Screening for Cervical Cancer in HIV Positive Kenyan Women: the Role of HPV Genotyping

by

Erin Elizabeth Dainty

Duke Global Health Institute
Duke University

Date:_______________________

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Nathan Thielman, Supervisor

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David Walmer

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Elkanah Orango Omenge

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Duke Global Health Institute in the Graduate School of Duke University

2013
ABSTRACT

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Abstract

Problem: Among HIV positive women in Kenya, cervical cancer has the highest incidence of any malignancy. In order to create effective screening strategies for both the primary and secondary prevention of cervical cancer in HIV-infected women, an understanding of the natural history of human papillomavirus (HPV) and HIV co-infection is critical.

Objectives: To describe the prevalence of HPV genotypes in HIV infected women with mild dysplasia and those with biopsy-confirmed severe dysplasia and invasive cervical cancer in Eldoret, Kenya. To determine how CD4 count, as a marker of immunocompetence, relates to HPV genotype distribution in patients with compared to those with severe dysplasia and invasive cervical cancer.

Methodology: This was a cross-sectional study recruiting from two groups: women who have invasive cervical cancer and women who have mild dysplasia. Cervical swabs were collected for genotyping, along with a recent CD4 count and relevant sociodemographic and medical data. HPV genotyping for types 6, 11, 16, 18, 26, 31, 33, 35, 39, 43, 44, 45, 51, 52, 53, 54, 56, 58, 66, 68, 69, 70, 71, 73, 74, and 82 was performing using the INNO-LiPA HPV genotyping assay, SPF10 system version 1 (Innogenetics, Ghent, Belgium). Crude and covariate-adjusted prevalence odds ratios were calculated using logistic regression analysis, and were performed separately for each HPV-type. In addition, modification of HPV-type prevalence ratios by CD4 count was analyzed. All data analysis was performed using Stata 12.1 (College Station, TX).

Results: HPV 52 had a significantly higher prevalence in this population than HPV 16 or 18. Neither HPV genotype nor CD4 count had changed the prevalence odds of severe
dysplasia and invasive cervical cancer. Participant age, history of syphilis, anemia, vaginal bleeding, and greater than five pregnancies increased the prevalence odds of severe dysplasia and invasive cervical cancer, compared to mild dysplasia.

**Conclusions:** These findings suggest that duration of HPV infection and host response to infection, rather than HPV genotype or CD4 count, are primarily responsible in the oncogenic transformation of infected cervical tissue. The overwhelming predominance of HPV 52 has implications regarding the efficacy of vaccination against HPV 16 and 18 in the primary prevention of cervical cancer in HIV infected women in Western Kenya.
Dedication

This thesis is dedicated to all of women in Kenya, for whom the research was conducted.
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All the wonderful nurses and research assistants who are a part of the CCSP
1. Introduction

1.1 Global Impact of Cervical Cancer

Cervical cancer is the second most common cancer in women globally, with 500,000 new cases and 250,000 deaths annually.\(^1\) Over 80% of worldwide invasive cervical cancers occur in developing world populations, largely as a result of the challenges in establishing effective screening programs. \(^2\)

![Figure 1: Global age-standardized incidence rates of cervical cancer](image)

The World Health Organization estimates that only about 5 percent of women have been screened for cervical disease in resource-poor countries, compared to 40-50 percent in the developed world.\(^3\) Additionally, the areas with the least expansive

---


\(^3\) World Health Organization, HPV Vaccine Position Paper, 2009.
screening programs also bear a large burden of HIV and AIDS, which is known as a risk factor for the development of cervical cancer. Globally, the mortality: incidence ratio of cervical cancer averages about 2:1, though this ratio is highest in Eastern Africa.

Figure 2: Global cervical cancer incidence: mortality ratios

1.2 The Burden of Cervical Cancer in Kenya

Cervical cancer has the highest incidence of any malignancy among women in Kenya and is second as cause of death.\(^5\) Kenya carries both a large population infected with HIV, as well as a very limited prevalence of cervical cancer screening. Of the 3902 women who were diagnosed with reproductive tract malignancies at Kenyatta National Hospital, the largest hospital in Kenya, 85% had invasive cervical cancer.\(^6\)

1.3 Human Papillomavirus and Cervical Cancer

Human papillomavirus (HPV) is the most common viral infection of the reproductive tract. Persistent infections with carcinogenic HPV cause virtually all cervical cancers.\(^7\) Persistent oncogenic HPV infection can lead to pre-cancerous cervical lesions and invasive cervical carcinoma. The International Agency for Research on Cancer has classified 12 HPV types as oncogenic: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82.

