0.3- 4.4	0.9
HPV 33	3.3	0.9- 11.9	0.07
HPV 35	2.4	0.7-8.3	0.2
HPV 45	3.3	0.3- 39.4	0.4
HPV 51	1.5	0.4-5.6	0.6
HPV 52	2.5	0.8 8.1	0.1
HPV 53	4.4	1.4-13.6	0.01
HPV 56	0.6	0.2- 2.2	0.4
HPV 58	2.6	0.7-8.7	0.1
HPV 66	3.1	0.8- 11.9	0.1
HPV 68	0.6	0.06- 6.2	0.7
Multiple HPV co-infection	3.7	1.2- 12.1	0.03
CD4 count ≥350 cells/ µl			
HPV 16	2.9	1.0- 8.3	0.05

HPV 18	0.4	0.08- 1.9	0.2
HPV 53	0.6	0.2-2.1	0.4
Multiple HPV co-infection	0.7	0.3- 2.0	0.5
Age ≥ 30 adjusted for sexual partners			
HPV 16	1.1	0.3- 4.1	0.8
HPV 18	2.3	0.5- 11.5	0.3
HPV 53	0.9	0.2- 3.2	0.8
Multiple pHR/HR HPV infections	2.4	0.7-8.9	0.2

*p-value from Wald test
Table 4: Age-Adjusted association between specific HR HPV genotypes and CD4 count < 200 µl and CD4 count ≥350 cells/ µl (upper part)**;** sex -adjusted associations between the three most prevalent pHR and HR HPV genotypes and age OR from Logistic regression

CD4 count:

35.1% women had low CD4 counts <200 cells/µl and 64.9% had high CD4 count ≥200 cells/µl. 70.3% had CD4 count <350 cells/µl and 29.7% had CD4 count ≥350 cells/µl (table 3). The OR obtained from the logistic regression yielded a statistically significant association between CD4 <200 µl and multiple HPV co-infections (OR: 3.7; p=0.03; 95%CI: 1.2-12.1 but a non significant association between CD4 count ≥350 cells/µl and multiple HPV infections (OR: 0.7; p=0.5; 95%CI: 0.3-2.0). Low CD4 counts <200cells/µl was found to be a significant predictor of only HPV 53, adjusted for age (HPV53 (OR=4.4, 95% CI: 1.4-13.6; p= 0.01) and HPV 16 adjusted for age (OR: 2.9; p=0.05; 95%CI: 1.0- 8.3). (table 4).

A Mann Whitney rank sum Test indicated that women with CD4 count <200 cells/µl have a higher median of prevalent HPV infections (p=0.01) and a non significant difference in women with CD4 count <350 cells/µl and CD4 count ≥350 cells/µl (p=0.6).

Micro-organisms:

68.9% had a concomitant STI, and 62.2% women had BV (table 1). With the exception of HPV 58 (OR=4.1; 95%CI: 0.8-21.0, p=0.07), no significant association was found between BV and HPV 16 and its phylogenetically-related genotypes, HPV 31, HPV 33, HPV 58, HPV 52 (table 5).

Discussion

Summary of results and comparison with other studies

Our analysis shows that the most common pHR/HR HPV genotypes, HPV 16, followed by HPV 53 and HPV 18, with a combined prevalence of 76%, were found in presence of other pHR/HR HPV infections. Strong associations were observed between HPV 53 and multiple HR HPV infections with CD4 count <200 cells/µl.

In agreement with studies showing HPV 16 to be the least affected by diminished immunity,[30] we found that women with CD4 <200 cells/µl had a 26.9% prevalence of HPV 16 compared to 37.5% in women with CD4 count >200/µl and a statically significant association between women with CD4 count ≥350/ µl when adjusted for age. One study in Kenya found a significantly higher prevalence of HPV 16 in an HIV clinic where the median CD4 count at recruitment was 407cells/µl. [31] Other studies in Kenya lacked stratification of HPV 16 and HPV 18 by HIV status and CD4 count. In a family planning unselected study conducted in Nairobi, it was observed that less than 10% had HPV 16 and/or 18 these types were present in about 40% of HSIL lesions.[32] In a study on FSW in Kenya, HIV infected women with normal cytology had an HPV 16 or 18 prevalence of 9.6%,[33] though the prevalence of either HPV is unknown in HIV infected women with abnormal cytology.[34] A hypothesis is that HPV16 may be more sensitive to

attack from other genotypes, and thus may be at higher risk of competition when there is more immune suppression.

In our study, we detected a marginal association between BV and HPV 58, a HPV 16- related types, which is line with a study that detected a significant association between BV and HPV16-related types.[35] The fact that we only found a marginal significance is likely to be due to the sample size.

In our study a HPV 18 prevalence of 17.6% was observed, of which 54% had CIN 1 and 46% had CIN2+. Our high HPV 18 prevalence was significantly higher than the 10.6% observed by de Vuyst et al in Kenya. Women with CD4 < 200 cells/µl appeared to have 20% higher odds of being infected with HPV 18, although these associations were not statistically significant, (OR=1.2; 95%CI: 0.3-4.1). However, a South African study suggested that the prevalence of HPV 18 is inversely correlated with the level of immunosuppression, (10.6%) in women with CIN 2/3 and 5% with women with normal cytology.

A high prevalence of HPV53 had also been observed in other studies from Kenya. A study in HIV positive women (median CD4 count 281/µl) from Nairobi found high numbers of HPV53 in normal smears, whilst rare in HIV negative women.[36] In a Nairobi cohort with a median CD4 count of 538cells/µl, HPV53 was among the most common HPV type (28.5%).[37]

Although, intuitively, it would seem reasonable that all HPV genotypes should increase in frequency in HIV positive individuals, strong statistically significant associations were only detected between CD4 count <200 cells/µl and HPV53, the second most prevalent potentially oncogenic HPV genotype in our study (aOR=4.4; p=0.01; 95%CI: 1.4-13.6) and the prevalence of multiple infection. Cervical HPV infection with multiple genotypes in HIV infected women has already been reported in regional studies.[38 39 40 41]

Whilst younger age has been linked with higher prevalence of HR-HPV genotypes in HIV-negative women,[42] we did not observe any statistically significant association between multiple HR HPV genotypes and age, in agreement with Luchters et al (2010).[43] One plausible explanation given was that the absence of age effects among HIV-infected women may have been due to a decreased ability to clear HPV infection, reactivation of HPV or re-infection with HPV types [43] previously cleared, all of which likely occur more commonly in HIV-infected women.

Estimates in BV range from 20% to 50% in African populations.[44] [45] A recent longitudinal study conducted in Kenya, Rwanda and South-Africa in HIV-negative women [46] reported a prevalence of 38%. [47] The higher prevalence of BV (62%) found in our study can be attributed to higher risk that women with BV face of contracting both HIV and HPV. In our study we found that 10.7% of women with BV harboured other STIs. Inflammation related to BV is of concern because BV prevalence is very high in this population. On bivariate analysis, the association between BV and cervicitis failed to reach significance, but this may reflect insufficient power due to the number of BV-affected patients enrolled.

Limitations and strengths of the study

A major strength of our study is that our analysis is limited to histological, considered to be the gold standard instead of cytological endpoints. However, the total sample size was rather limited; this may have occulted some plausible association of risk factors which may be evident with a larger sample size; for this reason, adjustment for various factors was limited. In addition, another limitation relates to the cross-sectional design, where the simultaneous data collection of BV and acquisition of HIV infection, as well as all other variables, the temporal criterion of causality is not fulfilled, thereby reversal causality cannot be excluded. These limitations may result in a sub-optimal internal validity of our study.

Our results can be generalized to other women receiving HIV care management. Our lack of association found between age and the most common HPV genotypes and multiple HPV genotypes do not appear to support the recommendation for a screen and treat protocol for women starting from thirty years of age. Also, a CD4 based triage may not be effective as HR HPV genotypes are seen at different levels of immunosuppression, at both CD4 < 200 cells/µl and CD4 count ≥350 cells/µl.

The new WHO 2014 HPV screen and treat recommendation comes with close timing with the WHO 2013 recommendation to initiate HAART when asymptomatic at CD4 count ≥350 cells/µl, which may offer opportunities to prevent HPV 53 and multiple HPV infections, thereby potential cervical dysplasia although it may not prevent HPV 16 and HPV 18. However, given the limitation of this study, including small sample size and its cross-sectional design, a prospective cohort or RCT with a larger size is warranted to elucidate potential relevance to plan screening according to the level of immunosuppression.

Biologic susceptibility to HPV acquisition and immune competence for clearance of an HPV infection could be affected by BV underscores the importance of prevention and successful treatment of BV. In light of this finding, the new WHO guideline to rescreen within ten years may be more effective if it is implemented in conjunction with a BV screening programme. Given the high prevalence of cervicitis in this population and its potential public health impact, it is important that BV clinical management becomes a major component for HIV-HPV management. To this effect, more health care personnel, including front line personnel, including nurses who make up the majority of the work force in sub Saharan Africa should be trained in miscroscope, Nugent score or Amsel reading of BV in HIV positive women. Furthermore, symptomatic management algorithm be established for cervicitis in this study population and its aetiology elucidated.

Effective treatment of cervicitis resulting in significant decreases in shedding of HIV-1 virus and infected cells in cervical secretions depends on the aetiology of BV and other pathogens involved implicated in cervicitis. In this study, cervicitis was common and predominantly non-gonococcal, although it is not possible to say that it was non-chlamydial in etiology as CT was not diagnosed and was found predominantly in women with BV.

Although high prevalence cervicitis has been reported in Africa, local epidemiological data regarding the prevalence, etiologies, and risk factors for cervicitis in HIV-HPV co-infected women in Kenya is lacking for guiding syndromic management of cervicitis in this group. The high prevalence in this study group may also affect sexual health at a population level as inflammation in the genital tract results in shedding of HIV in HIV-positive women may increase HIV to the general population. A recent meta-analysis including HIV viral load observations in blood plasma, showed the average effect of a STI co-infection on HIV viral load in individuals on HAART was unlikely to decrease the effectiveness of treatment as prevention, although there is evidence of HIV compartmentalization in some treated patients where viral loads as measured in genital secretions are congruent with a non-negligible risk of transmission despite very low blood plasma viral loads.[48] [49] [50]

Our marginal association between HPV 16 genetically related HPV 58 as well as the high prevalence of BV in HIV-HPV co-infected women along with a high prevalence of STIs underscores the need to elucidate its synergistic effects in increasing risk of HIV and HPV acquisition. Finally, the high level of co-infection with STIs underscores the value of screening for the simultaneous presence of different genital infections in HPV positive patients. This study also evidenced the lack of protective precautions against STIs, notwithstanding the lack of effectiveness of condoms reported in reducing HPV transmission.[51] [52]

Concluding remarks and recommendation for future research

Epidemiological considerations will be needed to determine the best screening approach for different geographical settings within Kenya. A screening interval for HPV 16, HPV 53, and HPV 18, the most prevalent HPV genotypes observed, triaged by age may not be optimal in this study population of HIV- infected women as these genotypes are often found in presence of other pHR/HR genotypes. The concomitant WHO recommendation to initiate HAART when asymptomatic at CD4 count ≥350 cells/µl, instead of at CD4 count ≥200 cells/µl, may offer women the possibility to ensure continuous cervical screening during regular follow up, thereby preventing HPV 16 and HPV 18 induced cervical dysplasia.

Given the high prevalence of HPV 53 in a HIV infected population with abnormal cytology, its cervical carcinoma genesis potential as a stand-alone genotype and as well as its synergism with multiple infections should be investigated.

The new WHO guideline to rescreen within ten years may be more effective if it is interspersed with BV management. Given the high prevalence of cervicitis in this population and its potential public health impact, it is important that bacterial ecology monitoring becomes an integral component of HIV-HPV management. To this effect, there is a need for training of health care personnel in validated point of care diagnostics for diagnosing BV in HIV infected women. In addition, it is pivotal that the aetiology of cervicitis be elucidated within this study population and a symptomatic management algorithm for cervicitis be established.

Furthermore, the association between BV and HPV 16 and its related genotypes must be further explored. A high BV prevalence, together with high concomitant STIs detected in our study underscores the need that the possible synergistic effects of co-infections be elucidated for public health interest. In addition, both high concomitant STIs prevalence and very irregular use of condoms make the case for strengthening STD counseling within HIV care.

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ASSOCIATIONS BETWEEN VAGINAL INFECTIONS AND POTENTIAL HIGH-RISK AND HIGH-RISK HUMAN PAPILLOMAVIRUS GENOTYPES IN FEMALE SEX WORKERS IN WESTERN KENYA

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Purpose:

Infection with and persistence of high-risk human papillomavirus (HR-HPV) are the strongest risk factors for cervical cancer. Little is known about the prevalence and role of concurrent STIs found in HPV-infected Female sex workers (FSW) in Africa. This study purports to test our a priori hypotheses that STIs are associated with genotypes pertaining to the alpha-group species 9. The objectives were to determine the prevalence of Bacterial Vaginosis, (BV) Trichomonas vaginalis (TV) and *Candida spp* in FSW, the association between these STIs and the prevalence of any potential HR/HR HPV genotypes in FSWs.

Design: A cross-sectional study design of 616 FSW from Western Kenya aged between 18 and 61 years during 2009-2015 using a peer recruitment sampling strategy. Inclusion criteria for the study entailed being female and > 18 years of age and having engaged in transactional sex in exchange for money, goods, services, or drugs in the last three months. Women were excluded if they were pregnant, < 18 years of age, had a history of cervical dysplasia or cancer, had current abnormal bleeding or had a hysterectomy.

Findings: 33.3% of FSW had HIV and 57.7% harboured a potential HR/HR HPV genotype. The two most prevalent pHR/HR genotypes were HPV 16 (16.10%) and HPV 59 (12.20%). BV was the most common infection (48.3%), followed by TV (31.4%) and *Candida spp* (19.9%). A multivariate regression revealed significant associations with both alpha group 9 and 6. BV and HPV 58 (aOR= 2.3; 95%CI: 1.0-5.2; p=0.05), TV and HPV 31 and HPV 35 (aOR=2.0; 95%CI: 2.0;95%CI: 1.0-3.8p=0.04) and (aOR (1.8;95%CI:1.0-3.3) respectively; between *Candida spp* and HPV 53 (aOR=2.0; 95%CI: 1.1-4.03.8; p=0.03) and 16 (aOR=1.9; 95%CI: 1.1-3.3; p=0.03).

Implications: Snowball sampling may have inadvertently excluded FSW less likely to benefit from a social network. Significant associations between BV and HPV 58 and between *Candida spp* and HPV 16 and 53 suggest the need for STD management within a cervical cancer prevention program. The probable synergistic effects of the vaginal microbiota should be elucidated, especially within this vulnerable population. Given the potential of FSW to transmit STIs, robust epidemiological sampling methods are urgently required that account for the heterogeneity of the FSW population.

Key words: Female Sex workers, potential high risk/high risk HPV, Bacterial vaginosis, Trichomonas Vaginalis, Candida spp, HIV

Abbreviations:

FSW= Female sex workers

HIV= Human immunodeficiency virus

HPV= Human Papilloma virus

pHR HPV= potential high risk

BV= Bacterial vaginosis

TV: Trichomonas vaginalis

1. Introduction

Human papillomavirus (HPV) is a sexually transmitted infection and High-risk (HR) HPV DNA has been shown to be present in 99.7% of cervical cancers spanning the globe.[1] Over 200 HPV genotypes have been identified and are divided into high-risk (HR) and low risk carcinogens depending on their capacity to induce cervical intraepithelial neoplasia (CIN) and invasive cervical cancer. HR types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Others are classified only as potential High risk (pHR) (types 53, 66, 68, 70, 73, 82). HPV 16 and HPV 18 are the most virulent HR-HPV genotypes, causing about 70% of all invasive cervical cancer (ICC) in the world. [2]The 15 HR oncological viral strains can be disaggregated into the HPV 16 group (alpha-9) of the alpha-papillomavirus genus (HPV 31, HPV 33, HPV 35, HPV 52, and HPV 58) and the HPV 18 group (alpha-7; HPV39, HPV 45, HPV 59, and HPV 68) and HPV 53, HPV 30, HPV 56 from the alpha-6 type species. [3] [4]

In Kenya, the Ministry of Public Health and Sanitation has conceived of a comprehensive cervical cancer prevention strategy, which encompasses plans for administrating quadrivalent vaccine, including HPV 16, 18, 6 and 11 to preteen girls in the near future. Currently the GAVI-supported HPV vaccine pilot programme is being implemented in Kitui County in Eastern Kenya, and is currently awaiting approval for nationwide rollout, and successful global funding.[5] In 2014, a nonavalent vaccine containing additional HPV types HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 antigens was licensed by the US Food and Drug Administration[6] which will represent a milestone in the global cervical cancer prevention landscape, as it is expected to prevent 90% of ICC cases worldwide.

However, despite these strides in the cervical cancer prevention landscape, concomitant STDs in HPV-infected women may hamper cervical cancer prevention by resulting in prolonged HPV infection and further increasing the risk of CIN.[7] [8] [9] [10]

African women have the highest prevalence of Bacterial vaginosis (BV) in the world, which is characterized by an overgrowth of vaginal anaerobic flora and reduction of H_20_2-producing lactobacilli.[11]A recent longitudinal study conducted in Kenya, Rwanda and South Africa revealed a BV prevalence of 38% in HIV negative women[12] in Kenya. It is posited that BV increases the risk for HR HPV infection due to its association with high levels of anaerobic microorganisms and their by products, which in turn can disrupt the vaginal epithelium, degrade cervical mucus, and cleave immunoglobulin A.[13] [14] [15] A recent meta-analysis of available literature reported a positive association between BV and HPV infection,[16] and a recent HIV Epidemiology Research study in Tanzania, an increased odds for incident of HPV as well as delayed clearance among women with BV.[17]

TV is the second most common cause of lower genital tract infection worldwide and a multiplicity of studies have demonstrated an association with previous and current TV infection and cervical dysplasia and HR HPV.[18] [19,20] [21] Similarly to mechanisms implicated in BV, TV produces microtrauma in the cervical epithelium that may increase the risk for HR HPV infection.[22]

Whilst *Candida albicans* is the most prevalent species in asymatomatic vulvo vaginal candidiasis, certain species of *Candida* are more pathogenic and capable of inducing hyphal and pseudo-hyphal formation, enhancing proteolytic activity and antigen modulation.[23]This would theoretically enable *Candida* to penetrate the mucosal surface and induce mucosal swelling, erythema, and exfoliation of cells[24]and consequently increase risk of HPV. [25]

In Kenya, as in many parts of sub-Saharan Africa, FSW bear the greatest burden of HIV infection and as early as 1985, a study reported that HIV prevalence was as high as 61 per cent among a group of FSW in Nairobi. [26] In Kenya, where the penal code specifically penalizes prostitution[27],

a recent study estimated that 5 per cent of the urban female population of reproductive age could be sex workers.[28]

This analysis purports to test our a priori hypotheses that STIs are associated with genotypes pertaining to the alpha-group 9. The objectives of this study are to assess the prevalence of pHR/HR HPV genotypes, BV, TV, Candida, the most important sexually transmitted infections in FSW women undergoing cervical cancer screening in a private clinic in Eldoret, Kenya, and explore associations between HR HPV genotypes and these vaginal microbiota.

2. Methods

2.1 Study design

A cross sectional design was used to explore associations between BV, TV, and Candida and different pHR/HR HPV genotypes. This cross-sectional study based on record reviews adhered to the methodological guidelines recommended in the STROBE document on observational studies.[29]

Participants were recruited between 2010 and January 2016. Inclusion criteria for the study entailed being female,> 18 years of age, giving consent after being explained the objectives of the study, and engaging in transactional sex in exchange for money, goods, services, or drugs in the last three months. Women were excluded if they were pregnant, < 18 years of age, had a history of cervical dysplasia or cancer, had current abnormal bleeding or bloody discharge, and/or had had a hysterectomy. Given the high stigma attached to FSW, randomized sampling could not be undertaken; instead, FSW were recruited using snowball sampling, a commonly used strategy for locating difficult-to-reach and stigmatized populations.[30]

Peer leader recruitment efforts were used to recruit women engaged in sex work through informational gatherings, snowballing sampling and neighborhood outreach. In order to reduce friendship bias, a limit of referral of 10 FSW has been established. This was undertaken through an extensive community outreach program by Gynocare Women and Fistula Hospital to identify women with obstetric fistula, and to perform STI and cervical screening in Western Kenya. The screening was supported by AML. University. Gynocare Women and Fistula Hospital is a private Non Governmental Organization (NGO) that specializes in Reproductive Health Matters.

Nurses collected the samples and were trained by the gynecologist, the principal investigator of the study. The cytology read the wet preps and if there was consensus about the positivity of the test result between the cytology and pathologist, was it considered to be positive. Women with TV were treated with Metronidazole and a see- and-treat approach was used for women with abnormal cytology. Cryotherapy was used for low grade squamous intraepithelial lesions and for high grade squamous intraepithelial lesions, loop electrosurgical excision procedure was performed.

2.2 Sample size:

The sample size was calculated to allow for a prevalence of at least 38 %[12] for BV and 15%[31] for TV or higher, with a confidence interval of 95% and a power of > 80%.

2.3. Data collection

2.3.1 Structured questionnaire

A structured paper questionnaire was privately administered by trained interviewers covering socio-demographic characteristics, and sexual behavior. Participants were offered testing for HIV and HPV, and participants and women infected with HIV received counseling and required treatment.

2.3.2 Specimen collection and laboratory testing

Women underwent a pelvic examination during which a cervical and high vaginal swab were collected. Cervical samples were collected using a Cervex Brush combo (Rovers Medical Devices, The Netherlands). Cervical cytology was assessed using conventional cytology and reported according to the Bethesda classification. Remaining cervical cells were preserved in Thinprep LBC medium (Hologic, USA) for HPV DNA genotyping. HPV genotyping was done using the Riatol HPV test (Antwerp, Belgium) as described in Micalessi et al. (2008). This test also probes for Trichomonas vaginalis. The high vaginal swab was processed for microscopic evaluation. A vaginal wet mount was prepared for detection of *Candida Albicans* prior to preparation of a second microscopic slide for Gram staining (BV). Candida was diagnosed using the KOH method (10% KOH), and slides were scored according to the Nugent criteria for BV.

A HIV diagnosis was performed using rapid immunoassays: Uni-Gold™ Recombigen® HIV (Trinity Biotech plc, Bray, Ireland) and Determine® HIV-1/2 (Abbott Japan co Ltd, Minato-Ku, Tokyo, Japan). In the case of indeterminate results, an enzyme-linked immunosorbent assay was used to confirm HIV status.

2.4 Ethical approval

Informed consent was sought by all participants. Ethical approval for the study was obtained from the Institutional Research and Ethics Committee at the MOI University in Kenya. Our research was funded by the VLIR IUC Moi University.

2.5 Statistics and Data Analysis

Data analysis was done using STATA version 12 (Stata-Corp LP, College Station, TX).

Due to incomplete information about the study samples, we checked to see whether the missing data (10%) was missing completely at random by performing the Little's MCAR test. Continuous variables were then converted into categorical. Age was dichotomized into ≥30 years and < 30 years; this categorization was used to reflect the WHO 2014 guideline concerning cervical screening.

The method employed for the first objective was to calculate the proportion of STIs and HPV genotypes, along with a 95%CI. For the second objective, univariate logistic regression analysis was performed to determine whether and which STI was a risk factor for the pHR/HR HPV. The resulting odds ratio was used to measure the strength of the association between HPV infection and the STI in turn.

The final multivariable logistic regression model was derived using a forwards stepwise modeling procedure where covariates, concomitant STIs, age, and the number of sexual partners in the past week were added to the model in an iterative manner. The same method was also used to explore the simultaneous effect of covariates. Statistical tests were considered significant when the p-value, derived from the LRT is 0.05 or less.

To assess the impact of HR HPV genotypes on the effects of the parameters, interaction terms were included and assessed in the models by doing a Likelihood Ratio Test. As no interaction terms were significant, no model reflecting the differential impact for that particular outcome was included.

3 Results

3.1Baseline measurements

The total sample of this study included 616 participants. Missing values were observed for BV, (61) TV (7) and *Candida spp* (7), which precluded them from being analyzed. The Little's MCAR test (p=0.4) revealed that the missing values were randomly distributed among the participants.

Socio-demographical characteristics

The mean age of study participants was 28 years and the median number of children was 2 (range: 0-7 children). Regular condom use, which was defined as always or almost always was reported in 21.7% of 369 women. (95% CI= 17.6-26.2%). The median number of partners in the past week was 4 (IQR: 2-7).

HPV genotype and Vaginal infection/STI prevalence findings

Of the 616 women tested for pHR/HR HPV genotypes, 357 (57.7%) harbored a pHR/HR genotype (95%CI= 53.7%-.61.6%). Of 607 women, 202 (33.3%) tested HIV seropositive (95%CI= 29.5%-37.2%). Of 555 women, 268 (48.3%) were classified as positive for BV (95% CI= 44.1- 52.5%). TV was found in 191(31.4%) of 609 women (95% CI= %) and 121 of 609 subjects (19.9%) had a positive test for *Candida spp* (95% CI= 16.8 – 23.3%).

3.2 Prevalence tables

pHR/ HR HPV Genotype	Frequency (n)	Percentage (N=616)	95%CI
pHR/HR HPV genotype	357	57.7%	53.7% 61.6%
HPV 16 (a, b)	99	16.1%	13.3%-19.2 %
HPV 18 (a, b)	68	11.0%	8.7%-13.8%
HPV 31 (b)	49	8.00%	5.9%-10.4%
HPV 33 (b)	2	0.3%	0.04%-1.2%

HPV 35	70	11.4%	9.0%-14.1%
HPV 39	48	7.8%	5.8%-10.2%
HPV 51	52	8.5%	3.7%-7.4%
HPV 52 (b)	89	14.5%	11.8%-17.5%
HPV 53	68	11.0%	8.7%-13.8 %
HPV 56	45	7.3%	5.4%-9.7%
HPV 58 (b)	30	4.9%	3.3%-6.9%
HPV 59	75	12.2%	9.7%-15.02%
HPV 66	60	9.7%	7.5%-12.4%
HPV 68	9	4.9%	3.3%-6.9%
HIV	202	33.3%	29.5%-37.2%
BV (N=555)	268	48.30%	44.06%-52.5%
TV (N=609)	191	31.4 %	28.0%-35.2%
Candida (N=609)	121	19.90%	16.8%-23.3%
BV § TV (N=555)	79	14.2%	11.4%-17.4%
BV & TV & Candida spp (N=555)	22	4%	2.5% - 5.9%

* (a) Covered by the quadrivalent vaccine; (b) covered by the nonavalent vaccine

See table 1 in annex for frequency and prevalence of HPV genotypes and STI. The following pHR/HR HPV types were found in BV, TV and Candida infections. Most HPV genotypes showed a prevalence of around 10%-15% across the three STI. HPV 33 had the lowest prevalence (less than 1% in all the three STIs), while the highest prevalence was for HPV16, which reached over 25% in patients affected by candida. See Table 2 in the annex for the frequency and prevalence of each HPV genotype in BV, TV, and *Candida spp*.

HR HPV genotype	BV infection (N= 268) n (%)	TV infection (N= 184) n (%)	Candida (N=121) n (%)
HPV 16	39 (14.8%)	29 (30.2%)	31 (25.8%)
HPV 18	31 (11.8%)	21 (31.3%)	17 (14.2%)
HPV 31	25 (9.5%)	22 (44.9%)	10 (8.3%)
HPV 33	1 (0.4%)	1 (50%)	1 (0.83%)
HPV 35	27 (10.3%)	29(44.6%)	16 (13.3%)
HPV 39	26 (9.9%)	17 (36.2%)	8 (6.7%)

HPV 45	16 (6.1%)	9 (29.0%)	7 (5.8%)
HPV 51	23 (8.8%)	22 (42.31 %)	13 (10.8%)
HPV 53	31 (11.8%)	30 (44.1%)	22 (18.3%)
HPV 56	19 (7.2%)	29 (45.5%)	10 (8.3%)
HPV 58	18 (6.8%)	9 (30%)	8 (6.7%)
HPV 59	38 (14.5%)	22 (29.3%)	16 (13.3%)
HPV 66	30 (11.4%)	17 (28.3%)	13 (10.8%)
HPV 68	5 (1.9%)	5 (55.6%)	1 (0.8%)

*****BV: Bacterial Vaginosis; §TV: Trichomona vaginalis

Table 2: Frequency and prevalence of each HPV genotype in BV, TV, and *Candida spp*

3.3 Univariate analysis

Univariate analyses of strength of association between the prevalence of pHR/HR HPV genotype and BV, reveal marginal associations between pHR/HR HPV genotype and BV and a marginal protection between the prevalence of pHR/HR HPV and TV.

A marginal association was observed between BV and HPV 58 and significant associations between TV and HPV 31, 35, 53, and 56. A univariate analysis also yielded a very strong significant association between *Candida spp* and HPV 16 (OR=2.2, 95%CI: 1.3-1.7) and 53 (OR=2.1, 95%CI: 1.2-3.6). See table 3, for crude associations between various HPV genotypes and BV, TV and Candida; measure of association is expressed as OR derived from univariate logistic regression

HPV genotype	**Bacterial vaginosis**		**TV**		**Candida spp**	
	OR (95%CI)	**p-value***	**OR (95%CI)**	**p-value***	**OR (95%CI)**	**p-value***
pHR/HR HPV	1.4 (1.0-2.0)	0.06	1.7 (1.3-2.4)	0.005	1.1 (0.7-1.7)	0.6
HPV 16	1.01 (0.6-1.6)	1.0	1.0 (0.6- 1.5)	0.9	2.2 (1.3-3.5)	0.002
HPV 18	1.2 (0.7-2.0)	0.6	1.0 (0.6-1.8)	0.9	1.4 (0.8-2.5)	0.3
HPV 31	1.4 (0.7-2.5)	0.3	1.9 (1.1-3.5)	0.03	1.02 (0.5-2.1)	1.0
HPV 33	1.1 (0.07-7.1)	1.0	0.4 (0.03-7.2)	0.6	4 (0.2-64.1)	0.3
HPV 35	0.9 (0.5-1.5)	0.7	1.9 (1.2- 3.3)	0.01	1.3 (0.7- 2.4)	0.3

HPV 39	1.7 (0.9-3.2)	0.1	1.3 (0.7-2.4) .	0.4	0.8 (0.4-1.8)	0.6
HPV 45	1.2 (0.6-2.6)	0.6	0.9 (0 .4-2.0)	0.8	1.1 (0.5-2.6)	0.8
HPV 51	1 (0.5-1.8)	0.9	1.7(1.0-3.1)	0.07	1.4 (0.7-2.6)	0.4
HPV 53	1.2 (0.7-2.1)	0.5	1.9 (1.1-3.2)	0.01	2.1 (1.2-3.6)	0.009
HPV 56	1.1 (0.6-2.1)	0.9	2.0 (1.1- 3.6)	0.03	1.2 (0.6-2.5)	0.7
HPV 58	2 (0.9- 4.4)	0.09	1.0 (0.4- 2.1)	0.9	1.5 (0.6-3.4)	0.4
HPV 59	1.4 (0.8-2.3)	0.2	0.9 (0.5-1.6)	0.7	1.08 (0.6-2.0)	0.8
HPV 66	1.2 (0.7-2.1)	0.5	0.9 (0.5-1.6)	0.6	1.1 (0.6- 2.1)	0.8
HPV 68	1.8 (0.4-7.5)	0.4	2.2(0.2-36.0)	1.0	0.5 (0.06-4.0)	0.5

*P-value from LRT

Table 3: crude association between various HPV genotypes and BV, TV and Candida; measure of association expressed as OR derived from univariate logistic regression

3.4Multivariate analysis:

As no interaction terms were significant, no model reflecting the differential impact for those particular outcomes were included.

In a multivariable logistic regression analysis controlling for concomitant TV, *candida spp*, HIV, ≥4 sexual partners, and ≥30 years of age, BV was associated with HPV 58 infection (aOR= 2.3; 95%CI: 1.0-5.2; p=0.05). A statistically significant borderline association was detected between TV and HPV 31 (aOR=2.0; 95%CI: 2.0; 95%CI: 1.0-3.8; p=0.04) and a borderline association between women with TV and HPV 51 and 53 remained after adjusting for the covariates (aOR=1.7; 95%CI: 0.9-3.3; p=0.08 and (aOR=1.7; 95%CI: 0.9-3.1; p=0.08 respectively.

Also, significant associations remained between *Candida spp* and HPV 53 (aOR=2.0; 95%CI: 1.1-4.03.8; p=0.03) and 16 (aOR=1.9; 95%CI: 1.1-3.3; p=0.03). See table 4 in annex which depicts ORs, adjusted (for age ≥30, ≥ 4 sexual partners in the previous week, concomitant STI/vaginal infection and HIV) association (expressed as OR) between the 3 STI/vaginal infections (as exposure) and each HPV genotype (outcome).

HPV genotype	Bacterial vaginosis		*Trichomonas vaginalis*		Candida spp	
	OR (95%CI)	p-value*	OR (95%CI)	p-value*	OR (95%CI)	p-value*
Any pHR/HR HPV	1.3 (0.9-1.9)	0.2	1.5 (1.0-2.2)	0.06	1.4 (0.9-2.3)	0.1
HPV 16	1.0.(0.6-1.6)	1.0	0.7 (0.4-1.2)	0.2	1.9 (1.1-3.3)	0.03
HPV 18	1.1 (0.6-1.8)	0.9	0.9 (0.5-1.7)	0.8	1.4 (0.7-2.6)	0.3
HPV 31	1.3 (0.7-2.5)	0.4	2.0 (1.0-3.8) 1.0)	0.04	0.9 (0.4- 1.9	0.7
HPV 35	0.8 (0.4-1.3)	0.3	1.8.0-3.3)	0.05	1.2 (0.6-2.3)	0.7
HPV 45	1.0 (0.5-2.2)	1.0	0.7 (0.3-1.6)	0.3	0.8 (0.3-2.2)	0.7
HPV 39	1.4 (0.7-2.8)	0.3	1.1 (0.5-2.1)	0.8	0.8 (0.3-1.8)	0.5
HPV 51	0.9 (0.5-1.7)	0.8	1.7 (0.9-3.3.)	0.08	1.2 (0.6-2.5)	0.6
HPV 53	1.2 (0.7-2.2)	0.5	1.7 (0.9-3.1)	0.08	2.0 (1.1-4.0)	0.03
HPV 56	0.9 (0.5-2.0)	0.9	1.4 (0.7-2.8)	0.3	1.3 (0.6-2.7)	0.6
HPV 58	2.3(1.0- 5.2)	0.05	0.8 (0.3-2.1)	0.7	1.4 (0.5-3.6)	0.5
HPV 59	1.5 (0.9-2.6)	0.1	0.9 (0.5-1.7)	0.7	1.3 (0.7-2.5)	0.4
HPV 66	1.2 (0 .7-2.1)	0.6	0.7 (0.4- 1.4)	0.4	1.1 (0.5-2.2)	0.9
HPV 68	2.3 (0.4-12.6)	0.3	1.8 (0.4-8.3)		Sample size too small	

P-value from LRT

Table 4: adjusted (for age ≥30, ≥ 4 sexual partners in the previous week, concomitant STI/vaginal infection and HIV) association (expressed as OR) between the 3 STI (as exposure) and each HPV genotype (outcome)

4 Discussion

4.1 Summary of findings

In our study, no statistically significant association was observed between BV, TV and *Candida spp* and the prevalence of pHR/HR HPV genotypes. The most prevalent vaginal infections/ STIs were pHR/HR HPV genotypes, followed by BV, HIV, TV and *candida spp*. A multivariate regression revealed significant associations with not only alpha group 9 but also alpha group 6. A statistically significant association was detected between BV and HPV 58, TV and HPV 31 and 35, and *Candida spp* and HPV 16 and 53.

4.2. Comparison with other relevant published studies

The prevalence of BV of 48.3% is less than the one found in our previous HIV-HPV study of 74 co-infected women in Mombasa, 62.2 % (95%CI: 50.9- 73.5%)[32]. Moreover, it is both slightly higher than the 43.1% exhibited in a cross sectional study in Kenya between 2010-2012[33] as well as the recent prevalence of 38% found in a recent longitudinal study conducted in Kenya, Rwanda and South-Africa in HIV-negative women. [12]

We have not detected a statistically significant association between TV and HPV 16 like the one a derived from a study in Tanzania [31], where patients with TV were 6.5 times more likely to have HPV type 16 than TV-negative patients (OR, 6.5; 95% CI, 1.1–37). However, we found significant associations between TV and HPV 31 and 35, both genotypes phylogenetically related to HPV 16. In disagreement with our findings is a study in Spain (2012), where the presence of TV was statistically significant, in women with HPV 18, 45, 66, and 68.[34]

However, our findings are also congruent with the findings of the Spanish study, which reported a positive association between BV and HR-HPVs with HPV 16 and its phylogenetically related alpha 7 clade genotypes 31, 33, 52, although not 58. Our statistically significant association detected between BV and HPV 58 is in agreement with our previous findings where a marginal association had been detected between HPV 58 and BV in 74 dually HIV-HPV infected women. [32]

In disagreement with the study in Spain, which reported that C*andida spp* was not a cofactor in the presence of HR-HPV, we observed a significant association between HPV 16, the pHR HPV 53 and *Candida spp*. Whilst some studies have only detected HPV 53 in low-grade lesions[35] [36] in two of our cross sectional studies with HIV infected women in Belgium [37] and in Mombasa (in press), HPV 53 was found as a stand alone genotype in a HSIL case in Belgium and in a ICC case in our HIV study population from Mombasa, Kenya.

4.3 Potential explanation for differences between studies

The lower BV prevalence found in this study population than the 64% found in our HIV-HPV co-infected study population,[32] suggests a potential synergistic interaction between HIV and BV. In addition, in light of a slightly protective effect of condom use for BV prevention, pooled (RR: 0.8; 95% CI, 0.8–0.9),[38] the irregular condom use in our FSW population may contribute to our higher prevalence.

Our high prevalence of TV (31.6%) is higher than the TV 10.4% reported in the study TV, which may be attributed to the higher number of sexual partners of our FSW population as well as the high prevalence of HIV-infected women in our study population. In addition, our very high TV prevalence may be attributed to the more significant PCR diagnosis of TV in this study.

The different significant associations between BV, TV, *Candida spp* and pHR/HR genotypes found in our study compared to both the Spanish and the Tanzanian study which included only HIV negative women suggests that synergistic interactions between HIV, HR HPV genotypes and BV, TV or *Candida spp* may differ.

4.4. Strengths and limitations

Our major strengths were the large sample size to explore risk factors for abnormal cytology and the sensitive screening HPV diagnostics employed. The study being cross sectional in nature, it was not possible to infer any causal association between the genotypes and BV, TV and *Candida spp* due to the non-fulfilment of the temporal criterion. In addition, information on risk factors were collected through a face-to face pre-coded questionnaire, therefore there may be underreporting and thereby misclassification of risky sexual behaviour, contending in residual confounding.

The participant selection could have introduced some selection bias, but unfortunately, approaching FSW with random sampling becomes more difficult; this factor further restricts the

generalizability of our results to other FSW within the region. Also, the non-randomness of snowball sampling precludes ascertainment of the representativeness of the sample to the FSW population in Western Kenya, and may have excluded FSWs without any social networks. Other studies have tested using more sophisticated sampling techniques, such as respondent driven sampling (RDS), in order to better reach FSWs in China, Brazil, Kenya, and Vietnam.[39] There is some evidence that this approach may provide better access to diverse and hidden types of FSW, though more work is still needed to understand just how well RDS measures disease prevalence, as well as its ability to capture variations in the social networks of hidden populations. [39] Additionally, by excluding FSW under 18 years of age, our findings may not be generalizable to adolescent FSW. Also, the non-random sampling nature of the snowballing sampling strategy precludes ascertainment of the representativeness of the population to the FSW population in Western Kenya and may have excluded FSW without any social network. By excluding FSW under 18 years of age, our findings may not be generalizable to adolescent FSW.

Moreover, the high percentage of missing data for condom use precluded us from introducing this variable into the model.

4.5. Potential clinical and/or public health implications

Cervical cancer remains the second most common cause of cancer morbidity/mortality among women of reproductive age in Kenya and sub-saharan Africa.[40]Given both our > 10 % prevalence of HPV 53 in HIV positive women and> 10% prevalence of *Candida spp*, and the lack of coverage of the HPV 53 within the forthcoming roll-out of the nonavalent vaccine, our findings underscore the urgent need to elucidate the synergistic interactions between HPV 53, *Candida ssp* and TV to corroborate these findings. In addition, the epidemiology and micro epidemiology of HPV 53, especially in HIV infected women needs to be explored.

In order to prevent cervical disease related to HPV 53, it might be of interest to include HPV 53 as well as other potential high-risk types in a primary HPV screening test. Currently these tests are available, but if the tests designed for developing countries could also include these types, it would be of important public health benefit. Development of novel assays is to be preferred over cytological detection. In addition, in vaccinated women, it is completely unknown how not-covered HPV types will be behave. It is postulated that either the phenomenon of cross protection could be detected or alternatively, type-replacement could occur, which in turn would require surveillance of HPV 53 along with other genotypes not found in the forthcoming nonavalent vaccine.

The significant association observed between HPV 16 and *Candida spp* in this study suggests that women not eligible for the Gardasil vaccine in Kenya may benefit from a simultaneous *Candida spp* screening for prevention of HPV 16. Moreover, the significant association observed between BV and HPV 58 also suggests that screening of all women for BV prior to the roll out of the forthcoming nonavalent vaccine may contribute to cervical dysplasia and possibly cancer prevention.

Also, our high prevalence of BV and TV in this study population and the potential association found between these STIs and abnormal cytology confirm the need to gain a greater understanding of the vaginal microbiota and its synergistic influence on HR HPV infections. In addition, our findings also include the public health benefit of making STDs screening and treatment as a crucial component of a cervical cancer prevention programme in order to potentially decrease the high rates of cervical cancer in Kenya.

Moreover, FSWs are a vulnerable population group, as they lack access to sexual and reproductive health services tailored to their needs, and are more prone to sexually transmitted infections and HIV/AIDS. The findings of this study suggest a public health approach that goes

beyond HIV screening with this population group, which involves concomitant screening for cervical cancer and other co-infections like BV and candida.

In light of the spreading of STIs in Sub Saharan African by FSWs to the general population, it is urgent that new epidemiological research methodology within this stigmatized and hard to reach population be enhanced to prevent possible selection bias that convenience sampling may introduce.

5. Conclusions

Our study suggested significant associations between HPV type 16 and 53 and *Candida spp* and between BV and HPV 58 underscoring both a need for future microbiological research on how these organisms interact at the cellular level and introducing BV and candida control within a cervical cancer prevention framework. As HPV 53 is covered by the future nonavalent vaccine, there is a need to elucidate the synergistic interactions between HPV 53 and *Candida spp,* and possibly between TV along with its involvement as a stand-alone genotype in high grade lesions.

The high prevalence of BV, TV, and *Candida spp* found in pHR/HR HPV infected women with a high HIV prevalence also underscores the beneficial public health impact that screening for concomitant genital infections would have in order to reduce the probable synergistic effects the vaginal microbiota within this vulnerable population and as a corollary to the general population. Furthermore, our findings expose both the high concomitant STIs prevalence and very irregular use of condoms in this vulnerable population, thus making the case for strengthening STD counseling.

Given the crucial role that FSWs also have in STI transmission, more robust epidemiological sampling methods are needed to make findings more generalizable to the general FSW population.