The great majority of sexually active women and men have been infected with HPV at least once in their lifetime. 90% of women with detectable HPV infection will spontaneously clear the infection within 1 to 3 years.\(^8\) Differences in the immune responses account for differences in viral clearance rate among individuals.\(^9\)

Based on available cross-sectional and short-term prospective data, the risk of persistence and neoplastic progression of precancerous cervical lesions differs markedly by HPV type. There is currently very limited data about HPV types associated with invasive cervical cancer (ICC) and severe dysplasia from most countries in Africa.

1.3.1 Variation in Distribution of HPV Genotypes

Current data suggests the disparate distribution of HPV genotypes among subgroups of women, with variations in geographic distribution, immune status, and cervical lesion severity. Among all women infected with HPV, the proportion of infections due to HPV16 and 18 is higher in Europe, North America and Oceania (74–100%).

---

77%) than in Africa, Asia and South/Central America (65–70%).12 A recent study from Nairobi, Kenya revealed that the most common HPV types detected among sex workers, regardless of HIV status, were HPV 53 (27%), HPV 33 (18%) and HPV 58 (18%), with HPV 16 and 18 detected in only 9%.13 Recent data from Haiti also confirms the existence of geographically disparate HPV types. In this population of 10,000 women in Leogone, of which 20% of the women are HPV positive, HPV 35, 58, and 45 were found to be more prevalent than other oncogenic types, even in those women found to have invasive cervical cancer.14

HIV-positive women with high-grade cervical lesions are more likely to be infected with high-risk types other than HPV16.15 Furthermore, low-risk HPV types are detected more frequently in HIV-positive women with precancerous cervical lesions than the general population.14

14 Personal communication with Dr. David Walmer, Duke University, founder, Family Health Ministries
Figure 4: Type specific prevalence of HPV in HIV infected women
1.3.2 HPV as a screening modality

Strong evidence now supports the adoption of cervical cancer prevention strategies that explicitly focus on persistent infection with HPV. HPV test results predict the risk of cervical cancer and its precursors better and longer than cytological or colposcopic abnormalities.\textsuperscript{16}

By contrast to HPV infections that clear, the cancer risk increases dramatically for the 5% of HPV infections that persist detectably for more than a few years. A negative HPV test provides long-term risk stratification: 5–10 years of reassurance of not developing high grade cervical dysplasia and even stronger reassurance of not developing invasive cancer among HPV DNA–negative women.\textsuperscript{15}

The greatest potential for reduction in cervical cancer rates from HPV screening is in low-resource regions that can implement infrequent rounds of low-cost HPV testing and treatment, where no preventive health-care structure is in place.

1.4 HIV and Cervical Cancer

Women infected with the HIV virus are more likely to have persistent HPV infection, and four times more likely than non-infected women to develop cervical cancer.\textsuperscript{17} Compared with HIV uninfected women, the prevalence of all HPV types was higher in HIV-infected women.\textsuperscript{18} Studies suggest that 20-60 percent of HIV-positive women show signs of pre-cervical cancer. Furthermore, there continues to be a limited

understanding of the natural history of HPV and cervical cancer in HIV infected women, though it is believed that HIV-induced immunosuppression impairs cell-mediated immune control of HPV infections.\textsuperscript{19}

HIV-infected women in different geographic regions, such as Africa, appear to be infected with less prevalent types of carcinogenic HPV as compared to the rest of the world where types 16 and 18 are more common.\textsuperscript{20} Immune response in HPV/HIV co-infection may lead to mechanisms for less virulent HPV to cause malignant transformation in HIV-infected women.\textsuperscript{21}

\textbf{1.4.1 HIV and cervical cancer in Kenya}

In 2009, the estimated prevalence of HIV in adults aged 15-49 was 6.3%. This prevalence varies significantly in different parts of the country, with the highest prevalence in Western Kenya.\textsuperscript{22} For persons aged 13-34 years, the HIV prevalence in Western Kenya is 15.4\% overall, 20.5\% among females and 10.2\% of males.\textsuperscript{23} HIV prevalence was highest in women aged 25-29 years, at 36.5\%, and men aged 30-34 years, 41.1\%.