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MULTIPLE HPV INFECTIONS IN FEMALE SEX WORKERS IN WESTERN KENYA: IMPLICATIONS FOR PROPHYLACTIC VACCINES WITHIN THIS SUB POPULATION

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Multiple HPV infections in Female Sex workers in western Kenya: Implications for prophylactic vaccines within this sub population

Whilst the imputed role of High Risk (HR) HPV infection in the development of cervical lesions and cancr has been established, the high number of HPV genotypes that Female Sex workers (FSW) harbour warrants that the synergistic effects of potential HR (pHR) and HR HPV genotypes be elucidated to assess the potential impact of prophylactic vaccines. This population in Kenya also harbours a number of other vaginal infections and STIs, including bacterial vaginosis (BV), trichomonas vaginalis (TV) and *candida spp*.

The aims of this cross-sectional analysis in Kenya are to explore the epidemiology of abnormal cytology and the pairing of pHR/HPV genotypes in HIV-negative and HIV-infected FSW.

Method: A cross-sectional study design of 616 FSW from Western Kenya aged between 18 and 61 years during 2009-2015 using a peer recruitment sampling strategy.

Results:

Of the 599 FSW who underwent cytological examination, 87 had abnormal cytology (14.5%; 95% CI: 12.0-17.6%). A combined prevalence of HPV16 and 18 (29.6%; 95%CI: 22.2- 37.8 %) was observed in abnormal cytology. HPV 53 and 51 were the most observed pairing in FSW with abnormal cytology. Significant adjusted associations were found between abnormal cytology and TV (aOR: 30; 95% CI: 14.1-62.9), multiple HR HPV (aOR: 3.7; 95% CI: 1.9- 7.3), HPV 51 (aOR 3.7; 95%CI 1.6-8.6) and HPV 52 (aOR 6.1; 95% CI: 2.8-13.3).

Conclusion:

HPV 51 and 52 were independently associated with abnormal cervical cytology. The strong association between TV and cervical dysplasia and the high percentage of FSW harbouring more

than one STI underscore the need for enhanced STI management within the framework of cervical cancer prevention.

Key words: FSW, HIV, high risk HPV, potential high risk HPV, multiple pHR/HR coinfections, vaccine efficacy

Introduction:

Human Papilloma Viruses (HPV) are double-stranded DNA viruses which are now deemed to be the chief etiological agents in cervical intraepithelial neoplasias and cancers.[1] High-risk (HR) genotypes, which are associated with cervical cancer include HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 while HPV types 26, 53, 67, 70, 73 and 82 are now classified as possible carcinogenic, and low risk (LR) HPV genotypes considered benign include 6, 11, 42, 43, and 44. [2] The 15 HR oncological viral strains, which have been identified can be broken down into different species: the HPV 16 group (alpha-9) of the alpha-papillomavirus genus (HPV 31, HPV33, HPV 35, HPV 52, and HPV 58) and the HPV 18 group (alpha-7) (HPV39, HPV 45, HPV 59, and HPV 68).

Cervical intraepithelial neoplasia (CIN) can be histologically graded into mild dysplasia (CIN 1), moderate dysplasia (CIN 2), and severe dysplasia to carcinoma in situ (CIN 3). Several studies have reported a robust association between HIV and HPV co-infection and therefore the development of CIN and genital cancer, [3] [4] along with a persistence and recurrence of pre-invasive cervical lesions, CIN 2 or CIN 3. [5]

It is well recognized that among the 14 HR HPV genotypes, HPV 16 and 18 are associated with approximately two thirds of all invasive cervical carcinomas. [6] After HPV16/18, data confirm

HPV31/33/35/45/52/58 as the most frequently detected genotypes in Invasive Cervical Cancer (ICC) worldwide.[7] [8]

Prophylactic vaccines against HPV 16 and 18 are currently being rolled out across the globe for the prevention of cervical cancer, which is likely to yield a significant impact on the future burden of cervical cancer, particularly in sub Saharan Africa, where screening is scarce. Although the bivalent/quadrivalent vaccines, including the LR HPV 6 and 11 constitute a crucial milestone in cervical cancer prevention in HIV-negative women, epidemiological data available suggest that in HIV positive populations, HPV 16 has shown to be frequent, but not as predominant as seen in most HIV negative populations.[9] [10] Moreover, HIV immunosuppression has been linked to multiple HPV infection. [11] [12] Concomitant infection with multiple HPV genotypes has been found to be attributable to the inability to clear HPV infections as well as to the reactivation of latent HPV infections; both occurring as a consequence of immune suppression.[13] [14]

Also, epidemiological knowledge of pHR HPV types is highly limited, mainly because commercial molecular assays focus on HR HPV genotypes. There is a paucity of data on these genotypes in HIV positive women with abnormal cytology, notwithstanding their potential enhanced role in cervical dysplasia development in HIV positive patients. [15] [16]

In Kenya, the Ministry of Public Health and Sanitation has developed a comprehensive cervical cancer prevention strategy, entailing plans for administrating quadrivalent vaccine to preteen girls in the near future. Currently they are running the pilot programme Kituwi in Eastern Kenya and awaiting approval for nationwide rollout and successful global funding.[17] The not yet commercialized nonavalent vaccine in Kenya, containing additional HPV types HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 antigens may have the ability to prevent 90% of ICC cases worldwide.

In Kenya, as in many parts of sub-Saharan Africa, where the penal code specifically penalizes prostitution[18], FSW bear the greatest burden of HIV and STI infections. Concomitant STIs and vaginal infections may lead to prolonged HPV infection, which may in turn increase the risk of CIN.[19 20] Bacterial vaginosis (BV) and *Trichomonas vaginalis* (TV), have been associated with an increased risk of squamous intraepithelial lesions and/or CIN based on biopsy results.[21 22]

As early as 1985, a study reported that HIV prevalence was as high as 61 per cent among a group of FSW in Nairobi. [23]A recent study reported that in Kenya, 5 per cent of the urban female population of reproductive age could be sex workers.[24]

The objectives of this analysis were primarily to assess genotype-specific distribution of pHR/HR HPVs in FSW with abnormal cytology, as well as the pairing prevalence of certain pHR/HR HPV genotypes found in HIV negative and HIV infected women with abnormal cytology; secondly, to investigate which HPV genotypes and other variables were associated with abnormal cytology.

2. Methods

2.1 Study design

A cross-sectional design was used to explore associations between abnormal cytology and pHR/HR HPV genotypes. This cross-sectional study based on record reviews adhered to the methodological guidelines recommended in the STROBE document on observational studies.[25]

Women were excluded if they were pregnant, <18 years of age, had a history of cervical dysplasia or cancer, had current abnormal bleeding or bloody discharge, and/or had a hysterectomy. Snowball sampling was undertaken instead of randomized sampling, which is an often used strategy for locating difficult-to-reach and stigmatized populations.[26] This involved a sample of women engaged in sex work being recruited through informational gatherings, snowball sampling

and neighborhood outreach. Inclusion criteria for the study entailed being female, giving consent after being explained the objectives of the study, and having engaged in sex in exchange for money, goods, services, or drugs in the last three months. In order to reduce friendship bias, a limit of referral of 10 FSW was established. This activity was undertaken by means of an extensive community outreach program by Gynocare Women and Fistula Hospital, two non-governmental organizations specializing in reproductive health, to identify women with obstetric fistula, STI screening and cervical screening in Western Kenya. The screening was supported by AML, Antwerpen in Belgium.

2.2 Sample size:

The sample size was calculated to allow for a prevalence of at least 15 % for abnormal cytology,[27] with a confidence interval of 95% and a power of 80%.

2.3. Data collection

2.3.*1 Structured questionnaire*

A structured paper questionnaire was privately administered by trained interviewers covering socio-demographic characteristics, and sexual behavior. Participants were offered testing for HIV and HPV. HIV results were disclosed to participants and women infected with HIV received counseling and treatment.

2.3.2 Specimen collection and laboratory testing

A gynaecological examination was performed using a swab. Candida colonization was diagnosed by Gram stain; bacterial vaginosis was scored according to Nugent's criteria. Infection with

Trichomonas vaginalis was diagnosed by PCR, using a validated method which was part of the HPV genotyping essay.

A HIV diagnosis was performed using rapid immunoassays: Uni-Gold™ Recombigen® HIV (Trinity Biotech plc, Bray, Ireland) and Determine® HIV-1/2 (Abbott Japan co Ltd, Minato-Ku, Tokyo, Japan). In the case of indeterminate results, an enzyme-linked immunosorbent assay was used to confirm HIV status.

2.3.3 Biologic specimens

Cervical samples were collected using a cervix brush (Cervex-brush®, Rovers®, Oss, The Netherlands), and cervical cytology was assessed with conventional Papanicolaou (Pap) smears. Slides were read by a cytologist with master level training, supervised by a pathologist. An external cytopathologist provided quality control. The Bethesda Reporting System was used for cytological classification.[28]

The cervix brush tips were preserved in a liquid-based cytology collection medium (SurePath®, Tripath Imaging Inc., Burlington, North Carolina, USA) and stored at 4°C until further processing.

2.4 Ethical approval

Ethical approval for the study was obtained from the Institutional Research and Ethics Committee at the MOI University in Kenya (No 000187) on August 11th, 2011.

2.5 Statistics and Data Analysis

Data analysis was done using STATA version 12 (Stata-Corp LP, College Station, TX). Due to incomplete information about the study samples, we checked whether the missing data (10%) were randomly distributed by performing the Little's MCAR test. Continuous variables were then

converted into categorical. Age was dichotomized into ≥30 years and <30 years; this categorization was used to reflect the WHO 2014 guideline concerning cervical screening. The number of pHR/HR HPV co-infections was also dichotomized as a categorical variable with 1 and ≥2 genotypes.

We first described the distribution of pHR/HR HPV types observed among women with both normal cytology and abnormal cytology, for which the overall prevalence and 95% confidence intervals (95%CI) based upon normal distributions were calculated.

To examine patterns of clustering of high-risk HPV types, the prevalence of pHR/HR HPV genotypes in presence of another pHR/HR HPV genotypes by abnormal cytology was calculated, which was defined as the proportion of women with abnormal cytology who were positive for the pHR/HR HPV genotypes. The prevalence of pairings detected in women with HSIL was also calculated.

The variables were explored by means of tabulation and cross-tabulation. The χ^2 test was used to assess whether there was an association between CIN 2+ and various risk factors. In building the regression models, age, STIs and multiple pHR/HR HPV genotypes tested were entered. A multivariable logistic regression analysis was performed to assess the association between pHR/HR HPV genotypes and abnormal cytology, ASC-US or higher, the main outcome of interest and to simultaneously control for potential confounders. The Likelihood Ratio Test (LRT) was used to measure the association of each variable with the outcome.

To assess for a possible interaction due to age, logistic regression models were fitted with and without the interaction term; significance for interaction was then checked through visual inspection of the OR and LRT. Statistical significance was considered at $p \leq 0.05$.

Results:

Out of the 616 participants, data from only 599 participants could be analysed because of the quality of the specimen**.** Missing values were observed for BV (61), TV (7) and *candida spp* (7). The Little's MCAR test revealed that the missing values were randomly distributed among the participants (p=0.4), therefore unlikely to have introduced information bias.

The mean age of study participants was 28 years and median parity was 2 (range: 0-7 children). Regular condom use, which was defined as always or almost always was reported in 21.7% of 369 women. (95% CI= 17.6-26.2%). The median number of partners in the past week was 4 (IQR: 2-7). Table 1 depicts the prevalence of categories for age and sexual behavior.

Socio demographic Variables	**Percentage (95%CI)**
>30 years ≤30 years	40.1% (95%CI: 36.2-44.1) 59.9% (95% CI: 55.9-63.8)
Sexual behavior:	
>4 sexual partners the past week ≤4sexual partners	1.9 % (95%CI:1.0-3.3) 98.1% (95%CI: 96.7-99.0)
Regular use of condom No regular use of condom	21.7% (95%CI 17.6-26.2) 78.3% (95%CI: 73.8-92.3)

Table 1 reports the prevalence of categories for age and sexual behaviour

Of the 616 FSW who underwent cytological examination, 512 (85.5%: 95% CI: 82.4- 88.2) had normal cytology, 87 had abnormal cytology (14.5%; 95% CI: 12.0-17.6%) and 17 were excluded due to poor quality of the sample, leaving 599 participants on whom we could perform the

analysis. Of the FSW population, 192 FSW were HIV positive, of which 27.1 % (95%CI: 20.9-34.0%) had abnormal cytology. Table 2 in annex reports the prevalence of cervical abnormalities observed in the sample (N=616).

Cytological status	n	% of FSW (95%CI)
Normal cytology	512	85.5% (82.4-88.2)
ASC-US	10	1.7% (0.8- 3.04)
LSIL	63	10.5% (8.2-13.3)
HSIL	14	2.3% (12.8-3.9)
Excluded samples due to poor quality	17	2.8%

Table (2) reports the prevalence of cervical abnormalities observed in the sample (N=599)

The prevalence of pHR/HR HPV and multiple pHR/HR co-infections in the 616 FSW was 57.7% and 32.8% respectively. In HPV infected FSW, HIV-infected FSW had a significantly higher number of co-infections (2.0) than HIV negative FSW (0.9), p<0.001.

The prevalence of BV in this population was 48.3 %, followed by TV 31.4 %, and *candida spp* 19.9 %. FSW with BV had the highest prevalence of multiple pHR/HR co-infections with 53.4%, followed by TV, and *candida spp*, 38.8%and 23.9% respectively. Table (3) reports the prevalence of each HPV genotypes and vaginal infections/TV.

pHR/ HR HPV Genotype	Frequency (n)	Percentage (N=616)
HPV 16 (N=616)	99	16.1%
HPV 18 (N=616)	68	11.0%
HPV 31 (N=616)	49	8.0%
HPV 33 (N=616)	2	0.3%
HPV 35 (N=616)	70	11.4%
HPV 39 (N=616)	48	7.8%
HPV 51 (N=615)	52	8.5%
HPV 53 (N=616)	68	11.0%
HPV 56 (N=616)	45	7.3%
HPV 58 (N=616)	30	4.9%
HPV 59 (N=616)	75	12.2%

HPV 66 (N=616)	60	9.7%
HPV 68 (N=616)	9	4.9%
BV and STIs		
BV* (N=555)	268	48.3%
TV (N=609)	191	31.4%
Candida (N=609)	121	19.9%

Table (3) reports the prevalence of each HPV genotypes and STIs

Normal and Abnormal cytology and each HPV genotypes.

The combined prevalence of HPV 16 and HPV 18 was 27.1%, and of the two pHR HPV genotypes tested, HPV 53 and 66, 20.8 % (95%CI: 17.6- 24.2%). In women with abnormal cytology, we observed a multiple pHR/HR HPV genotype prevalence of 65.5%.

HPV genotype	Abnormal cytology	%(N=84)
HPV 16	24	28.6%
HPV 18	15	17.9%
HPV 31	13	15.5%
HPV 33	1	1.2%
HPV 35	18	21.4%
HPV 39	13	15.5%
HPV 45	9	10.7%
HPV 51	18	21.4%
HPV 52	26	31.0%
HPV 53	21	25.0%
HPV 56	13	15.5%
HPV 58	4	13.3%
HPV 59	17	23.6%
HPV 66	9	10.7%
HPV 68	4	4.8%

Table (4) reports the prevalence of each HPV genotype among HPV-positive women with abnormal cytology

Potential HR HPV in abnormal cytology

HPV 53 and HPV 66 were found as a stand-alone pHR HPV genotype in two LSIL cases.

Pairings of pHR/HR HPV genotypes in abnormal cytology

A higher prevalence of pairings in abnormal cytology were observed in HIV infected FSW than HIV negative FSW. The most frequently observed pairings in HIV- negative FSW were HPV 18 and 31 (n=3) occurrences, and HPV 31 and 52 (n= 2), involving genotypes phylogenetically related to the HPV 16.

In HIV-infected women, HPV 53 and HPV 51 are observed in 5 of the most prevalent pairings, followed by HPV 16, observed in 4 of the most prevalent pairings. Whilst in HIV negative FSW, the most prevalent co-infection pairings exhibited a mixed alpha 9-alpha 7 combination or a homogenous alpha 9 pattern, in HIV infected women, the non-alpha 9/7 pHR HPV genotypes, HPV 53 (alpha 6) and HPV 51 (alpha 5) figure prominently. See Table 5 in annex which depicts the most prevalent pairing occurrences in women with abnormal cytology.

Prevalent pairings in abnormal cytology in HIV-negative women: N=35)	**(n= in normal cytology)**	**n= abnormal cytology**
HPV 18 and 31	2	3
HPV 31 and 52	7	2
Prevalent pairings in HIV infected women with abnormal cytology: N=52)		
HPV 16 and 39	2	6
HPV 16 and 52	9	7
HPV 16 and 51	4	5
HPV 16 and 53	10	7
HPV 18 and 52	12	5
HPV 18 and 53	8	5
HPV 31 and 51	2	5
HPV 35 and 51	2	5

HPV 35 and 53	4	7
HPV 45 and 53	0	6
HPV 45 and 59	2	5
HPV 51 and 53	2	7
HPV 51 and 56	1	6
HPV 52 and 56	3	6
HPV 53 and 56	1	5

Table (5) most prevalent pairing occurrences in women with abnormal cytology

Risk factors for abnormal cytology:

In the univariate analysis, age did not appear to be associated with abnormal cytology: women under 30 years of age have a crude OR 1.1 (9 5%CI: 0.7-1.8 p=0.6) of having abnormal cytology compared to older women.

A very strong significant association was found between TV and abnormal cytology OR: 30.0 (95% CI: 14.1-62.9) adjusting for age and pHR/HR HPV genotypes, BV and *candida spp* and HIV.

A statistically significant OR, adjusted for age was found for multiple HPV and abnormal cytology compared to single HPV genotype infection. This association decreased but remained significant (OR; 3.9; p< 0.001; 95%CI: 1.9-7.8) when adjusted for HIV.

Whilst all pHR/HR HPV genotypes were significant predictors of abnormal cytology, when adjusted for age pHR/HR HPV genotypes co-infections, HIV, BV, TV, and *candida spp* these associations became statistically insignificant, except for HPV 51 and 52. Table 6 in annex depicts the age, BV, TV and *candida sp*p adjusted association between specific pHR/HR HPV genotypes and abnormal cytology.

As no interaction terms were significant, no result reflecting the differential impact for that pacular outcome was presented.

STI or HPV genotype	OR Model 1 (95%CI)	p-value	OR Model 2 (95%CI)	p-value
BV	0.9 (0.6-1.5)	0.8	0.8 (0.5-1.4)	0.5
TV	24.8 (12.7-48.3)	<0.001	30.0 (14.1-62.9)	<0.001
Candida spp	1.0 (0.5-1.7)	1.0	0.9 (0.5-1.7)	0.7
Multiple HPV infection	5.3 (2.9-9.7)	<0.001	3.7 (1.9-7.3)	<0.001
HPV 16	1.9 (0.8-4.5)	0.1	1.2 (0.5- 3.2)	0.5
HPV 18	0.8 (0.3-2.1)	0.7	1.04 (0.4-2.8)	0.9
HPV 31	0.5 (0.2-1.5)	0.2	0.6 (0.2-1.7)	0.3
HPV 33	3.9 (0.05-293.9)	0.5	2.8 (0.03-254.6)	0.6
HPV 35	1.3 (0.6-3.0)	0.5	1.1 (0.5-2.7)	0.9
HPV 39	3.3 (1.3-8.7)	0.03	2.5 (0.9-7.1)	0.09
HPV 51	3.7 (1.6-8.6)	0.002	3.7 (1.5-9.0)	0.004
HPV 52	6.1 (2.8-13.3)	<0.001	4.0 (1.6-8.2)	0.002
HPV 53	2.0 (0.8- 4.9)	0.1	1.4 ; (0.5-3.8)	0.5
HPV 56	2.5 (1.0-6.6)	0.06	2.0 (0.7-5.7)	0.2
HPV 58	0.9 (0.2-3.6)	0.9	1.1 (0.3-5.2)	0.9
HPV 66	1.2 (0.5-3.0)	0.7	1.0 (0.4-3.0)	0.9
HPV 68	1.7 (0.2-17.0)	0.7	0.8 (0.1-7.4)	0.8

Table 5: association between STI, specific pHR/HR HPV genotypes and abnormal cytology; OR from Logistic regression; p-value from LRT
Model 1: OR adjusting for age, pHR/HR HPV genotypes, STIs
Model 2: OR adjusting for age and pHR/HR genotypes, STIs and HIV

Discussion

Summary of results

In the present study, we observed a high prevalence of women harbouring more than two pHR/ HR HPV genotypes, which was significantly higher in HIV-1–infected women, consistent with results of several studies illustrating that HIV-1–infected women not only have a greater prevalence of HR-HPV infection but multiple coinfections. [29] [30]

Our observations are in agreement with those of a large study on multiple HPV infections in Costa Rica in which young healthy women with multiple infections were at significantly increased risk of CIN 2+, Although our findings of an association between TV and abnormal cytology are congruent with other observations [31] demonstrating an association between TV and abnormal cytology, [32] [33] [34]our study suggest an unprecedented strong association.

After adjusting for age and multiple pHR/HR HPV co-infections, BV, TV and *candida spp*, no significant association was observed between any single pHR/HR HPV and abnormal cytology, except for HPV 51 and 52 in both HIV negative and positive women. This is incongruent with our previous findings in an exclusively HIV-infected study population in Belgium, [35] in which only the association between HPV 39 and abnormal cytology became statistically significant.

In contrast to HPV 31 and 58 being the most observed frequent pairing in Brazil in HIV infected women, and HPV 31 and 66 and HPV 39 and 52 in our Belgian study population, in this FSW study population, HPV 31, 52 and 66 do not figure prominently within pairings observed in HIV-infected women despite a higher prevalence of HPV 52 than HPV 53.

Also, our high prevalence of women with abnormal cytology without any detected HPV genotypes can be attributed to our testing of only 18 genotypes out of the over 200 HPV types described. Whilst some types may be inducing low grade lesions, they may also lack a cancer-initiating capacity**.**

Strengths and limitations:

Our major strength was that all cervical smears were histologically confirmed and the high sensitivity of the HPV DNA diagnostics employed. However, whilst our sample was large, the number of FSW with abnormal cytology was small, which precluded us from exploring particular co-infection patterns as a risk factor. Moreover, due to our lack of behavioral and clinico-

epidemiological data, including smoking, CD4 count, HAART use or the presence of other co-infections, we have not been able to adjust for these potential confounders, nor assess whether these factors were associated with cervical abnormalities. Also, there may be potential for selection bias as FSW with similar characteristics may have been sampled, as a result of the convenience sampling method used. However, a convenience sampling strategy may have inadvertently excluded those FSW operating in a more clandestine fashion and thereby not benefitting from a social network.

A limitation related to a cross sectional study design may be the lack of data concerning age of acquisition of HIV infection since it is possible this may have occurred too late in life for some of the women in our study to influence abnormal cytology. Similarly, an analysis of a cross sectional study for exploring associations between multiple HPV genotypes and abnormal cytology may have inherent limitations as infections may have been acquired concurrently or sequentially, therefore, resulting in the criterion of temporality for causation not being met. This may have an impact as immunologic responses may differ following concurrent acquisition of multiple HPV genotypes from infections that are acquired sequentially.

Implications for vaccination programs

The only significant association observed between abnormal cytology and HPV 51 and HPV 52 underscore the need for post quadrivalent and nonavalent vaccine surveillance in both HIV negative and HIV infected FSW.

In light of a high prevalence of multiple HR HPV infections in this FSW HIV-negative and infected population, it will need to be elucidated whether cross protection may be hampered by the additional burden due to other synergistic relationships among HR HPV genotypes present. A recent systematic review and meta-analysis[36] found that bivalent Cervarix© vaccine from GlaxoSmithKline had better cross protection against HPV 31 in persistent infection, but that

efficacy against persistent infections with types 31 appeared to decrease with longer follow-up, suggesting a waning of cross-protection. It still remains to be determined whether a cross protection can be extrapolated to HIV-infected women and in presence of multiple HR HPV genotypes.

Moreover, with a high prevalence of the pHR HPV 53 in pairings with the vaccine preventable HPV 16 and HPV 18 in HIV infected women, it will need to be determined how HPV 53 will fare within the vacuum that ensues the quadrivalent vaccine. [37] In addition, given the high median number of pHR/HR HPV genotypes harboured by this population, the synergies between not only two pHR/HR HPV genotypes need to be determined but within a context of other prevalent genotypes, capable of inducing cervical cancer genesis. Whether Gardasil and Cervarix can attain a 70% reduction of cervical cancer may be contingent upon the natural history of the imputed pHR/HR HPV genotypes in cancer genesis along with their synergistic effects

Our very high association between TV and cervical dysplasia underscores the need for STI management to be integrated within cervical cancer prevention program. Furthermore, the biological interaction between TV and HPV and its subsequent capacity to induce progression of cervical dysplasia should be further explored. Moreover, the impact of immune modulating infections, such as tuberculosis, malaria and helminthic infections on cervical disease progression should be elucidated in HIV triple co-infected women with HPV and TV.

Conclusion:

Co-infection with pHR/HR HPV genotypes was more strongly associated with abnormal cytology than any single high-risk HPV. In light of the high prevalence of multiple pHR/HR HPV genotypes harboured by FSW and especially HIV infected women, its micro epidemiology in cervical carcinoma in HIV positive women needs to be explored in order for the vaccine efficacy to be assessed.

Whilst the quadrivalent vaccine may be effective in reducing the prevalence of abnormal cytology in HIV negative and HIV infected FSW, the high presence of multiple infections with HPV 16 requires that the micro epidemiology of concurrent be elucidated. In particular, the high prevalence of the non alpha 9 and 7 genotypes, HPV 51 and HPV 53 observed in pairings with HPV 16 and HPV 18 in HIV infected FSW requires further characterization.

These current gaps in epidemiology underscore the need for FSW, HIV negative or positive to be regularly monitored in the post quadrivalent /nonavalent vaccine era.

The strong association observed between TV and cervical dysplasia as well as the high percentage of FSW harbouring more than one vaginal infection/STI begs for the elucidation of synergistic interactions between multiple STIs to be better assessed as factor(s) for cervical dysplasia and for a wider encompassing cervical cancer prevention framework.

Abbreviations:

FSW= Female sex workers

HIV= Human immunodeficiency virus

HPV= Human Papilloma virus

LR HPV= low risk HPV

pHR HPV= potential high risk

ASC-U= Atypical cells of undetermined significance

LSIL= Low grade squamous intraepithelial lesion.

ASC-H= Typical squamous cells-cannot exclude high-grade squamous intraepithelial lesion

HSIL= High grade squamous intraepithelial lesion.

Declaration

Ethics approval and consent to participate:

Informed consent was sought by all participants. Ethical approval for the study was obtained from the Institutional Research and Ethics Committee at the MOI University in Kenya.

Consent for publication: yes

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HUMAN PAPILLOMA VIRUS CORRELATES OF HIGH GRADE CERVICAL DYSPLASIA IN HIV-INFECTED WOMEN IN MOMBASA, KENYA: A CROSS-SECTIONAL ANALYSIS

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Abstract

Background

Women living with HIV are at increased risk to be co-infected with HPV, persistent high-risk (HR) human papillomavirus (HPV) infection and increased HR HPV viral load, which make them more at risk for cervical cancer. Despite their inherent vulnerability, there is a scarcity of data on potential high risk (pHR) and HR HPV genotypes in HIV- infected women with cervical dysplasia and HPV-type specific viral load in this population in Sub Saharan Africa.

The aim of this analysis of HIV-infected women was to explore the virological correlates of high-grade cervical dysplasia (CIN 2+) in HIV-infected women, thereby profiling HPV genotypes.

Method

This analysis assesses baseline data obtained from a cohort study of 74 HIV-infected women with abnormal cytology attending a Comprehensive Care Centre for patients with HIV infection in Mombasa, Kenya. Quantitative real-time PCR was used for HPV typing and viral load.

Results

CIN 2 was observed in 16% (12/74) of women, CIN 3 in 23% (17/74), and, invasive cervical carcinoma (ICC) in 1% (1/74) of women. In women with CIN 3+, HPV 16 (44%), HPV 56 (33%), HPV 33 and 53 (HPV 53 (28%) were the most prevalent genotypes. HPV 53 was observed as a stand-alone HPV in one woman with ICC.

A multivariate logistic regression adjusting for age, CD4 count and HPV co-infections suggested the presence of HPV 31 as a predictor of CIN 2+ (adjusted odds ratio [aOR]:4.9; p=0.05; 95%

(Confidence Interval) [CI]:1.03-22.5). Women with CIN2+ had a significantly higher viral log mean of HPV 16, (11.2 copies/ 10 000 cells; 95% CI: 9.0-13.4) than with CIN 1.

Conclusion

The high prevalence of HPV 53 in CIN 3 and as a stand-alone genotype in the patient with invasive cervical cancer warrants that its clinical significance be further revisited among HIV-infected women. HPV 31, along with elevated means of HPV 16 viral load were predictors of CIN 2+.

Key words: human papilloma virus, potentially high risk/ high-risk HPV genotypes, HPV viral load, co-infections, pairings, CIN 2+, Kenya

Background:

Kenya is home to the world's fourth-largest HIV epidemic in the world. In 2013, an estimated 1.6 million people were living with HIV and roughly 57,000 people died from AIDS-related illnesses.[1] Cervical carcinoma, an AIDS-related cancer, is the most common female cancer in sub-Saharan Africa;[2] it has become the second most prevalent cancer among women in Kenya, after breast cancer, and its incidence is increasing.[3]

Distinct precancerous stages or pre-invasive precursor lesions called cervical intraepithelial neoplasia (CIN), or dysplasia can be discriminated before becoming invasive cervical cancer (ICC). CIN can be histologically graded into mild dysplasia (CIN 1), moderate dysplasia (CIN 2), and severe dysplasia to carcinoma in situ (CIN 3).[4]

Human Papilloma Viruses (HPV), a sexually transmitted DNA virus are double-stranded DNA viruses, considered the primary etiological agents in cervical intraepithelial neoplasia and cancers. **"H**igh-risk", (HR) include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and due to lack of evidence of biological activity in tumour tissues, HPV26, 53, 66, 67, 70, 73, and 82 are classified as probably or possibly high risk.[5]

It is well recognized that among the 15 HR HPV genotypes, HPV 16 and HPV 18 confer the greatest risk for CIN 2 or worse because these two genotypes are associated with approximately two thirds of all invasive cervical carcinomas.[6] Several countries, including Kenya, have licensed and adopted the bivalent HPV vaccine (Cervarix™) that protects against HPV genotypes 16 and 18 and the quadrivalent vaccine (Gardasil™) that protects against HPV genotypes 6, 11, 16 and 18.[7] In 2014, a nonavalent vaccine, not yet commercialized in Kenya, containing the most frequently detected types in ICC worldwide, HPV types 31, 33, 45, 52, and 58 antigens,[8] will have direct implications for cervical cancer incidence and prevention in all regions of the world with the potential to prevent almost 90% of ICC cases worldwide.

If viral persistence is established, a variety of host cofactors may act upon the immune system and the tissue microenvironment in the cervix to induce development of cervical lesions.[9] A relationship has been established between HIV immunosuppression and multiple HPV infection,[10] [11] which has been attributed to the inability to clear HPV infections and to reactivate latent HPV infections,.[12] [13] [14] [15] Moreover, certain viral risk factors may also play a role in establishing viral persistence. It has been suggested that high HPV viral load may be aetiologically associated with cervical disease pathogenesis, although studies have yielded conflicting results.[16] [17] [18] [19] [20] [21]

Moreover, epidemiological knowledge of potential high-risk (pHR) HPV types is limited, mainly because commercial molecular assays focus on HR HPV genotypes. Data on pHR genotypes in HIV-infected women are even scarcer, although it can be hypothesized that they might play a role in HPV related diseases in HIV positive women.[22] [23] This can be attributed to the fact that HIV infected women harbor a higher prevalence and broader range of HR HPV, and HPV 16 does not figure as prominently in HIV positive women.[24] [25]

Our analysis purported to test our two a priori hypotheses: first that single pHR and HR HPV genotypes in HIV-infected women are not independent predictors of factors of CIN 2+, but involve synergistic mechanisms, and second that HPV 16 viral load may be correlated with CIN 2+. The objectives of this secondary analysis were to determine the most prevalent genotype-specific distribution of HPV among women with CIN 2+, and to assess whether specific pHR and HR HPV genotypes and their respective viral load are associated with CIN 2+.

Methods

To examine the epidemiology of type-specific HPV infections, we carried out a cross-sectional analysis of all 74 HIV-infected women. This cross-sectional analysis based on primary data

collection and record reviews adhered to the methodological guidelines recommended in the STROBE document on observational studies.[26]

Between November 2005 and April 2006, women attending the Comprehensive HIV Care Centre (CCC) at Coast Provincial General Hospital in Mombasa, Kenya were informed about the study and were offered on site cervical cancer screening with conventional Pap smear, in addition to a general medical examination and routine blood tests, including CD4 cell count. Women were enrolled if they were HIV positive and diagnosed with squamous intra-epithelial lesions (SIL) by Pap smear, were between 20 and 50 years of age, not pregnant and did not have a history of hysterectomy or cervical cancer. Cervical sampling for HPV testing was done. During the enrolment visit, socio-demographic data were collected.

Written informed consent was obtained from all participants. Illiterate women elected a person who signed on their behalf after thorough explanation. Ethical approval for the study was given by the Ethics Committee of Ghent University Hospital and from the Ethics and Research Committee of the Kenyatta National Hospital (Ref number: Ref: KNH-ERC/01/3618). Six hundred HIV infected women were tested to reach a cohort of 74 HIV women with abnormal cytology.

Biologic specimens

Blood plasma samples were taken and a gynaecological examination was done with speculum insertion, prior to collection of endocervical and high vaginal swabs. Cervical samples were collected using a cervix brush (Cervex-brush®, Rovers®, Oss, The Netherlands), and cervical cytology was assessed with conventional Papanicolaou (Pap) smears. Histology of the biopsy specimens were processed and read by a qualified histopathologist. An external cytopathologist provided quality control by reviewing all cases. A diagnosis to each case was assigned according to the Bethesda 2001 criteria.[27] The cervix brush tips were preserved in a liquid-based cytology

collection medium (SurePath®, Tripath Imaging Inc., Burlington, North Carolina, USA) and stored at 4°C and shipped to Belgium for HPV testing.

Sample

Samples were collected according to the method described by Micalessi et al 2012.[28] Cervical cells were collected into an ethanol-based preservative (Surepath TM , Tripath Imaging, Burlington, NC, USA) using the Cervex-Brush ® or Cervex-Brush ® Combi (Rovers Medical Devices B.V., KV Oss, The Netherlands), and were processed into thin-layer LBC preparations using the fully robotic Autocyte PREP system (Tripath Imaging, Burlington, NC, USA)[29]. Upon finalizing the LBC preparations, 800 µL of the remaining cell suspension was used for DNA extraction.

HPV DNA Extraction, Detection and Typing

HPV testing was done as described by Depuydt et al (2006) in an accredited laboratory (ISO certification: ISO15189).[30] Briefly, HPV DNA was extracted from exfoliated cervical cells using the standard proteinase K-based digestion protocol. Cells were incubated with proteinase K solution (100 µg/ml) for three hours at 55°C. DNA was then further purified by spin column chromatography. HPV types were determined using a series of real-time PCR reactions with specific primers and TaqMan® (Invitrogen, La Jolla, USA) probes for HR- HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, [31] including the pHR HPV genotypes 53 and 66**.** Low-risk HPV types 6, and 67 were also detected. HPV viral load was detected by the use of HPV type-specific real-time TaqMan PCR assays.

Assays were normalized to a reference gene. A calibrator was included in every run and a standard curve was used to convert the signal to viral load. Detection limits were described by Micalessi et al (2012) for each primer or probe set.[28]

A standard national testing algorithm was used for HIV diagnosis using rapid immunoassays: Uni-Gold™ Recombigen® HIV (Trinity Biotech plc, Bray, Ireland) and Determine® HIV-1/2 (Abbott Japan co Ltd, Minato-Ku, Tokyo, Japan). In the case of indeterminate results, an enzyme-linked immunosorbent assay was used to confirm HIV status. CD4 count was performed using the Becton Dickinson automated FACS count system.[32] Cervicitis was diagnosed from inflammatory cells by means of microscopic evaluation.

Statistical analysis

Data were checked and cleaned as per standard processes without substantial implications to the data, and analysis was undertaken using STATA version 12 (Corporation, College Station, TX, USA).

We first described the distribution of pHR and HR HPV types observed among women with > CIN 2, CIN 2, CIN 3 and ICC.

Women over 30 years have a higher risk of abnormal cytology, hence, age was dichotomized into two categories, ≥30 years and <30 years. CD4 cell count was transformed into categories including CD4 <200 cells/µl and CD4 ≥200 cells/µl. This breakdown was used to assess severe immunosuppression. The number of pHR and HR HPV co-infections was treated both as a categorical variable, no HPV, one HPV and two or more HPV co-infections**.** As the outcome of interest, patients' histology result was dichotomized into lower than CIN2 and CIN 2+. For the univariate analysis, a logistic regression was fitted to measure the strength of the association between pHR and HR HPV genotype separately and CIN 2+. A multivariable logistic regression analysis was performed to simultaneously control for potential confounders, including age, low CD4 count, and the presence of co-infections.

Based on women with CD4 count < 200 being more at risk for abnormal cytology, we tested this variable as a potential effect modifier. We fitted a regression model to assess the association between pHR and HR HPV genotypes on CIN2+; in the same model, we assessed the potential role of confounding and/or interaction of age and CD4 count. Statistical significance of an odds ratio (OR) was considered at $p \leq 0.05$.

The log of the pHR/HR HPV viral load was taken as the data were not normally distributed.

Results:

Characteristics of the population

This study consisted of 74 HIV+, non-pregnant women with an abnormal cytology, of which 81% (60/74) were on HAART. Our study population had a mean age of 34.2, and 73% of women were 30 years of age and older. The median CD4 count was 236 cells/µl [interquartile range (IQR) 158-374], and 35 % had CD4 count of 200 cells/µl or lower. The median age at first sexual intercourse was 18 years (IQR=15.5-20), and the median number of sex partners was 2 (IQR: 1-4).

Prevalence of cervical and histological abnormalities

LSIL was detected in 43/74 (58%), Atypical Squamous cells of undetermined significance (ASC-US) in 12/74 (16%), Atypical Squamous Cells cannot rule Out High-Grade Squamous Intra-epithelial Lesion (ASC-H) in 3/74 (4%), (HSIL) in 15/74 (20%) and 1/74 was inconclusive (1%).

Histological results in the 74 women with cytological abnormalities were CIN 1 in 58% (41/74), CIN 2 in 16% (12/74), CIN 3 in 23% (17/74), and one participant (1/74) had invasive cervical carcinoma. Cervicitis was detected in 15% (11/74) and normal histology in 4% (3/74). Two (3%)

biopsies were inconclusive. Overall, 30 (40.5%) women had a cytology of CIN2+ (CIN2, CIN3 and ICC combined).

Prevalence of pHR and HR HPV genotypes

To assess the prevalence of, potential high risk (pHR) and high-risk (HR) HPV genotypes in the 74 HIV+ women with abnormal cytology, we assayed for specific genotypes listed in Table 1 by qPCR. In our study, 48 harbored (65%) at least one pHR/HR HPV genotype. The median number of concurrent HR HPV genotype infections was two (IQR: 2-4).

Among the 30 women with CIN 2+, over half of women (57%) had either HPV 16 or HPV 18 infection. The combined prevalence of intermediate HPV risk types in CIN 2+ was 30%, of which HPV 53 represented (7/30) 23 % and HPV 66 (2/30) 7%. In women with CIN 3+, (18/30), HPV 16 was the most prevalent 8/18 (28%), followed by HPV 53 5/18 (33%) and HPV 56 5/18 (33%). The only case of ICC had a stand-alone HPV 53 infection (Table 1).

	Histological results			
HPV Genotype	**< CIN 2 (n=44)**	**CIN 2 (n=12)**	**CIN 3 (n=18)**	**ICC**
HPV 16	32% (14/44)	25% (3/12)	44 %(8/18)	
HPV 53	25% (11/44)	17 % (2/12)	28% (5/18)	1
HPV 52	16% (7/44)	42% (5/12)	17 % (3/18)	
HPV 56	18% (8/44)	8% (1/12)	33 % (6/18)	
HPV 58	16% (7/44)	42% (5/12)	6% (1/18)	
HPV 18	16% (7/44)	25% (3/12)	17 % (3/18)	
HPV 35	14% (6/44)	25% (3/12)	18 % (4/18)	
HPV 31	7 % (3/44)	33% (4/12)	18 % (4/18)	
HPV 33	7 % (4/44)	25% (3/12)	28 % (5/18)	
HPV 39	7 % (3/44)	17% (2/12)	17% (3/18)	
HPV 45	0%	8% (1/12)	11% (2/18)	
HPV 59	2 % (1/44)	8% (1/12)	11% (2/18)	
HPV 66	20 % (9/44)	8% (1/12)	9 % (1/18)	
HPV 68	7 % (3/44)	0% (0/12)	9 % (1/18)	
Multiple HPV	60% (26/44)	83% (10/12)	67 % (12/18)	

Table 1: Prevalence of pHR and HR HPV genotypes according to histological results

HPV correlates of CIN 2+

Only one genotype, HPV 31, was found to be statistically significant in association with CIN 2+ adjusted for CD4 count, age and co-infections, (AOR=4.9, 95%CI: 1.1-22.6). No interaction with CD4 count and age was noted (Table 2).

Variables	**adjusted OR for CIN 2+ (95%CI): model 1**	**P-value**	**adjusted OR for CIN 2+ (95%CI): model 2**	**P-value**
Age 30 and above	0.9 (0.3 -2.3)	0.8		
pHR and HR co-infections prevalence	1.6 (0.6-4.5)	0.4		
CD4 count < 200	2.2 (0.8-5.8)	0.2		
HPV 16	1.3 (0.5-3.6)	0.6	1.2 (0.4-3.5)	0.7
HPV 18	1.2 (0.3-4.2)	0.8	1.1 (0.3-3.9)	0.9
HPV 31	5.0 (1.1-21.6)	0.03	4.9 (1.03-22.5)	0.05
HPV 33	3.0 (0.8-11.5)	0.1	2.9 (0.7-11.1)	0.1
HPV 35	1.6 (0.5-4.4)	0.5	1.4 (0.4-5.0)	0.6
HPV 39	2.6 (0.6-12.3)	0.2	2.4 (0.5-11.6)	0.3
HPV 51	1.7 (0.4-6.4)	0.4	1.6 (0.4-6.1)	0.5
HPV 52	1.6 (0.5-5.1)	0.5	1.3 (0.4-4.7)	0.6
HPV 53	0.6 (0.2-2.0)	0.4	0.5 (0.1-1.7)	0.3
HPV 56	1.6 (0.5-5.6)	0.4	1.4 (0.4-5.2)	0.6
HPV 58	1.0 (0.3-4.0)	1.0	1.0 (0.3-3.5)	1.0
HPV 66	0.2 (0.04-1.0)	0.06	0.2 (0.03-0.9)	0.04
HPV 68	0.5 (0.05-5.4)	0.4	0.2 (0.04-4.5)	0.5

Model 1: adjusted for CD4 count, age
Model 2: adjusted for CD4 count, age, and pHR and HR HPV coinfections
P-value from Likelihood Ratio Test
Table 2: Association between various pHR and HR HPV genotypes and CIN 2/+. Odds Ratios (OR) from logistic regression

We found a non-significant inverse association between HPV 53 and CIN 2+, and a significant inverse effect against HPV 66 when adjusted for co-infection (Table 3). Seventy-three percent (22/30) of women with CIN 2+ harbored 2 or more pHR and HR HPV genotypes. A univariate logistic regression yielded a statistically non-significant association (OR: 1.9; p= 0.2; CI: 0.7- 5.3) between CIN 2+ and multiple pHR and HR HPV genotypes (Table 2).