At Kenyatta National Hospital, HIV-seropositive women with invasive cervical cancer were 10 years younger at presentation than HIV-negative women with invasive cervical cancer, even when accounting for education, number of sexual partners and

\begin{thebibliography}{9}
\bibitem{20} McKenzie ND, Kobetz EN, Hnatyszyn J, Twigg LS, Lucci III JA. Women with HIV are more commonly infected with non-16 and -18 high-risk HPV types. Gynecol Oncol. 2010 3;116(3):572-7.
\bibitem{22} World Health Organization, Epidemiological Fact Sheet on HIV and AIDS-Kenya, 2008.
\end{thebibliography}
previous history of sexually transmitted diseases.\textsuperscript{24} As the proportion of women infected with HIV continues to increase in Kenya, it is crucial to obtain a better understanding of HIV/HPV co-infection in this population, as well as to develop effective screening programs in this region.

2. Objectives/Hypotheses

2.1 Objective 1

Objective #1: To describe the prevalence of HPV genotypes in HIV infected women who have evidence of acetowhite lesions on visual inspection with acetic acid and those with biopsy-confirmed severe dysplasia and invasive cervical cancer in Eldoret, Kenya.

Hypothesis #1: Epidemiology of oncogenic HPV types

- HIV infected women in Western Kenya with cervical dysplasia harbor different HPV types than those commonly seen in North America.
- There is a further difference in HPV types in those with severe dysplasia and invasive cervical cancer compared to those without.
- Rates of HPV 16 and 18 will be lower in this population than reported rates in North America.

2.2 Objective 2

Objective #2: To determine how CD4 count, as a marker of immunocompetence, relates to HPV genotype distribution in patients with only acetowhite lesions compared to those with severe dysplasia and invasive cervical cancer.

Hypothesis #2: HPV epidemiology and pathology by immunologic status

- Among HIV-positive women in Kenya, those with lower CD4 counts will have infections with a greater number of HPV types, including high risk types, compared to women with higher CD4 counts.
- Women with CD4 counts < 300 will have a higher prevalence of high risk HPV types than those with CD4 counts > 300.
3. **Study Design and Methodology**

This was a cross-sectional study conducted among HIV positive women in Western Kenya.

3.1 **Study site**

Participants were enrolled through the gynecologic health services affiliated with Moi Teaching and Referral Hospital in Eldoret, Kenya. The reproductive health department at Moi Teaching and Referral Hospital has 17 obstetrician-gynecologists, 5 medical officers, 2 clinical officers, 100 nurses, and a Mother and Baby Hospital with a capacity of 150 beds. The AMPATH clinic in Eldoret is the primary site where cervical cancer screening currently takes place. Study patients were enrolled from the AMPATH clinic site in Eldoret, as well as from Turbo and Mosoriot, two outlying clinic sites.

3.1.1 **AMPATH**

In 1989 Indiana University School of Medicine joined Moi University School of Medicine and Moi Teaching and Referral Hospital in a collaborative effort to advance health care and education in Kenya. In 2001, along with USAID, Moi University, Brown University Medical School and Indiana University School of Medicine developed AMPATH, the Academic Model Providing Access to Healthcare (AMPATH), in western Kenya. AMPATH is a working model of urban and rural HIV care. To date more than 115,000 HIV-infected patients have been enrolled in AMPATH and more than 70,000 are currently receiving care at 23 locations.
In 2008 the AMPATH cervical cancer screening and prevention program (ACCSPP) began with research funding from Fogarty International Center to compare the accuracy of visual inspection with acetic acid (VIA) versus Pap smear as a screening test for cervical dysplasia in HIV positive women. After the completion of this initial study, PEPFAR funded the ACCSPP, allowing for a scale up of cervical cancer screening services throughout the entire AMPATH catchment population. Currently, nurses are trained for visual inspection and cryotherapy procedures, seeing approximately 50-60 patients per week. Ten to fifteen patients per week are referred to the weekly colposcopy clinic for further evaluation, including biopsies, which are performed by 4 OB/GYN consultants. Women receive their results 4-6 weeks later and are then referred to AMPATH-Eldoret if loop electrode excision procedure or hysterectomy is indicated. A full time outreach worker collects each patient’s phone number so that an outreach worker can trace each patient should they fail to return for follow up.