To assess the specific pHR/HR HPV load as a type-dependent risk marker for CIN 2+, we measured the log viral load copies/ 10^3 cells for specific genotypes in the 74 HIV+ women. Among the 30 women with CIN 2+, HPV 16 and its phylogenetically related HPV 31 and 33 were found to have the highest mean viral load, with HPV 16 having a mean of 11.2 (9.0- 13.4) (Table 3).

pHR and HR HPV genotypes	**Mean VL copies in CIN 1 (95% CI)**	**Mean VL copies in CIN 2+ (95% CI)**	**P value**
HPV 16	8.5(6.6-10.4)	11.2 (9.0- 13.4)	0.05
HPV 18	7.1 (4.4- 9.8)	6.4 (2.8-9.9)	0.7
HPV 31	6.2 (0-14.8)	10.4 (7.0-13.8)	0.1
HPV 33	9.5 (6.3-12.7)	11.4 (9.8-13.1)	0.1
HPV 35	11.1 (6.1- 16.0)	8.5 (6.4-10.5)	0.2
HPV 39	9.2 (0.1-18.2)	7.8 (5.3-10.3)	0.5
HPV 45			
HPV 51	9.8 (3.5-16.1)	9.3 (5.9-12.6)	0.8
HPV 53	6.0 (3.9-8.2)	3.4 (1.3-5.4)	0.07
HPV 56	11.0 (8.7-13.3)	9.1 (5.8-12.5)	0.3
HPV 58	8.1 (6.1-10.0)	8.3 (4.3-12.1)	0.9
HPV 66	6.4 (0-19.4)	8.9 (6.6-11.2)	0.3
HPV 68	6.0 (0-15)	NA	NA
Total pHR/HR HPV VL	10.7 (9.6-11.8)	11.8 (10.7-12.9)	0.2

Table 4: Mean log viral load copies/ 10^3 cells per pHR and HR HPV genotypes

Discussion

We observed a combined HPV 16 and HPV 18 prevalence of 57% in women with CIN 2+ and a prevalence of pHR HPV genotypes of 30%. In line with recent findings of a meta-analysis on HPV distribution in HIV-infected women disaggregated by cytological status in Africa, we found a higher prevalence of HPV 16 in women with CIN 3 (56%) than CIN 2 (25%), although our percentage for CIN 3 was higher than the one reported (41%-47%).[33]

Contrary to our first hypothesis, our data do not suggest a significant association between multiple pHR/HR HPV infections and CIN 2+. This study suggests that HPV 31 is the only independent predictor of CIN 2+, and an inverse relationship was detected between HPV 66 and CIN 2+. However, in agreement with our hypothesis, our study suggests that HPV 16 viral load correlated with CIN 2+. Our combined HPV 16 and HPV 18 prevalence of 61 % in CIN 3+ suggests the need for a wider protection that the nonavalent vaccine would confer.

There is a presumed link between HIV-positivity and the prevalence of multiple HPV infections.[34] [35] Our non-significant association between 2 or more coinfections and CIN 2+ contrasts with findings of a recent large study on multiple HPV infections in Costa Rica in which 5,871 young healthy women with multiple infections were at significantly increased risk of CIN 2+ when compared with those with single infections.[36]

Contrary to our hypothesis of required synergistic mechanisms between pHR/HR HPV genotypes for cervical cancer genesis, it may be that in our study population with a low median CD4 count of 236 cells/µl (IQR: 158- 374), single pHR HPV genotypes are capable of inducing cervical cancer genesis.

Our lack of association between pHR HPV genotypes and CIN 2+ is in agreement with an observation made by Rahman et al (2011) that infection with pHR HPV genotypes in HSIL became

non-significant when HIV status was included in the multivariate analysis. In women with CIN 3+, HPV 53 was the third most prevalent genotype. (28%) The stand-alone HPV 53 in the only ICC case recorded is incongruent with those from a recent study in Kenya, where pHR HPV genotypes were only detected in low-grade lesion.[37] The woman with ICC was severely immunocompromised and had a very low CD4 count of 2 cells/µl, which we hypothesize may make her more at risk for a potential oncogenic capacity of a pHR HPV genotype.

When examining associations between specific pHR and HR HPV genotypes and CIN 2+, a multivariate analysis suggested the presence of HPV 31 as an independent predictor of CIN 2+. Furthermore, a high number of concomitant pHR and HR HPV infections in women was observed in the presence of HPV 31.

Our finding suggesting that HPV 16 viral load may correlate with the severity of lesions is congruent with those found in the literature pertaining to sub Saharan Africa. [38] [39]Notwithstanding, a high viral mean load found for HPV 31 and 33 in women with CIN 2+, a statistically significant association was not demonstrated for these HR HPV genotypes. From an epidemiological perspective, this finding suggests a higher replicative capacity for HPV 16, which is known to be persistent, along with its phylogenetically related genotypes HPV 31 and 33 in HIV-infected women with CIN 2+ and may result in an increased transmission rate. As a corollary, this underscores the public health impact of monitoring unvaccinated HIV-infected women more regularly than once every three years as recommended by the WHO.[40]

Our findings can be extrapolated to a HIV population that is moderately to severely immunosuppressed and has had few sexual partners within the region. The relatively high median of concurrent pHR and HR HPV infections suggests an inability to either clear HPV infectious or a propensity to reactivate latent HPV infections. However, it is unknown how many sexual

partners their spouses have had, nor can a social desirability bias in reporting sexual behavior be excluded.

Strength and limitations

A major strength of our study is that our samples have been histopathologically confirmed. A recent systematic review suggests that most studies on cervical dysplasia on the continent, have cervical cytology as endpoints,[41] which only has a clinical sensitivity between 55%-65% for detection of histopathologically confirmed 'true disease status.[42] Furthermore, the real-time TaqMan PCR assays we used for detecting HPV genotypes were validated.[43]

We recognize that our study has certain inherent methodological limitations. The small sample size compromised our power to assess correlates solely for CIN 3, which is the best proxy for ICC, despite type distribution in CIN 3 not being completely representative of cancer. [33]

Additionally, the cross-sectional design, which does not allow the fulfillment of the temporal criterion for causality, bases its analysis on a single measure of pHR and HR HPV viral load for prevalent infections at the baseline screening phase, which may not be able to capture the transient nature of pHR and HR HPV infections. Consequently, it may not be possible to disentangle the risk posed by recently acquired infections along with its elevated viral load from viral load deriving from older infections.

A further limitation related to a cross sectional study design may be the lack of data concerning age of acquisition of HIV infection, since it is possible this may have occurred too late in life for some of the women in our study to influence CIN 2+. Moreover, lack of data on HIV viral load and on the recombinant strains present in HIV-1 infected women, precludes us from fully exploring synergistic mechanisms between the two viruses.

Research gaps

The epidemiology of pHR HPV genotypes in HIV women is still poorly characterized, as HPV 26, 53, 67, 70,73, and 82 are not included in any HPV DNA screening protocols in sub Saharan Africa. Our findings warrant that the potential carcinogenesis of HPV 53 be better elucidated, especially in severely immunosuppressed women. According to Padalko et al (2015) the role of pHR HPV genotypes will also need to be assessed in the post vaccine era, in case type replacement leads to pHR HPV genotypes becoming more prevalent in ICC. [44]

A systematic review and meta-analysis[45] found that the bivalent vaccine from GlaxoSmithKline had better cross protection against HPV 31 in persistent infection, but that efficacy against persistent infections with type 31 appeared to decrease with longer follow-up, suggesting a waning of cross-protection. It still remains to be determined whether a cross protection can be extrapolated to HIV-infected women and in the presence of multiple HR HPV genotypes.

The kinetics of different genotype viral load must also be assessed amidst, not only different levels of immunosuppression, but also in the presence of different levels of HIV viral load, multiple pHR and HR HPV infections and other concomitant sexually transmitted infections harbored by HIV-infected women. This would help to elucidate the aetiologic role of pHR and HR HPV viral load in cervical dysplasia pathogenesis and determine virological parameters to predict high-grade lesion in HIV infected women in sub Saharan Africa.

Conclusion

Our small sample suggest a high prevalence of HPV 16, 53, 56 and 33 in women with CIN 3+. Furthermore, a HPV 31 was found to be an independent predictor of CIN 2+ and HPV 16 viral load significantly higher in women with CIN 2/+.

Whether the available bivalent prophylactic vaccine will be able to meet its objective of reducing cervical cancer incidence by 70% may depend on the efficacy of cross protection against HPV 31 in HIV-infected women and the synergies between HPV genotypes in inducing cervical cancer genesis. The high prevalence of non-HPV 16 and 18 genotypes underscore the benefits of the nonavalent vaccine within this population.

Our high prevalence of HPV 53 in CIN 3+ and as a stand-alone HPV genotype in ICC, suggests a need for enhanced HPV 53 detection and its incorporation into a local screening protocol. Given the potential public health impact of pHR HPV in HIV-infected women and its exclusion from prophylactic vaccines, future research efforts are needed to investigate the epidemiology of these genotypes in HIV-infected women in the role of cervical cancer genesis. Moreover, large protective studies assessing the impact of the kinetics on different genotype viral load in HIV infected women in sub Saharan Africa, are needed to elucidate the tripartite relationship between HIV viral load, CD4 count and pHR and HR HPV viral load.

List of abbreviations

HIV= Human immunodeficiency virus

HAART= Highly active antiretroviral therapy

HPV= Human Papilloma virus

pHR HPV= potential high risk

ASC-U= Atypical cells of undetermined significance

LSIL= Low grade squamous intraepithelial lesion.

ASC-H= Typical squamous cells-cannot exclude high-grade squamous intraepithelial lesion

HSIL= High grade squamous intraepithelial lesion

ICC= Invasive Cervical Cancer

CIN= Cervical intraepithelial neoplasia

LEEP= loop electrosurgical excision procedure (LEEP)

ECC= endocervical curettage

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ASSOCIATIONS BETWEEN HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON THE PRESENCE OF HPV, PRE-MALIGNANT AND MALIGNANT CERVICAL LESIONS IN SUB-SAHARAN AFRICA, A SYSTEMATIC REVIEW: CURRENT EVIDENCE AND DIRECTIONS FOR FUTURE RESEARCH

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Key words: Sub-Saharan Africa, HAART, systematic review, cervical disease

Abstract:

Objectives: In Sub-Saharan Africa, substantial international funding along with evidence-based clinical practice have resulted in an unparalleled scale-up of access to antiretroviral treatment at a higher CD4 count. The role and timing of Highly Active Anti-Retroviral Therapy (HAART) in mediating cervical disease remains unclear. The aim of this article is to systematically review all evidence pertaining to Africa and identify research gaps regarding the epidemiological association between HAART use and the presence of premalignant/malignant cervical lesions.

Method: Five databases were searched until January 2017 to retrieve relevant literature from sub-Saharan Africa. Publications were included if they addressed prevalence, incidence or clearance of HPV infection in women undergoing HAART as well as cytological or histological neoplastic abnormalities.

Results: 22 studies were included, of which 7 prospective studies. Women receiving HAART are less likely to develop squamous intraepithelial lesions (SIL). There is evidence that duration of HAART along with the CD4 count may reduce the prevalence of high–risk HPV (HR-HPV), suggesting that without HAART, severe immunosuppression increases the risk of becoming or remaining infected with HR-HPV. Furthermore, according to existent literature, the CD4 count, rather than HAART coverage or its duration, plays a central role in the prevalence of CIN 2 and CIN 3

Conclusion: Our findings suggests a positive impact of HAART duration, in conjunction and interaction with CD4 count, on reducing the prevalence of HR-HPV. The greatest treatment effect might be seen among women starting at the lowest CD4 count, which may have a more instrumental role in cervical oncogenesis than either HAART use or the treatment duration on the prevalence of CIN 2 and CIN 3. There is still insufficient evidence to show a clear association

between HAART coverage and the incidence of invasive cervical cancer. Enhanced surveillance on the impact of HAART treatment, is crucial.

Strengths and limitations

Strengths:

- Comprehensive review within 5 databases on a major public health concern for women's health
- Contributes to and summarizes the evidence of the role of CD4 count and HAART use in the progression to cervical disease for HIV-positive women in Africa

Limitations:

- High variability in the sample size of the retrieved studies, therefore difficult to give the right weigh to the current evidence
- Heterogeneity in the sensitivity or specificity of screening methods may also render results less comparable; in some studies, HIV status of participants may not have been accurately measured, leaving the possibility of misclassification
- Lack of prospective studies, with clear strategy to deal with the loss in follow-up, retrieved by the literature search using the same definition of regression and progression of cervical disease; therefore the criterion of temporality for causality is not well fulfilled

Introduction:

Cervical cancer is the second most common female malignancy and the leading cause of death from cancer in women worldwide, of which 85% occur in low and middle income countries.[1] The Human papillomavirus (HPV), one of the most common sexually transmitted infections, is now well-established as the etiological agent. "High Risk" (HR) HPV DNA has been found to be present in 99.7% of cervical cancers worldwide.[2] Over 200 HPV genotypes have been identified and are divided into high risk (HR) and low risk (LR) carcinogens, depending on their potential to cause cancer.

ICC and its precursor, cervical intraepithelial neoplasia (CIN), are associated with persistent infection with HR HPV genotypes. Products of HPV oncogenes E6 and E7 alter normal genetic and cellular functions and induce malignant transformation. HPV infections are usually transient, and even those that persist for a few months are usually cleared naturally, however HIV-infected women are at higher risk of contracting HPV and developing squamous intraepithelial lesions (SIL), the precursor of cervical cancer.[3] [4] In this population, this type of lesions tends to be more aggressive, persistent and more likely to recur following cervical disease treatment.[5] [6]

In Sub-Saharan Africa, the region with highest prevalence of HIV 1 and HIV 2 in the world, the current standard recommendations for first-line adult antiretroviral therapy include two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI).[7] Protease inhibitors (PI) are primarily reserved for second-line treatment due to issues of cost, dosing frequency, drug–drug interactions, potential for long-term side effects and higher pill burden. Persons not responding to first-line regimens are usually switched to a cocktail of two NRTIs plus a boosted PI.[8]

Whilst the use of HAART decreased the risk of Kaposi Sarcoma and non-Hodgkin lymphoma, both AIDS-induced cancers,[9] there was no significant change in the incidence of cervical cancer.[10] [11] [12] Moreover, studies on the impact of HAART on the natural history of cervical squamous intraepithelial lesions (SILs) have yielded inconsistent results.[13] [14]Moreover, most studies have been conducted in industrialized settings and not in Sub-Saharan Africa, the area hardest hit by the global HIV/AIDS pandemic, home to nearly 25 million people living with HIV/AIDS.[15]

Whilst the use of HAART might be expected to reduce the incidence of cervical cancer and progression of cervical dysplasia as a result of improved immunologic function, as women are accessing HAART, their life expectancy will increase, due to less competing risk of dying from an opportunistic infection, which in turn may put them more at risk for ICC.[16] These two reasons will

augment their risks of a premalignant condition to transform into an ICC. Inversely, a prolonged use of HAART might potentially prevent the acquisition of HR-HPV and/or evolution to invasive cervical cancer.

In the HAART policy landscape, there have been recent shifts towards earlier initiation of treatment. Until 2009, the WHO recommendations for Sub-Saharan Africa stipulated initiation of antiretroviral treatment at a CD4 count threshold of 200 cells/mm^3 in order to further reduce the risk of HIV transmission to others. However, this guideline was revised in 2009 and initiation of ART recommended at CD4 counts of 350 cells/mm^3, only to be revised in 2013 and the treatment threshold set to 500 cells/mm^3 or less.[17] The policy was again recently revised and treatment initiation recommended for all HIV infected adults, regardless of WHO clinical stage or CD4 cell count, following the results of the START trial.[18]

As a research gap, it remains to be determined whether HAART could improve control of and reduce the burden of HPV infection and the associated precancerous states if initiated even earlier in the course of HIV infection.[19] The role and timing of HAART in mediating this relationship has been difficult to study and remains unclear.[20 21] The objectives of this paper are to review the exiting evidences on the association between the use of HAART and presence of HPV infection and premalignant/malignant cervical lesions.

Methodology:

We conducted this systematic review based on a pre-defined search protocol that conformed to the criteria set out by the PRISMA statement (see Research Checklist).[22] CINAHL, SCOPUS, Global Health, PubMed and Embase databases were searched (Figure 1).

Search terms:

The domains of the search terms were Antiretroviral therapy, Human Papillomavirus, abnormal cytology, cervical dysplasia, cervical cancer and Sub-Saharan Africa (Supplementary file).

Inclusion criteria:

Article selection criteria included any clinic-based Randomised-Controlled Trials (RCTs), meta-analysis/systematic reviews, observational or population-based linkage studies documenting both HAART status and HPV/Cervical Intraepithelial Neoplasia/Invasive Cervical Cancer (CIN/ICC) rates in Sub-Saharan Africa. The five databases mentioned above were searched in January 2017 for articles published from January, 1997 onwards to limit the publications to the combined HAART era. The following types of articles were excluded from our search: case reports, articles that did not include combination antiretroviral therapy, risk factors for CIN 2+ or did not stratify data by HAART users. We limited our search to English language literature.

Study selection:

We considered the following four components (Population, Intervention, Comparison, Outcome - PICO) to assess and categorize studies to be included in this review.

Population: The population eligible for this study includes HIV infected women or women with AIDS with concomitant HPV infection and/or with various degrees of abnormal cytology diagnosed through a recognized method such as PCR HPV genotyping, cytology, VIA, VILI, and histologically confirmed biopsies and living in Sub-Saharan countries.

Intervention: The intervention examined in this study is the administration of HAART, defined as at least 3 antiretroviral drugs belonging to 2 drug classes (NRTI, NNRTI or PI) and (if possible) the duration of treatment.

Comparison: A comparison is made between women not receiving HAART and women receiving HAART.

Outcome(s): The wide spectrum of cervical diseases, ranging from the HPV infection (and its potential clearing) through cervical oncogenesis and precursor lesions at the lower end of the neoplastic spectrum, to invasive cervical dysplasia.

All potentially relevant publications were evaluated by 2 reviewers (SM and RR) and were included in this review if they addressed any of the following outcomes: (1) prevalence, incidence of onset or clearance of HPV infection in women on HAART; (2) prevalence of cervical cytological or histological neoplastic abnormalities or invasive cervical cancer in HAART users. Possible cases of disagreement about the inclusion or exclusion of articles were resolved on an individual basis via thorough discussion among the authors of the justification for exclusion/inclusion.

With the exception of steps concerning the quantitative synthesis (meta-analysis), the PRISMA 2009 checklist and Flow diagram[22] was used to screen and include studies. We also enriched the search by reviewing reference lists from retrieved publications to identify additional manuscripts not captured by the searches. In reporting the results, we used the original terminology as stated in the original articles.

Duplication removal: The original searches from all the databases were combined in an EndNote® library (EndNote® version 8, San Francisco, CA, USA) and all duplicates removed.

Extraction of relevant information from the selected studies:

For all publications, SM and RR recorded the following variables: study location, year of study, study design, study population, main exposure of interest, main outcome of interest, confounding factors adjusted for and main findings.

Data assessment:

Because of the lack of randomized trials retrieved (due to ethical considerations), we considered prospective/longitudinal studies as of highest quality. We reported an overall score (from 3 to 1) based on our assessment of the overall quality of evidence for the following parameters: 1) minimization of selection bias: (Score "3"= strong), the participants were likely; (Score "2"= moderate), somewhat likely; (Score "1" = weak), not likely, to represent the target population. 2) Study design: (Score "3" = strong), prospective study and high sample size (higher than 200)[23]; a prospective study with a sample size lower than 200 and a cross sectional study with a sample size > 200, (Score "2" = moderate), and a cross sectional study with a sample size under 200 as (Score "1") weak; 3) Diagnostic accuracy: (Score "3" = strong), histopathological diagnosis, moderate (Score "2"): VIA/VILI using colposcopy with biopsy solely in cases when lesions appeared suspicious for CIN, and weak (Score "1"): cytological diagnosis. 4) Confounders: (Score "3") strong, controlled for all relevant confounders; (Score "2") moderate, controlled for some confounders; (Score "1") weak, control for confounders not specified; 5) withdrawals, loss in follow-up and drop-outs: (Score "3") strong, description of a strategy to minimise the loss in follow-up, reported withdrawals and drop-outs (80–100% of participants completed the study)[24]; (Score "2") moderate, reported withdrawals and drop-outs; (60% of participants completed the study); (Score "1") weak, withdrawal and drop-out rates not specified.

We assessed the methodological quality of evidence by calculating the mean of each study and attributing the mean one of the qualitative attributes, strong, moderate, and weak.

Results:

On January 15th, 2017, we included 22 studies in this review (figure 1). No disagreement appeared between the 2 main co-authors about the studies to be included. The summary of the study is presented in table 1.

Study population:

A total of 19,345 HIV infected women were included in the studies retrieved for and included in this review (one study was an ecological which did not report the individual sample size). The sample sizes for these studies ranged from 70 to 3241 HIV infected women.

Study design:

The published literature on HAART and HR HPV presence and cervical dysplasia is characterized by a lack of standardization in study designs. Twelve studies were cross sectional, two case-control, seven were prospective cohorts and one was a time-series ecological study.

Geographical location:

Two studies were conducted in Nigeria; four in Kenya, seven in South Africa; one in Ethiopia; two in Uganda, two in Cameroon, one in Benin, one in Tanzania, one in Ivory Coast and one in Burkina Faso (see table).

Quality assessment of studies (table 1):

The overall quality of evidence ranged from weak/moderate (Score of 1.5) to strong (Score of 3). 5 studies had an average score below 2 and the quality of their evidence was considered as "weak to moderate"; 15 studies had a score above 2 but less than 3 and the quality of their evidence was considered as "moderate" or "moderate to strong. Just one study had a full score of 3 and was considered as "strong". One study was ecological in design and could not be assessed in the same way as per the other studies that analysed individual data. The quality of evidence was mostly downgraded due to cross sectional nature of the studies as well as the lack of studies where samples have been histopathologically confirmed.

We discuss these differences by stage of disease: 1) HAART and HR HPV infection 2) HAART and SIL/CIN 3) HAART and ICC and outline trends and differences.

Summary of studies on the epidemiological association between HAART and HR HPV infection/prevalence

Four studies found a significant and inverted association between HAART use and HR HPV infection/prevalence. De Vuyst et al (Kenya, 2012)[25] reported that the prevalence of HR-HPV linearly decreased with use and duration of HAART (p=0.011). This association becomes more evident when this analysis is stratified by CD4 count categories. Whilst the prevalence of HR-HPV among non-HAART patients linearly increased with each decrease of CD4 count category, CD4 <200; CD4 200-349; CD4 350-499; and CD4 >= 500 (PR for linear trend 5.03, p=0.025), this trend was only marginally significant among women receiving HAART for <2 years (PR for linear trend 2.98, p=0.084) and insignificant for women on HAART therapy for ≥2 years (PR for linear trend 1.19, p=0.275). Zejer et al (South Africa 2015)[26] reported a significant reduction of risk of HPV in women on HAART, (OR: 0.23, CI 95% 0.15-0.37) in the 204 patients eligible for HAART, and for every additional month of HAART since initiation, the risk of detection of any HPV type decreased by 9%.

Table 1: summary of the quality of evidence assessment for the included studies

First author (year)	Minimization of selection bias	Study design	Diagnostic accuracy	Control of confounders	Loss to follow up	Overall methodological score & quality
Mogtomo M. (2009)	strong	weak	weak	weak	N/A	1.5 (weak to moderate)
Ononogbu U. (2013)	strong	moderate	weak	weak	N/A	1.8 (weak to moderate)
Ezechi O. C (2014)	strong	moderate	weak	strong	N/A	2.3 (moderate to strong)
Ezechi O. C (2014)	strong	moderate	Strong	strong	N/A	2.8 (moderate to strong)
MacKenzie KP (2011)	strong	moderate	weak	weak	N/A	1.8 (weak to moderate)
Huscko M. (2014)	strong	moderate	moderate	strong	N/A	2.5 (moderate to strong)
De Vuyst H. (2012)	strong	moderate	strong	strong	N/A	2.8 (moderate to strong)
Gedefaw A. (2013)	strong	moderate	weak	strong	N/A	2.3 (moderate to strong)
Mutyaba I. (2015)*	N/A	N/A	N/A	N/A	N/A	N/A
Firnhaber C. (2012)	strong	strong	weak	strong	weak	2.2 (moderate to strong)
Memiah P. (2012)	strong	moderate	moderate	weak	N/A	2.0 (moderate)
Rositch A. (2013)	strong	Strong	strong	strong	weak	2.6 (moderate to strong)
Omar T (2012)	strong	strong	weak	strong	strong	2.6 (moderate to strong)
Adler DH. (2012)	strong	strong	weak	strong	weak	2.2 (moderate to strong)
Zeier MD (2015)	strong	strong	strong	strong	weak	2.6 (moderate to strong)
Bekolo CP (2016)	strong	moderate	moderate	strong	N/A	2.5 (moderate to strong)
Capo Chichi (2016)	strong	weak	strong	weak	N/A	2.0 (moderate)
LJ van Bogaert (2013)	strong	moderate	strong	weak	N/A	2.0 (moderate)
AM Mwakigonja (2012)	strong	moderate	weak	weak	N/A	1.8 (weak to moderate)
A Jacquet (2014)	strong	moderate	moderate	strong	N/A	2.5 (moderate to strong)
Katumba et al (2016)	strong	moderate	weak	weak	N/A	1.8 (weak to moderate)
Kelly et al (2017)	strong	strong	strong	strong	strong	3.0 (strong)

* Ecological study, not amenable to quality assessment since this study is population-based and not individual-based

Table 2: Summary of Studies exploring the epidemiological association between HAART and HR HPV

First author	Year of publication & setting	Study design and sample size	Main exposure(s) and outcome(s) of interest	Main results concerning HAART and Remarks	Diagnosis	Confounding factors adjusted for
Ezechi O. C	Nigeria 2014	Cross sectional study among 231 HIV positive and 305 HIV negative	HIV infection and HAART on HR HPV prevalence and distribution.	A multivariate logistic regression analysis showed a lower hr HPV prevalence in HIV positive women on antiretroviral drugs (OR = 0.4; 95% CI: 0.3-0.5)	HPV PCR	Age, type of community, marital status and life time sexual partners
De Vuyst H. *	Kenya 2012	Cross-sectional study of 498 HIV-positive women	HR HPV genotypes and CIN 2+ in HIV infected women	HAART users (⩾2 year) had lower hr HPV prevalence (PR vs non-users=0.77, 95% CI: 0.61–0.96) and multiple infections (PR=0.68, 95% CI: 0.53–0.88),	HPV PCR	Age-adjusted
Rositch A.	Uganda 2013	Longitudinal study of HIV and HSV-2 co-infected individuals (N=440)	Detection of HPV before and after Initiation of HAART	No effect noticed of HAART on monthly HPV DNA detection (PR: 1.0; 95% CI: 0.96, 1.08), regardless of immune reconstitution or HIV viral suppression	HPV PCR.	CD4 counts and HIV viral load were analyzed using both time-invariant pre-HAART and time-varying measurements based on the corresponding pre- and post-HAART period values.
Zeier MD	South Africa 2015	HIV-infected women on HAART (N=204)	Effect of the initiation of HAART for HPV genotype detection on cervical samples in HIV-	HAART significantly reduced the risk for detection of HPV by 77% (OR 0.23, 95% CI: 0.15–0.37).	HPV PCR	All models were adjusted for excision treatment of cervical, sexual

		Prospective cohort study	infected South African women.			activity and the CD4 cell count as time-dependent variables. Age was included as a non-time-dependent variable due to the relatively short follow-up time.
Kelly H.A*	Burkina Faso and South Africa (2017)	Prospective cohort of 1238 HIV infected women in Burkina Faso and South Africa	Effect of HAART on HR-HPV in HIV-infected women	HR-HPV prevalence was higher among those on short duration HAART in both countries. However, when adjusted for CD4 cell count, this association was observed in Burkina Faso only (65.1 vs. 52.1% for <2 years compared with >2 years; (adjusted PR: 1.24, 95% CI 1.04– 1.47)	HPV PCR	The site and sociodemographic and behavioral factors that were independently associated in univariate analyses (P<0.10) were adjusted for.

*studies with >1 outcomes of interest

Table 3: Summary of Studies exploring the association between HAART and SIL/CIN and ICC

First author	Year of publication & setting	Study design and sample size	Main exposure(s) and outcome(s) of interest	Main results concerning HAART and Remarks	Diagnosis	Confounding factors adjusted for
Mogtomo M.	Cameroon 2009	A cross sectional design of 70 HIV-infected women	Cervical abnormalities in women on HAART and without HAART	Among the 22 HSIL-positive women, 63.6% (14/22) were not on HAART, while 36.4% (8/22) were under HAART. HIV infected women under HAART with positive HSIL, showed a median CD4+ count of 253.7 +/- 31.7 higher than those without therapy (164.7 +/- 26.1).	Pap smears were interpreted and classified according to the Bethesda System	N/A (Univariate analysis)

Ononogbu U.	Nigeria 2013	Cross sectional study 2,501 HIV-positive women	Explore risk factors leading to VIA/VILI positivity	HAART and positive cervical screening: TDF-containing HAART 1.4 (1.0–2.0); DDV-containing HAART 1.5 (1.0–2.2); d4T-containing HAART 0.9 (0.4–2.1)	Results of VIA or VILI were classified according to the IARC manual and recorded after each test	N/A (univariate analysis)
Ezechi O. C	Nigeria 2014	Cross sectional study among 1140 (531 HIV positive; 609 HIV negative)	Explore the association between HIV, HAART use and SIL	Not using HAART was found to be associated with an increased risk of SIL (aOR: 2.1; 95% CI: 1.4–3.5) and HSIL (aOR: 2.6; 95% CI: 1.1–6.4).	Pap smear were interpreted and classified according to the Bethesda system. 31.4% slides were reviewed for cytology quality control.	Age, viral load, marital status,CD4 count, age at first intercourse and life time sexual partners
MacKenzie K.P	Kenya 2011	Cross sectional 267 HIV-infected women	Risk factors for SIL	SIL is prevalent among women on HAART and is associated with immunosuppression. Duration (14 vs 11 months; p=0.17) and type (NVP-based [74%] vs EFV-based [72%]; p=0.8) of antiretroviral regimen were not significantly associated with SIL.	Pap smear were interpreted and classified according to the Bethesda System	N/A (no OR calculated)
Huscko M.	Kenya 2014	Cross sectional design. 3241 HIV infected women were screened for cervical cancer	Explore risk factors for CIN2+	Combined oral contraceptives were associated with detection of CIN2+ among women on HAART (AOR 1.84, CI 1.20-2.82), and not on HAART (AOR 1.72, 95% CI 1.08-2.73), use of a progesterone implant was associated with increased detection of CIN2+	Those with a positive VIA underwent immediate colposcopy with biopsy for confirmation of any lesions	Age-adjusted

				(AOR 9.43, 95% CI 2.85-31.20) only among women not on HAART.	suspicious for CIN2+.	
De Vuyst H.	Kenya 2012	Cross-sectional study of 498 HIV-positive women	Explore risk factors for CIN 2+ in HIV infected women	The prevalence of CIN 2 and CIN 3 did not vary across HAART non-users, HAART users for <2 years or ≥2 years (p for linear trend=0.416). However, when stratified for CD4 count categories, the prevalence of CIN2/3 among HAART non-users linearly increased per each decrease of CD4 count category, CD4 <200; CD4 200-349; CD4 350-499; and CD4 >= 500 (p for linear trend=0.013) but this is not seen among women receiving HAART for <2 years and ≥2 years (p for linear trend=0.9 and 0.5, respectively	Histologically confirmed cytology	Age-adjusted
Gedefaw A.	Ethiopia 2013	cross-sectional study (hospital-based) among 448 HIV-infected women	Risk factors for precancerous cervical cancer lesion among HIV-Infected Women	Being currently on HAART had a protective effect for precancerous lesions (AOR=0.52, 95%CI: 0.35, 0.92).	Results of VIA were classified as negative, positive, or suspicious for ICC.	Adjusted for age, education, occupation, parity, lifetime history of pelvic infection, STD, ulcerative genital lesion, age at first marriage, age at first sexual intercourse, and life time number of sexual partners
Firnhaber	South Africa 2012	prospective cohort study of 601 HIV-seropositive women	Incidence and progression of cervical lesions among HIV-positive women.	HAART use was associated with reduction of incidence and progression of cervical lesions, adjusted IRR=0.55 (95%CI 0.37, 0.80).	Pap smear were interpreted and classified according to	Adjusted for age, CD4 count, age at first intercourse, lifetime number of sexual

					the Bethesda System	partners, history of sexual transmitted diseases, hormonal contraception, condom use, employment, smoking and education
Memiah P.	Kenya 2012	Cross sectional design, 715 HIV-infected	Prevalence and risk factors associated with precancerous cervical cancer lesions among HIV-infected women	Non-HAART users were 2.21 times more likely to have precancerous lesions than HAART patients (95% CI 1.28–3.83)	VILI was used as the screening technique. A positive VILI test necessitated a cervical biopsy	Not adjusted
Omar T	South Africa 2012	Longitudinal study 2,325 HIV infected women	Progression and regression of cervical dysplasia in HIV-infected women in Soweto	HAART reduced the risk of SIL progression (aHR 0.72; 95 % 0.52-0.99)	Conventional Pap smears are performed	CD4, Baseline weight, smoking exposure, age, baseline smear results
Adler DH.	South Africa 2012	HIV-infected women (N=1123) from Soweto; prospective cohort study	Risks of progression, regression, and incidence of HPV-related cervical lesions between those on HAART and not on HAART	Increased likelihood (adjusted OR 2.61; CI 1.75–3.89; P< 0.0001) of regression of cervical lesions among women on HAART	Pap smear were interpreted and classified according to the Bethesda System and verified by a second reader	BMI, number of sexual partners, number of STI.
Bekolo CP	Cameroon 2016	Cross-sectional study on 302 women	Risks for cervical disease in HAART-experienced women with	After controlling for age and other covariates, women in the HAART group had a 67% reduction in the odds of	Each woman was screened using three	Age, place of residence, occupation,

		of whom 131(43.4%) were HIV-infected and receiving HAART	that in women in the general population of Cameroon.	cervical lesions compared with the community group (adjusted OR = 0.33, 95%CI: 0.15–0.73, p = 0.006). The authors could not however, account for differences in methods and quality assurance in the ascertainment of cervical lesions as well as other risk factors for cervical cancer that must have changed over time.	different methods in series: visual inspection VIA, VILI and conventional Pap smear cytology	religion, education, marital status, smoking, pregnancy history and family history of cancer
Capo Chichi	Benin 2016	86 women cases and 86 women controls	Association of HR - HPV to cervical dysplasia among women under stringent HAART treatment and in controls without HIV.	cervical dysplasia was observed in 4/86 (5 %) of women living with HIV on HAART Among the HAART group, no new cases of cervical dysplasia were reported after 2 years.	VILI and video colposcopy	No adjustment for potential confounders
van Bogaert LJ	South Africa (2013)	Case control study of 1,023 HIV-infected and 1,023 uninfected women.	Influence of the CD4-cell count and of HAART on the relative distribution of cervical pathology.	Patients on HAART had less CIN1 (*P* = 0.018), 2 (*P* = 0.18).	Histologically confirmed cytology	No adjustment for potential confounders
AM Mwakigonja	Tanzania, 2012	Prospective unmatched, case-control study of HIV-seropositive 120 HIV-seropositive cases on HAART and 50 seronegative	Frequency of cervical cancer and pre-cancerous lesions in the general compared to the HIV-infected populations in Tanzania	The cytological findings in this study suggest that the frequency of SIL and carcinoma appeared to be higher among HIV-infected women on HAART compared to seronegative controls and as expected increased with age. SIL and carcinoma constituted 28.3% (34/120), 38.3% (46/120) and 5.8% (7/120) among cases, and 28% (14/50), 34% (17/50) and 2% (1/50) for controls respectively, (P-value = 0.6)	Pap smear were interpreted and classified according to the revised Bethesda System	Not adjusted

A Jacquet	Ivory Coast, 2014	Cross sectional 2,998 HIV-infected women, of which 76 % were on HAART	Preventable determinants of SIL in HIV positive women	Non-significant (p=0.26) decrease of CIN with increased exposure to HAART	Positively screened women by VIA and VILI were scheduled for colposcopy. Directed biopsies were performed in case of positive findings with colposcopy	Stepwise descending procedure for adjustment of confounders in the multivariate analysis. Variables adjusted for not mentioned.
Katumba	South Africa (2016)	A cross-sectional descriptive study of 390 HIV-positive women	Correlation between HAART with abnormal Pap smear results of HIV-infected women	Participants who did not use HAART had more abnormal results compared to those who used HAART (p < 0.028, 95% CI 0.28-0.93)	Pap smear were interpreted and classified according to the Bethesda System	No adjustment for potential confounders
De Vuyst H*	Kenya (2012)	Cross-sectional study of 498 HIV-positive women	CIN 2+ in HIV infected women	The prevalence of CIN 2 and CIN 3 did not vary across HAART non-users, HAART users for <2 years or ≥2 years (p for linear trend=0.416). However, the prevalence of CIN2/3 among HAART non-users linearly increased per each decrease of CD4 count category, CD4 <200; CD4 200-349; CD4 350-499; and CD4 >= 500 (p for linear trend=0.013) but this is not seen among women receiving HAART for <2 years and ≥2 years (p for linear trend=0.9 and 0.5, respectively	Histologically confirmed cytology	Age-adjusted
Kelly H.A*	Burkina Faso and South	Prospective cohort of 1238 HIV infected women in	Effect of HAART on CIN 2+ in HIV-infected women	CIN2+ prevalence was higher among short-duration HAART users (aOR): 1.99, 95% CI 1.12–3.54) and HAART-	Biopsies were analysed by histology	Stratification by site, HAART use and duration (or >2 years), HIV-1

	Africa (2017)	Burkina Faso and South Africa		naive participants (aOR 1.87, 95% CI 1.11–3.17) in SA.		viral suppression (< or _1000 copies/ml) and CD4 cell counts
ICC						
Mutyaba I.	Uganda 2015	Ecological Population based study	Correlation between % of HAART coverage and incidence of AIDS-related malignancies	HAART coverage was not associated with incidence of invasive cervical cancer	Unknown	Age-adjusted
Van Bogaert LJ *	South Africa (2013)	Case control study of 1,023 HIV-infected and 1,023 uninfected women.	Relative distribution of ICC among HIV-infected (treated or not) and uninfected women	There was a significantly higher proportion of CIN1 (*P* = 0.012) and 2 (*P* = 0.01) but a lower proportion of ICC (*P* = 0.015) among HIV-infected women. Patients on HAART had less CIN1 (*P* = 0.018), 2 (*P* = 0.18) and ICC (*P* = 0.019) that their untreated counterparts.	Histologically confirmed cytology	No adjustment for potential confounders

*studies with >1 outcomes of interest

Kelly et al (Burkina Faso and South Africa, 2017) observed that, compared with long-duration ART users (>2 years), HR-HPV prevalence was higher among those on short duration HAART in both countries. However, when adjusted for CD4 cell count, this association was observed in Burkina Faso only (65.1 vs. 52.1% for <2 years compared with >2 years; aPR¼1.24, 95% CI 1.04– 1.47). Ezechi et al (Nigeria, 2014) reported lower hr HPV prevalence in HIV positive women on antiretroviral drugs (OR = 0.4; 95% CI: 0.3-0.5).

Whereas four studies found a significant epidemiological association between HAART and HR HPV genotypes, one did not. Rositch et al. (Uganda, 2013)[27] did not find any impact on monthly HPV DNA detection (prevalence ratio: 1.0; 95% CI: 0.96, 1.08), regardless of immune reconstitution or HIV viral suppression. Almost all test subjects (92%) had detectable HPV in the 6 months preceding HAART initiation and the cumulative prevalence remained high following initiation of therapy (90%) after a follow up of 6 months. Table 2: Summary of studies exploring the epidemiological association between HAART and HR HPV.

Summary of studies on the association between HAART and the frequency of SIL/CIN 1/2/3 (table 3)

The review revealed heterogeneity between studies in the utilization of the screening/diagnostic methods for cervical pathology. Nine studies used cytological changes with reporting based on the revised Bethesda classification, two studies used histopathological confirmation, and five studies used VIA/VILL screening methods of which three ascertained disease using colposcopy with biopsy solely in cases when lesions appeared suspicious for CIN.

Seven cross-sectional studies reported on the frequency of SIL and risk factors for SIL. Memiah et al. (Kenya, 2012)[28] observed that patients not receiving HAART treatment were 2.2 times more likely to have precancerous lesions than patients receiving HAART (adjusted OR = 2.21, 95% CI 1.28–3.83). Similarly, Mogtomo et al (Cameroon, 2009)[29] reported that, of 22 HSIL positive

women included in the study, 63.6% (14/22) were not on HAART, whereas 36.4% (8/22) were receiving HAART. The study revealed that total cervical abnormalities (HSIL and LSIL) had a prevalence of 48.6% (n=17) among HAART users compared to 62.9% among women not receiving HAART (p=0.034).

In line with the association between the absence of HAART treatment and development of SIL, O. Ezechi et al (Nigeria, 2013) showed an increased risk of both SIL (adjusted OR: 2.0; 95% CI: 1.2–3.5) and HSIL (adjusted OR: 2.6; 95% CI: 1.0–6.7) [30] among 1140 participants not on HAART, adjusted for age, marital status, age at first intercourse and lifetime sexual partners. Nevertheless, Ononogbu et al (Nigeria, 2013) found only marginal or insignificant associations with different HAART regimens. [31] Their study reported that 2501 women on Tenofovir-containing HAART had a borderline significant RR of 1.4 (95% CI: 1.0-2.0), and a RR of 1.5 (95% CI 1.0-2.2) on Zidovudine-containing HAART, whereas women on Stavudine-containing HAART showed a RR of 0.9 (95% CI: RR 0.4-2.11) of having cervical disease compared to women not receiving HAART.[31]

Hushko et al (Kenya, 2014) [32] observed that combined oral contraceptives remained significantly associated with detection of CIN2+ in women on HAART (adjusted OR 1.84, 95%CI 1.20-2.82), compared to in women not HAART (adjusted OR 1.72, 95% CI 1.08-2.73), whereas the use of progesterone implant was associated with increased detection of CIN2+ (adjusted OR 9.43, 95% CI 2.85-31.20) only among women not on HAART. Mc Kenzie et al (Kenya, 2011) [33], who explored risk factors for SIL development in women on HAART, reported no significant difference in the use of Neviparine-based regimens compared to Efavirenz-based regimens between women with normal cytology and those with SIL (74% *vs* 72%; p=0.80). Similarly, there was no difference in the prevalence of nucleoside reverse transcriptase inhibitors backbones containing Zidovudine compared to those containing Stavudine between women with SIL and those without (12% *vs* 15%; p=0.41).

Bekolo et al (Cameroon 2016)[34] also reported a reduction a decreased risk of cervical lesions among HAART receivers. After controlling for age and other covariates, women in the HAART group had a 67% reduction in the odds of cervical lesions compared with the community group (aOR 0.33, 95%CI: 0.15–0.73, p = 0.006).

Mwakigonja et al (2012 Tanzania)[35] identified immunodeficiency as the main determinant of the presence of CIN+ in HIV-infected women: CD4 count >350 cells/mm3 (OR: 0.3; 95% CI: 0.2–0.6) or ≥200–350 cells/mm3 (OR 0.6; 95% CI 0.4–1.0) (Ref: <200 cells/mm3 CD4).

One study, Katumba et al (2016, South Africa) reported that women who did not use HAART had more abnormal results compared to those who used HAART ($p < 0.028$), although no confounders were adjusted for.

Two longitudinal studies reporting on progression/regression of cervical disease in HIV infected women on HAART were identified. There was a lack of harmony in how regression/ progression was considered. A prospective observational study conducted in South Africa by Firnhaber et al (2010) [36] suggested that HAART was associated with a robust reduction in the incidence and progression of cervical disease (adjusted IRR= 0.55, 95% CL 0.37, 0.80). The study sensitivity analyses confirmed that the result was not dependent on the length of HAART exposure and this significant effect of HAART was only seen in women with CD4 counts of <350 cells/mm.[3, 36] ASC-US (atypical squamous cells of undetermined significance) results were classified as LSIL results, while ASC-H results were classified as HSIL results.

A smaller magnitude of effect was found by Omar T et al (2011)[37] in South Africa, (HR= 0.72; 95% CL 0.52-0.99) using time-varying CD4 counts as a covariate in the multivariate model of progression. Progression was defined either as a subsequent smear with a cytological diagnosis

of ASCH, HSIL or worse in women who had a previous normal or LSIL smear with an interval between smears of >5.5 months.

One study was identified on HAART and regression of cervical disease. A large prospective cohort study from Soweto, South Africa undertaken by Adler et al (2012) [38] reported that HAART positively affected the natural history of cervical disease in HIV-infected women and a multivariate marginal models analysis identified a significantly increased likelihood (OR 2.61; 95%CI 1.75–3.89; p<0.0001) of regression of cervical lesions among women on HAART. Regression was defined as a subsequent improvement in cytological results from normal, ASCUS, low-grade SIL, ASC-H, HSIL, and cancer.

Other studies reported specifically on the duration of HAART and SIL. Kelly et al (South Africa 2017) reported that CIN2 + prevalence was higher among short-duration ART users <2 years (adjusted OR=1.99, 95% CI 1.12–3.54) and ART-naive participants (adjusted OR: 1.87, 95% CI 1.11–3.17) in SA. De Vuyst et al (Kenya, 2012)[25] reported that the prevalence of CIN 2 and CIN 3 did not vary across HAART non-users, HAART users for <2 years or ≥2 years (p for linear trend=0.416). However, when stratified for CD4 count categories, the prevalence of CIN2/3 among HAART non-users linearly increased per each decrease of CD4 count category, CD4 <200; CD4 200-349; CD4 350-499; and CD4 >= 500 (p for linear trend=0.013) but this is not seen among women receiving HAART for <2 years and ≥2 years (p for linear trend=0.9 and 0.5, respectively). Still, CD4 nadir before initiating HAART was unknown and HAART may have been started at a CD4 count too low to prevent or reverse CIN2/3. Similarly, Huchko et al (Kenya, 2014) [32] detected a non-significant association between the duration of HAART and CIN 2+ (adjusted OR=0.98, 95%CI 0.95-1.00) although it was not adjusted for CD4 count. McKenzie (Kenya, 2011)[33] did not find any significant correlation between the duration and type of HAART and SIL

and the median number of months on HAART did not differ between women with normal cytology and those with SIL (14 versus 11 months; p=0.17).

Nevertheless, Mogtomo et al (Cameroon 2009) observed that whilst LSIL incidence in HIV infected women under HAART therapy decreased from 8.6% (3 cases) to 5.7% (n= 2 cases) the first 10 months of HAART use, it increased to 11.4% (n= 4) after 10 months. In contrast, HSIL cases decreased from 14.3 % (n=5) to 5.7% to 2.8% (n=1) after 10 months.

Capo Chichi et al (2016)[39] in Benin reported that cervical dysplasia was observed in 4/86 (5 %) of women living with HIV on HAART, in whom after 2 years no new cases of cervical dysplasia were reported, nor were the HPV genotypes present at baseline there after 2 years.

A cross-sectional study from Ethiopia (Gedefaw et al. 2013) showed that presence of precancerous lesions were inversely associated with HAART: patients under HAART at the moment of the study (not specifying the duration of the treatment) were 48% less likely to have precancerous cervical cancer lesion than those who were not on HAART (aOR=0.52, 95%CI: 0.35, 0.92, p=0.015).[40] One prospective study assessed CIN 2+ incidence. Kelly et al (South Africa 2017) reported that CIN2+ incidence was reduced among women on HAART (adjusted OR 0.39, 95% CI 0.15–1.01).

Four studies contrasted in their reported results concerning the protective effect of higher CD4+ count nadir, prior to HAART initiation, on the onset of cervical disease. Huchko et al (Kenya, 2014)[32] reported that CD4+ nadir over 500 cells/mm^3 was associated with a reduced detection of CIN2+ (adjusted OR=0.61, 95%CI: 0.38, 0.97) in the overall group, but current CD4+ was only associated with reduced detection of CIN2+ among HAART non-users (adjusted OR=0.42, 95%CI: 0.22, 0.80).[41] This finding is compatible with the immune driven trend between CD4+ and a positive cervical screening among HIV-infected women observed by Ononogbu et al (Nigeria,

2013),[42] which reported a decreasing risk with increasing CD4 count: RR 0.5 (95% 0.3–0.7) for CD4 count 300–<450; 450–<650 RR 0.5 (95% 0.3–0.7) and ≥650 RR: 0.3 (95% CI: 0.2–0.6).

A prospective observational study of 601 HIV-seropositive women conducted by Firnhaber et al (South Africa, 2012) suggested that the effect of baseline HAART exposure was strongly modified by baseline CD4 count when initiating HAART in women with CD4 counts < 350 cells/mm^3. A RR 0.47 (95% CL 0.30, 0.73) was found in women initiating HAART with CD4 counts < 350 cells/mm^3 compared to RR of 0.84 (95% CI: 0.42, 1.72) in women with a baseline CD4 counts ≥ 350 cells/mm^3.

Only one study reported baseline CD4 count not to be significant. Jacquet et al (Ivory Coast, 2014)[43] reported that the baseline CD4 count at the time of first clinical follow-up, which could be interpreted as a proxy for nadir CD4 count, was not found to be significantly associated with CIN+ in the multivariate model. Table 3: Summary of studies exploring the epidemiological association between HAART and SIL/CIN and ICC.

Epidemiological association between HAART and ICC (table3)

Only two studies were found that assessed the effect of HAART on cervical cancer. An age-adjusted population-level evaluation of the effect of antiretroviral therapy on cancer incidence in Kyadondo County, Uganda (1999-2008), revealed that the increase of population-level HAART coverage over time did not affect the incidence of invasive cervical cancer (IRR: 1.02 95% CI 0.98 to 1.05).[44] However, Bogaert et al (South Africa 2013) reported that patients on HAART had not only less CIN1 (P = 0.018) and CIN2 (P = 0.18) but also less ICC (P = 0.019) that their untreated counterparts, despite similar mean CD4 count. Table 3: Summary of studies exploring the epidemiological association between HAART and SIL/CIN and ICC.

Discussion:

The findings of our systematic review suggests a positive impact of HAART duration, in conjunction and interaction with CD4 count, on reducing the prevalence of HR-HPV. Our review also illustrates how untreated people living with HIV with low CD4 count may be at increased risk of being or remaining infected with HR-HPV compared to those treated with HAART. Moreover, our findings support the hypothesis that the greatest treatment effect might be seen among women starting at the lowest CD4 count, since higher CD4 count might be associated with lower incidence of CIN2+ thereby reducing the possible treatment effect of HAART.

The literature identified in this systematic review suggests that CD4 count may have a more instrumental role in cervical oncogenesis or the integration of the latent reservoir throughout the body than either HAART use or the treatment duration on the prevalence of CIN 2 and CIN 3.

Surprisingly, we were unable to retrieve any studies that assess the impact of HAART on cervical disease and HPV infection across different types of HIV infections. It therefore remains unknown whether the effect of HAART differs between patients with HIV-1, HIV-2 or dual infection. HIV-2 infection and dual infection are highly prevalent in Sub-Saharan Africa, predominantly in Western Africa in countries like Guinea Bissau, Ivory Coast, Mali and Burkina Faso.[45]

The sample size of the studies included in this review greatly varies, rendering it difficult to assess and compare strength of the associations. Due to various difficulties, such as financial and ethical considerations linked to the conduct of RCTs, evidence is mostly based on retrospective or cross-sectional studies, which do not fulfill the causal criterion of temporality. Heterogeneity in the sensitivity or specificity of screening methods may also render results less comparable. Cervical cytology, which has a clinical sensitivity between 55%-65% for detection of histopathologically confirmed 'true disease status'[46] along with low inter- or intraobserver' true correlation can result in even one grade of misclassification introducing a bias in studies assessing regression or

incidence of SIL in women on HAART. Moreover, a recent study in Kenya has reported that the sensitivity of VIA is affected in women on HAART and by CD4 Count,[47] which in studies ascertaining disease using colposcopy with biopsy solely in cases when lesions appeared suspicious for CIN 2+, may have led to under-ascertainment of true disease, sensitivity 89.9% (82.2-95.0) in women under 35 and 78.6% (63.2-89.7) in women above 35.[47]

Our findings derived from the sub Saharan context contrast with the conclusion of a systematic review of studies from industrialized settings suggesting a largely inconsistent impact of HAART on reducing the incidence and progression and facilitating the regression of HPV infection and cervical abnormalities.[48] This discrepancy may be attributed to the greater access in industrialized settings to frequent cytological screening, thereby making the potential benefits of HAART less apparent.

One of the few retrieved prospective studies exploring the impact of HAART on SIL incidence and progression only reported a significant association in women with CD4 counts <350 cells/mm^3. Apart from the inclusion criteria providing heterogeneity in baseline profiles of women, different potential confounders have been adjusted for in studies addressing the same outcome. Not all studies control for hormonal/barrier contraception use, HIV viral load, CD4 count baseline or the number of sexual of partners, therefore residual confounding cannot be excluded. In addition, the duration of follow up may be too short and not always detailed in articles.

Clinical and public health implications:

The lack of statistically significant associations observed in this review between duration of HAART intake and reduction of CIN 2+, in women initiating HAART at an earlier CD4 count initiation suggests the importance of continued follow up with cervical cytological and/or histological screening, even after HAART has been initiated and immune function restored. Even

after HAART-initiation and immune reconstitution, lesions remain prevalent and progression of disease should be studied further to allow best timing of screening schedule.

Although current HAART guidelines in industrialized countries promote biannual cervical cancer screening in the first year following HIV diagnosis and annually thereafter, in the absence of evidence-based knowledge in sub Saharan African women, it may be advisable to address the following research gaps before suggesting the relevance of the guideline in this population. In the meantime, a cautionary approach may be employed and biannual cervical screening continued, if feasible.

Research gaps:

The dearth of studies on duration and initiation of HAART at an earlier CD4 count and the consensus on findings preclude policy makers from developing local guidelines. Moreover, due to the changes in recommendations for HAART start, women in sub Saharan Africa at the time of the studies were more likely to have low CD4 counts, whereas in the future they will be initiating HAART in a far less immunosuppressed state.

Future studies should preferably be prospective in design. This will allow a better follow-up of patients at different points in time and fulfill the criterion of temporality for causality. Moreover, including nested case-control studies within cohorts follow-up will allow robust intermediary evidence for such research questions. Studies should also elucidate the association between cervical HIV DNA and HPV incidence/prevalence, or cervical dysplasia.

The exploration of an association between HAART and cervical cancer warrants a large cancer epidemiological studies. Previously, the lack of effect of HAART on the prevalence on cervical cancer can be ascribed to the high mortality associated with AIDS although in the future, with improved longevity, and in settings with a life expectancy that exceeds the average age of cervical

cancer, available data suggests that a decrease in cervical cancer incidence in sub Saharan countries with effective and extensive HAART programs can be expected.

Finally, women with HIV-HPV coinfections may be at risk for immune-modulating helminthic, tuberculosis, malarial infections[49], which may compromise the positive impact that HAART may have on clearing cervical lesions. Their synergistic interactions in the post HAART era should be elucidated.

Conclusion:

Access to HAART in Sub-Saharan Africa has dramatically increased over the past decade, improving life expectancy for women living with HIV. This in turn requires urgent clarification of the impact of HAART on the development of cervical precancerous states and cancer.

The preponderance of studies suggests that women on HAART are less likely to have SIL, not ICC. However, our review imputes a more significant role to CD4 count or the CD4 count at which HAART is initiated, the timing of HAART in relation to immunosuppression on HR HPV genotype and CIN 2/3 prevalence, than to HAART use, irrespective of its duration. The scarce evidence shows that the positive impact of HAART is only felt in women with CD4 counts of <350 cells/mm.[3] Given the current limited evidence available to assist policymakers in developing local guidelines and prevention and treatment strategies for cervical cancer among HIV infected women on HAART, a tailored screening protocol cannot be established. Longer term surveillance data on HIV positive women at different levels of immunosuppression is crucial to determine the effect of HAART on CIN 2+.

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IV Discussion

9. Discussion of results

The review article summarizes the risk factors to be considered when designing a primary and secondary cervical prevention program in a post-vaccination era for HIV-infected women in Kenya

PUBLIC HEALTH APPROACH TO PREVENT CERVICAL CANCER IN HIV-INFECTED WOMEN IN KENYA: ISSUES TO CONSIDER IN THE DESIGN OF PREVENTION PROGRAMS

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Key words: HIV, HPV, Bacterial vaginosis, Kenya, prevention, primary health care

Abstract:

Women living with HIV in Africa are at increased risk to be co-infected with Human Papilloma Virus (HPV), persistent high risk (HR) HPV infection and bacterial vaginosis (BV), which compounds HPV persistence, thereby increasing the risk for cervical dysplasia. New guidance from WHO in 2014 advocating for a "screen and treat" approach in resource poor settings is becoming a more widely recommended screening tool for cervical cancer prevention programs in such contexts. This review article summarizes the risk factors to be considered when designing a primary and secondary cervical prevention program in a post-vaccination era for HIV-infected women in Kenya.

This review article is based on our prior research on the epidemiology of pHR/HR-HPV genotypes in HIV-infected women and CIN 2+ in Kenya and other sub-Saharan contexts. In order to contextualize the findings, a literature search was carried out in March 2017 by means of four electronic databases: PUBMED, EMBASE, SCOPUS, and PROQUEST

Risk factors for potential (pHR)/HR HPV acquisition, including CD4 count, HAART initiation, Female Sex Worker status (FSW) and BV need to be considered. Furthermore, there may be risk factors for abnormal cytology, including FSW status, multiple potential (p)HR/HR HPV genotypes, which may require that HIV-infected women be subjected to screening at more frequent intervals than the three year recommended by the WHO. The quadruple synergistic interaction between HIV, HPV and BV and its related cervicitis may need to be reflected within a larger prevention framework at the community level. The opportunities brought forth by the roll

out of HAART could lead to task shifting of HIV-HPV-BV care to nurses, which may increase access in poorly-served areas.

Background

Kenya is home to the world's fourth-largest HIV epidemic. In 2013, an estimated 1.6 million people were living with HIV and roughly 57,000 people died from AIDS-related illnesses.[1] In 2007, the World Health Organization (WHO) included Invasive Cervical Cancer (ICC) to stage "4" of the HIV/AIDS clinical classification of staging for resource-constrained settings.[2]

More than 200 types of human papillomaviruses (HPV) have been identified, of which 40 can progress to high-grade precancerous squamous intraepithelial lesions (HSILs) and subsequent invasive cervical cancer (ICC).[3] Although most HPV infections clear without intervention within 1 year, certain High Risk HPV (HR-HPV) genotypes have a propensity to persist and are consequently the chief risk factor for the development of ICC.[4] The 15 HR oncogenic viral strains, which have been identified, can be broken down into different species: the HPV 16 group (alpha-9) of the alpha-papillomavirus genus (HPV 31, 33, 35, 52, and 58), the HPV 18 group (alpha-7; HPV 39, 45, 59, and 68) and the alpha-6, genotype HPV 56. [5] [6]

Sexually transmitted infections (STIs) have been associated with longer HR-HPV persistence in previous studies.[7] The prevalence of Bacterial Vaginosis (BV) in African women , characterised by an overgrowth of vaginal anaerobic flora and reduction of H_2O_2-producing lactobacilli, is among the highest worldwide.[8] The high BV prevalence is of particular concern as there is evidence that BV is a risk factor for acquisition and transmission of many STI's, including HIV and HPV.[9] [10] A meta-analysis also suggested a positive association between BV and cervical pre-cancerous lesions.[11] Cervical inflammation has been associated with cervical intraepithelial neoplasia (CIN)

and may be a cofactor for high-grade cervical lesions in HPV-infected women.[12] BV frequently coexists with cervicitis[13] and was found to be an independent risk factor for cervicitis,[14 15 16]which may be a risk factor for cervical dysplasia in its own right.[17 18]

The WHO recommends the inclusion of HPV vaccination in national immunization programs as HPV represents a public health priority, and vaccine delivery is feasible and cost-effective.[19] Whilst immunogenicity trials have shown the effectiveness of a two-dose HPV vaccine against HPV 16 and HPV 18 in HPV-naive girls aged 9-14,[20] the WHO still recommends that immunocompromised/HIV-infected girls aged 9-14 be administered a third dose.[21] However, this will not obviate the need for secondary prevention in Kenya, where our recent meta-analysis (2016) on the distribution of pHR/HR HPV genotypes in HIV-infected women in Kenya found a pooled estimated prevalence of HPV 16 and 18 of 61% in HIV-infected women with ICC[22]. This finding underscores a need for a broader primary prevention program than the quadrivalent vaccine (Gardasil™) that protects against HPV genotypes 6, 11, 16 and 18 that is currently being rolled out. [23]In addition, in light of the limited vaccine uptake in Kenya[24] in tandem with poor cervical cytology infrastructure, Kenyan women with undiagnosed or untreated severe cervical lesions may be at high risk of developing ICC.[25]

The more feasible, and WHO-approved, strategy for cervical cancer screening in low resource settings, like Kenya is visual inspection with acetic acid (VIA) or visual inspection with Lugol's iodine (VILI), although cytological screening is available in Kenyan urban settings. Women with HIV infection are recommended by the Centers of Disease Control (CDC) to have more frequent screening with cervical cytology: twice in the first year after diagnosis of HIV and, if normal, annually thereafter.[26] However, the WHO recommends that women living with HIV be screened within 3 years if tested negative by VIA or cytology.[27]

The regular immunological and/or virological followup that accompanies the latest WHO recommendation to initiate HAART regardless of the CD4 count provides a golden opportunity to increase cervical screening. However it is expected that Kenya will need to utilize more public health resources to expand laboratory facilities to monitor HIV viral load and, by default, CD4 count to monitor treatment efficacy.[28] As a corollary, this may leave even fewer resources for cervical cancer preventive measures while HIV-infected women will live longer, thereby potentially experiencing an increased incidence of ICC. This review firstly discusses clinical, virological and behavioural risk factors for pHR/HR-HPV genotypes to take into consideration when designing a triage-based cervical cancer prevention programme; secondly, it discusses programmatic issues related to implementation.

Methodology:

We contextualized our prior research on Kenya and on the sub Saharan African continent, consisting of systematic reviews, meta analyses, and cross sectional studies, by carrying out a literature search in April 2017 by means of four electronic databases: PUBMED, EMBASE, SCOPUS, and PROQUEST.

Association between BV and cervical pre cancerous lesions in Africa

Relevant studies on the association between BV and cervical pre-cancerous lesions were identified through an extensive search of the electronic databases based on the following key words: 'bacterial vaginosis', 'bacterial infections and vaginitis' in combination with 'cervical intraepithelial neoplasia' (CIN), 'squamous intraepithelial lesions' (SIL), 'cervical lesions', 'cervical dysplasia', and 'cervical screening AND Africa'.

Distribution of HPV genotypes in HIV-infected women in Kenya

The domains of the search terms were HIV, HPV, cervical cancer, incidence or prevalence, and Kenya. We combined HPV and cervical cancer with the Boolean operator "OR", and the result was combined with the other terms with "AND".

Association between BV and alpha 9 related HPV genotypes

'bacterial vaginosis', 'bacterial infections and vaginitis' in combination with alpha 9 related HPV genotypes

Association between vaginal inflammation and VIA specificity

'vaginal inflammation' AND VIA OR visual inspection with acetic acid AND specificity

Task shifting of cervical cancer prevention in Africa

Task shifting AND cervical cancer screening OR prevention AND HIV treatment infrastructure AND Africa

Treatment of Bacterial vaginosis in HIV-infected women

Metronidazole OR treatment AND Bacterial vaginosis OR 'bacterial infections and vaginitis' AND HIV

Self-sampling of HPV in Africa

HPV self-test AND sub Saharan Africa

Results:

Fifteen studies were included, one of which is still in press.

Risk factors for HPV acquisition

Triage according to HIV, HAART and CD4 count

Our meta-analysis demonstrated a high burden of HR-HPV genotypes with 64% (95%CI: 50%-77%) in a general HIV population in Kenya[22], significantly higher than a recent meta-analysis on pooled HPV prevalence in the general female population in eastern Africa (42.2%).[29] This finding suggests that a compromised immune system may be a risk factor for HR-HPV acquisition. Our recent systematic review of HAART and its association on the presence of HPV, pre-malignant and malignant cervical lesions in Sub-Saharan Africa suggests that there is evidence that duration of HAART treatment along with the CD4 count may reduce the prevalence of high–risk HPV (HR-HPV).[30]

However, the immuno-epidemiology of each HPV genotype remains inadequately elucidated. This can be prone to variations based on the culturally different follow-up algorithms and endpoint evaluations, quality of cytological specimens and biopsies taken along with heterogeneous HPV genotyping methods available.[31] In Kenya, our exploration of the association between different levels of immunosuppression and pHR/HPV genotypes suggests that the WHO 2013 recommendation to initiate HAART regardless of CD4 count may offer opportunities to prevent HPV 53 and multiple HPV infections, and thereby reduce the potential for cervical dysplasia in HPV-vaccinated women.[32] However, the significant association between CD4 count ≥ 350 µl and HPV 16 we observed suggests that for HIV positive unvaccinated women, a CD4 based triage may not be as effective as they may still be at risk for this oncogenic genotype.[32]

Synergistic interactions

BV-based triage - Association between BV and alpha 9 related genotypes

Our research in the clinical epidemiology of pHR/ HR-HPV genotypes indicates the vulnerability of HIV-infected women with BV. Our findings suggest a potential beneficial impact of BV treatment to reduce the risk of HR-HPV genotype acquisition. In line with a study in Spain having found BV to be a predictor for alpha 9 related genotypes,[33] we have found BV to be a predictor of the HPV 16 genetically related HPV 58 in HIV-positive women. [32 34] This finding underscores the need to consider a potential synergistic effect between BV and phylogenetically related HPV 16 genotypes. It may be hypothesized that treatment of BV may be an effective intervention to prevent subsequent HPV infection.

Risk factors for abnormal cytology

A Bacterial Vaginosis-based triage

Apart from its benefits for preventing HR-HPV acquisition in HIV-infected women, a BV based initial triage can be an effective component of a secondary cervical cancer prevention program. The vaginal inflammation caused by BV may result in increasing HIV viral load, which in turn can lead to enhanced cervical dysplasia progression.[35] This relation may accentuate the positive association that a meta-analysis suggested between BV and cervical pre-cancerous lesions (pooled OR: 1.51).[36]

Moreover, in a clinical setting, inflammation may result in a false positive VIA if the appearance of leukocytes in the submucosa mimics white epithelium.[37] A BV-based primary triage for HR-HPV acquisition in HIV-infected women and treatment thereof may have the additional benefit of diminishing cervicitis, which studies have shown its confounding nature on VIA results, resulting in women with cervicitis being more likely to have false positive VIA results than women without cervicitis.[37 38]

Our study on HIV-infected women in Kenya, which found a cervicitis prevalence of 15%[32] highlights the impact that BV-related cervicitis might have on diagnosis by rendering VIA interpretation less specific. In turn, a VIA based "screen and treat" approach without histological confirmation may be too sensitive, leading to overtreatment of women.[39] A study of a HIV-infected population in Uganda reported that a VIA based "see and treat" strategy may have resulted in overtreatment by 72% (439 out of 625).[40]

In contrast to the significant association (OR 4.0; 95% CI, 1.07–15.1)[41] observed between BV and abnormal cytology in HIV-negative women in South Africa, our study did not find a statistically significant association between BV and abnormal cytology.[34] It may be that the vulnerability of our FSW clandestine population exposed them to competing risk factors, which in turn may have diluted the association between BV and abnormal cytology. Furthermore, BV in the South African study was diagnosed using the Amsel criteria, which had lower sensitivity and higher specificity than the Nugent scoring system, the gold standard[42] that we have used.

Triage based on HIV-infected Female Sex Workers (FSW)

In light of the financial constraints that preclude universal screening, as well as the high pooled prevalence of pHR/HR-HPV genotypes among the HIV-infected population of 64% (95%CI: 50%-77%),[22]enhanced screening may need to be devised for certain groups.

A borderline statistically significant difference in our recent meta-analysis in Kenya between pooled estimates of HR-HPV genotypes in FSW and the general HIV population suggested that a cost-effective approach for cervical cancer prevention may warrant a triage based on this risk factor.[22] Moreover, our study found that of the 192 HIV-infected FSW, 27.1% (95% CI: 20.9–34.0%) had abnormal cytology.[34] However, given the penal code specifically penalising prostitution in Kenya,[43] failure to guarantee anonymity of this group may preclude the implementation of prevention programmes targeting this population at risk [44] Also, the non-

randomness of snowball sampling of FSW, which is used in most of the research for this group, precludes generalizing findings to the larger FSW population in Western Kenya, as the sampling strategy may have excluded FSWs without any social networks.

Immunosuppression:

According to our systematic review, in sub Saharan Africa, the CD4 count, rather than HAART coverage or its duration, plays a central role in the prevalence of CIN 2 and CIN 3, which suggests the importance of earlier immune reconstitution.[30] Therefore, with CD4 count appearing in the causal pathway between HAART and CIN2+, HAART may have an indirect effect on decreasing the prevalence of CIN2+

Multiple pHR/HR-HPV genotypes

Our meta-analysis on Kenya found that the prevalence of multiple pHR/HR-HPV genotypes was very prominent not only in normal to abnormal cytology but also in ICC (48 %; 95 % CI: 37%-60 % and 35%; 95 % CI .25%-45%, respectively). [22] This association suggests that a triage for further cytological screening based on the presence of multiple HPV genotypes may be warranted.

Programmatic issues of preventive programmes

Whilst early diagnosis and treatment of cervical pre-cancerous lesions prevent up to 80 % of cervical cancers in high resource countries where cervical cancer screening is routine,[45] in sub Saharan African, regular follow up of cervical cytology is not feasible. A study in Kenya found that a significant percentage of women (56–80.6 %) [46] is only identified once their cervical cancer is at an advanced stage. Furthermore, a study in Tanzania reported that women co-infected with HIV are less likely to be treated. [47]

A roll out of cervical preventive programmes throughout underserved rural setting Kenya will necessitate a change in the cervical cancer prevention landscape. Such a programme should be

designed considering the limited resources. Indeed, a recent survey among health care workers found that the main barriers to service provision were staffing shortages and insufficient staff training.[48]

Task shifting of cervical cancer prevention using HIV care

While in sub-Saharan Africa nurses greatly outnumber doctors and are often on the frontline in primary care services, their expanded role in the post-HAART roll out era has been redefined. The decentralisation process of HIV care launched in Kenya has considerably increased services from 15 health facilities in 2003 to over 700 facilities by December 2008.[49] A crucial aspect of this process has been the establishment of HIV clinics at health centres and dispensaries, close to those in need, as well as the integration of HIV programs with other services provided at the health facilities. Concurrently, the roll out of HAART access to underserved communities gave impetus to a task shifting approach in HIV care to deal with the severe shortage of health workers in the front line.

Albeit poor access to services has resulted in the low cytological screening coverage rates in Africa, new strategies facilitate screening in resource-limited settings. Screening services have been expanding since the WHO 2014 guideline advocating for a VIA or HPV-based "screen and treat" approach, with mobile units reaching more rural areas and cervical cancer prevention integrating HIV and family planning services.[50] This approach contrasts with the previous screening and diagnosis by the standard sequence of cytology, colposcopy, biopsy, and histological confirmation of CIN.

Building on previous successful public health initiatives, which integrated TB and antenatal care within HIV care, studies have shown how the use of the HIV care infrastructure in sub-Saharan Africa has been capitalised to integrate cervical cancer prevention in HIV-infected women. In rural Mozambique, clinics were characterised by shortages of human resources, equipment, poor

paper record systems and a limited ability to follow-up with patients. These rural clinics managed to piggyback on prior HIV infrastructure to implement chronic disease screening and management for cervical screening.[51] In Zambia, a study demonstrated the feasibility of implementing a referral and management system for cryotherapy-ineligible women in a "screen-and-treat" cervical cancer prevention program targeting HIV-infected women.[52]

In Kenya, where there are only 40 registered and 81 enrolled nurses per 100,000 people, outpatient treatment services, where cryotherapy and loop electrosurgical excision procedure are performed, are largely unavailable throughout the country.[53] In a rural setting in Western Kenya, a recent study showed that the established infrastructure of an HIV treatment program was successfully used to build capacity for cervical screening.[54]Task-shifting and population-based cervical screening was found to be feasible, nevertheless loss to follow-up and poor cytology infrastructure remained important obstacles.[54]

However, the availability of doctors in Kenya, which is 20 per 100,000 inhabitants,[55] is even lower than the availability of nurses and mostly concentrated in urban areas. These barriers to cervical cancer prevention will undoubtedly hamper the enhanced responsibility that nurses should be granted in cervical cancer prevention in HIV-infected women within a high BV prevalence setting. Sufficient and well trained human resources are necessary to optimise investments made in diagnostic and treatment equipment and infrastructure. In Sub Saharan Africa, diagnostic testing for STIs and BV is often not available and most pregnant women are managed using syndromic algorithms.[56] However, a study in Kenya reported poor detection of BV using syndromic diagnostic algorithms by health care workers.[56] This underscores the need to enhance microscopy proficiency or Amsel reading of BV in HIV-positive women, who possibly harbour several co-infections. In addition, there is a need to develop nurses' skills in applying a management algorithm for BV associated cervicitis.

Discussion:

Secondary prevention programs in Kenya will need to be tailored to the local epidemiology of cervical dysplasia and ICC within the HIV-infected Kenyan female population. In light of some shortcomings of primary prevention for HIV-infected women in Kenya, risk factors for pHR/HR HPV acquisition, including CD4 count, HAART use, FSW status and BV presence may need to be considered when determining who requires a more intense follow up. Furthermore, there may be risk factors for abnormal cytology, including FSW status, multiple pHR/HR HPV genotypes, which may require HIV-infected women to undergo screening at more frequent intervals than the every three years WHO 2014 recommendation for HIV-infected women with negative VIA and cytology tests. Until the advent of rapid, point of care and low-cost HPV screening options, regular BV monitoring and its treatment may also be sought to improve the specificity of VIA.

Strength and limitations:

The studies used to draw our recommendations have the strength of stemming from a wide array of settings, which enabled us to capture a more representative HIV positive female population. However, our recommendations are subjected to the limitations of the cross-sectional study design, which does not allow the fulfillment of the temporal criterion for causality.

Generalizability:

Our recommendations may be extrapolated to other clinical environments where women present similar levels of immunosuppression. However, our recommendations may not be generalizable to HIV-infected women who have scarce access to health care, as being of poorer socio-economic status, they may be more at risk for co-infection of poverty, including other STIs, TB, malaria, and helminthic infections. These are infections, which may affect persistence of certain pHR/HR HPV genotypes and/or cervical dysplasia progression.[30]

In order to determine a tailored screening interval, there are first a number of epidemiological, clinical and then programmatic research gaps, which need to be addressed

Research gaps

Research gaps for risk factors for pHR/HR HPV acquisition:

Longitudinal studies should be undertaken to determine if treatment of BV may reduce pHR/HR-HPV in genotype acquisition and/or cervical dysplasia progression in HIV-infected women. Furthermore, the epidemiology of BV recurrent BV within this population is still poorly elucidated. A recent study on the epidemiology of BV in a cohort of women at high risk for STI and HIV infection in Kampala, Uganda, showed that of the HIV+ women who were treated for BV, 72% had a second episode within 3 months.[57]

Since the bivalent vaccine covers species α7 and α9, the burden of HPV carcinogenesis in a fully vaccinated population might shift to the potentially high-risk genotypes that are not currently covered by either vaccine. Given the relatively high prevalence of HPV 53 as stand-alone and in pairings in HIV-infected FSW with abnormal cytology, its potential public health impact has yet to be estimated.

FSW are a major risk group for HPV, STIs and HIV. At the same time, it is very difficult to conduct methodologically-sound research for this group due to the difficulties encountered in sampling methods and to avoid exposing them to stigma and legal actions. It is therefore recommended that innovative methods to conduct epidemiological research for FSW are explored, tested and validated.

If a causal relationship between treatment of BV and prevention of HPV infection can be assumed, the population attributable fraction of BV due to synergistic interactions with HIV and HPV should be estimated. Although it has been shown that HAART use may reduce the prevalence of various HPV genotypes, it is still unclear whether this effect is also valid for HPV16, which is, among all the genotypes, the one with the highest potential to induce ICC. Due to the increasing number of HIV-infected women accessing HAART at any CD4 count, it is essential that this hypothesis is confirmed.

Risk factors for abnormal cytology

Prospective studies need to be carried out to establish the relationship between BV and abnormal cytology within sub Saharan HIV-infected women.

The high prevalence of concomitant STIs in HIV-infected women warrant that their biological interactions and their subsequent capacity to induce progression of cervical dysplasia be further explored. Furthermore, the synergistic effect of multiple pathogens implicated in cervicitis must be elucidated.

While one of our studies in Kenya, (Menon et al in press) has found a propensity to cluster between phylogenetically similar α9 genotypes in HIV-infected women with CIN 2+/HSIL, another study exhibited a prominent role for non-alpha 9 and 7 genotypes, HPV 51 and 53.[34] In order to target HIV-infected women harbouring more than one pHR/HR HPV genotype most at risk for progression, a large prospective study should be undertaken to investigate clustering between phylogenetically similar or dissimilar phylogenetic groups in HIV-infected women with CIN 2+/HSIL so that a less crude triage than one based merely on the presence of more pHR/HR HPV genotypes can be designed.

The screening of the pHR/HR-HPV genotypes 26, 53, 67, 70, 73 and 82 are currently not included in any HPV DNA screening protocols in sub-Saharan Africa. The emergence of these serotypes will need to be monitored as well as their synergistic/antagonistic interactions with other HR-HPV genotypes. At the population level, affordable point of care nucleic acid amplification methods to detect pHR HPV genotypes are required throughout Kenya to explore how these genotypes will behave in post-vaccination era. A personal level approach for secondary prevention may be used if pHR HPV gentoypes as stand alone genotypes are a risk factors for ICC.

Programmatic research gaps:

Once there is a wider body of scientific evidence, there are a number of programmatic research gaps which should be addressed.

In Kenya, a cluster randomised non-inferiority trial should be undertaken in rural HIV management clinics to explore whether task shifting to nurses of symptomatic management of BV and cervicitis in HIV-HPV co-infected women is at least as effective as when carried out by physicians in secondary health care facilities. Moreover, a cost-benefit analysis should be undertaken to estimate whether bacterial ecology monitoring by nurses in the front line may be a valuable component of a primary cervical cancer prevention program.

Additionally, the population attribution fraction of STI due to synergistic interactions with BV, as well as their increased combined cervical dysplasia genesis capacities, should be estimated. A cost-benefit analysis should be undertaken to assess whether there may be a need to scale up STI prevention and management within the FSW population, in addition to BV prevention within a cervical cancer prevention framework.

As HIV self-testing has been found to be an acceptable method among the general population and FSWs,[58] several countries are beginning to introduce HIV self-testing as a promising innovation to achieve faster scale up, prompting the WHO to develop enabling guidelines.[59] A recent study found that tampon-based self-collection for hrHPV mRNA, based on molecular diagnostics aimed at detecting hrHPV viral integration, via the production of oncogenic E6 and E7 messenger-RNA, in South Africa could function as a viable method for cervical cancer screening among HIV-infected women in low-resource settings.[60] Provided the linkage between HIV and cervical cancer care in Kenya is established, the acceptability and success of HIV self-testing may set the stage for a concurrent roll out of HPV self-testing kits in this hard to reach population, if it can be shown that there is an overall positive public health impact.

Concluding remarks:

The new WHO guideline to rescreen HIV infected women within three years if tested negative by VIA or by cytological screening may be more effective if BV and its related cervicitis management becomes an integral component of primary and secondary prevention.

With earlier HAART initiation requiring regular immunological and virological follow up, there are opportunities for a more regular cervical screening provided that front line nurses are better equipped to deal with challenges. The quadruple synergistic interaction between HIV, HPV and BV and its related cervicitis may need to be reflected within a larger prevention framework at the community level. The potential synergistic interactions between BV, HIV, and HPV begs for an integrative cervical cancer prevention framework, with algorithms easy to follow.

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9.2 Strength and limitations

The findings of the meta-analysis, systematic review, and two cross sectional studies used to explore the four research questions should be seen in light of certain strengths and limitations

Epidemiology of pHR/HR HPV genotypes in HIV-infected women:

A major strength of the two studies was the sensitive TaqMan-based qPCR assays used to detect 17 HPV genotypes. However, the cross-sectional nature of the study design, which resulted in the simultaneous data collection of BV, pHR/HR HPV genotypes and acquisition of HIV infection does not allow reversal causality to be excluded. Hence, these limitations may result in a sub-optimal internal validity of the study.

Epidemiology of HPV Genotypes among HIV Positive Women in Kenya: A Systematic Review and Meta-Analysis: The high heterogeneity may be attributed to the different settings from which the study population was derived, including family planning facilities, HIV clinics and community-based settings. However, the vast array of settings of studies may be seen as a trade-off as it was able to capture a more representative HIV positive female population in Kenya.

Epidemiology of CIN 2+ in HIV-infected women: A major strength of the two studies exploring this research question was the histopathological confirmation of samples. However, a limitation related to a cross sectional study design may be the lack of data concerning age of acquisition of HIV infection since it is possible this may have occurred too late in life for some of the women in our study to influence CIN 2+. Furthermore, the cross-sectional design, which does not allow the fulfillment of the temporal criterion for causality, based its analysis on a single measure of HPV, which may not have been able to capture the transient nature HPV infections. Also, because of the lack of clinic-epidemiological data in the 593 women, potential confounders could only be adjusted for in the small cross sectional study of 74 women.

Systematic review: ART and the presence of HPV, pre-malignant and malignant cervical lesions in Sub-Saharan Africa: The findings of the systematic review should be seen in light of the heterogeneity in the sensitivity or specificity of screening methods may in turn may render results less comparable as well as the lack of prospective studies retrieved by the literature search using the same definition of regression and progression of cervical disease.

Strength: A strength of the systematic review was that a wide search was performed in order to capture as many studies as possible. (PUBMED, PROQUEST, EMBASE, and SCOPUS)

9.3 Generalizability/external validity

Findings derived from HIV-infected women receiving HIV care may be extrapolated to other clinical environments, with similar catchment areas with women presenting at similar CD4 count counts. However, it may not be possible to extrapolate to non-clinical environments, as HIV-infected women with poorer socio-economic backgrounds may have less access to health care and be more at risk for co-infection of poverty, including other STIs, TB, malaria, and helminthic infections. These are infections which may affect persistence of certain pHR/HR HPV genotypes and/or cervical disease progression.

Also, the non-randomness of snowball sampling of FSW precludes ascertainment of the representativeness of the sample to the FSW population in Western Kenya, as the sampling strategy may have excluded FSWs without any social networks.

9.4 Recommendations to policy makers

Primary prevention:

- Pooled estimates of HPV 16 and 18 of 61% in HIV-infected women with ICC suggest the need for a wider protection that the nonavalent vaccine would confer.

- There is evidence that HAART increases CD4 count, which in turn is associated with lower HR HPV prevalence
- These two primary prevention strategies should be implemented in tandem with educational interventions, which are imperative to prevent misperceptions and risky sexual behavior after vaccination.

Secondary prevention:

- A secondary prevention program will be necessary as this HIV-infected population harbors pHR/HR HPV co-infections, which may not be covered by current vaccines. Also, older women ineligible for vaccine administration will still need to be monitored.
- A triage for more frequent follow up based on FSW as an indicator may be warranted due the higher prevalence of pHR/HR HPV prevalence harbored by this population. However, given the illegality of prostitution in Kenya, measures should be taken to ensure confidentiality of this clandestine population.
- The relatively high prevalence of HPV 53 and other undiagnosed pHR/HR HPV HPV 26, 53, 67, 70,73, genotypes not covered by the nonavalent vaccine along with their lack of elucidation of their post vaccine "behavior" underscores the need for these to be considered in a screening protocol in Kenya.
- Given the lack of elucidation of post vaccine pHR/HR HPV genotypes 'behavior', a future algorithm for affordable point of care HPV testing may need to consider co-testing with VIA
- If resources permit, more regular cytological screening of HIV-infected women on HAART than once every three years, especially for non-vaccinated women

Programmatic needs:

- Front line nurses must be trained in validated point of care diagnostics for diagnosing BV in HIV infected women
- Programmatic, infrastructural integration of HIV and cervical cancer prevention should be prioritized

9.5 Recommendations to researchers

Primary prevention/ risk factors for pHR/ HR HPV genotype acquisition:

- In light of the high prevalence of multiple HR HPV genotypes harbored by HIV positive women, their micro epidemiology in cervical carcinoma in HIV positive women needs to be explored in order for the vaccine efficacy to be assessed.
- Large prospective studies should be undertaken to investigate clustering between phylogenetically similar or dissimilar phylogenetic group in HIV-infected women with CIN 2+/HSIL so that a less crude triage than multiple pHR/HR HPV genotypes can be designed.
- More longitudinal studies are required to explore the impact of HAART duration on pHR/HR HPV infection and clearance.

HPV 31:

- It still remains to be determined whether a cross protection to HPV 31 can be extrapolated to HIV-infected women and in presence of multiple HR HPV genotypes and

Secondary prevention/ risk factors for abnormal cytology

- A HPV-based screening interval for HIV-infected women needs to be determined

HPV 53:

- Given the high prevalence of HPV 53 in a HIV infected population with abnormal cytology, its cervical carcinoma genesis potential as a stand-alone genotype and as well as its synergism with multiple infections should be investigated. This should also be investigated in women who have received the bivalent/quadrivalent/nonavalent vaccine.

HPV genotypes not covered by the nonavalent vaccine:

- The future role of HPV 53 along with other non-vaccine targeted genotypes amidst a vacuum ensuing the bivalent/quadrivalent/nonavalent vaccine will need to be elucidated.

Associations between ART and the presence of HPV, pre-malignant and malignant cervical lesions

- Future research should consider CD4-stratified effect estimates to evaluate if women with complete immune reconstitution are still at higher risk of SIL progression than HIV-women.
- HPV 16 clearance in HIV-infected women initiating HAART at CD4 above 350 cell/mm3 as well as the synergistic interactions between HPV 16 and other pHR/HR HPV genotypes should be explored.

Programmatic research:

- In Kenya, randomized non-inferiority trials should be undertaken in rural HIV management clinics to explore whether task shifting to nurses of both, the screen and treat of cervical

lesions as well as a BV in HIV-HPV co-infected women.

- With a combined pooled prevalence of HPV 16 and 18 in HIV-infected women of 33 % if a viral endpoint is used, a cost-effective study should be carried out to assess the cost effectiveness of launching a catch-up campaign among HIV-infected women and HIVinfected FSWs below 26 years.

Future research:

- Using demographic, epidemiological, and cancer data from Kenya, a cost effectiveness study should be undertaken to assess the value of including boys in an HPV vaccination campaign.