In the year 2011, 6,427 women were screened for cervical cancer at 4 different sites: Eldoret, Turbo, Mosoriot, and Webuye. 1101 women were found to be VIA
positive, with 81% of these women undergoing colposcopy and/or cryotherapy. Approximately 70% of patients with biopsy confirmed dysplastic disease requiring further treatment actually obtained recommended treatment, leaving a 30% loss to follow-up rate.

### 3.2 Study population

Patients were recruited from the pool of women who attend the AMPATH cervical cancer screening clinics in Eldoret, Turbo, and Mosoriot. Women were enrolled from two primary patient groups:

1. Women who presented for VIA and had acetowhite lesions noted at the time of exam, without a clinical suspicion for cancer.
2. Women who had biopsy proven severe dysplasia or invasive cervical cancer.

Inclusion criteria: Female, age >= 18 years, documented HIV infection for any duration, able to give informed consent and agrees to procedures and follow-up visits.

Exclusion criteria: Patients physically or medically unable to participate, pregnant or within 3 months of pregnancy, have a history of invasive cervical cancer (ICC), mucopurulent discharge or active vaginal bleeding, history of sexually transmitted infection or cervical infection in the last 2 weeks.

### 3.3 Sampling

Two trained nurses identified patients who had acetowhite cervical lesions at the time of VIA. A research assistant was available in the clinic at all times and was responsible for obtaining informed consent from participants. Informed consent was
obtained in Kiswahili or English, as appropriate to the patient, using forms at the appropriate reading level.

Patients with severe dysplasia and ICC were identified from those who had undergone biopsy at the AMPATH colposcopy clinic, as well as those seen in the gynecologic oncology clinic that had a histologically-confirmed diagnosis. Informed consent was obtained in the same way from this patient group.

3.4 Procedures

Cervical swabs were collected for HPV genotyping in the two patient groups. Swabs were collected as appropriate per the manufacturer’s recommendations for the INNO-LiPA assay. Specimens were kept in a secure location until batch transport to the lab for full HPV genotyping. CD4 counts from within the last 6 months were determined from the AMPATH client chart. Relevant sociodemographic and medical data was collected from the patient’s AMPATH chart at the time of initiation into the study (see appendix A for data collection sheet). All data collected apart from CD4 count was per patient report only, as it was not possible to verify all information from a medical record.

Researchers from Ghent in Belgium have established a precedent of HPV genotyping in Kenya through the International Council on Reproductive Health lab in Mombasa, Kenya using the INNO-LiPA HPV genotyping assay, SPF10 system version 1 (Innogenetics, Ghent, Belgium, manufactured by Labo Bio-Medical Products, Rijswijk, The Netherlands). INNO-LiPA has been validated by multiple comparison studies as an appropriate ‘gold-standard’ testing mechanism for the determination of HPV genotypes in cervical specimens.\(^1\),\(^2\),\(^3\) In this assay, genotype-specific probes for HPV genotypes 6,

Of the genotypes that are tested for 16, 18, 26, 45, 31, 33, 35, 39, 51, 52, 53, 54, 56, 58, 66, 68, 69, 70, 71, 73, 74, and 82 are considered ‘high risk’ for the development of cervical cancer, while types 6, 11, 43, 44, 54, and 70 are considered ‘low risk’.

In-country testing was beneficial for multiple reasons, including reduced cost of in-country transport and greater possibility of sustainability in settings outside of research protocols.

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4. Data Management/Statistics

4.1 Data Collection and Entry

De-identified patient data was collected using a data instrument, which was IRB approved. Participant data was then entered into a Redcap database for data compilation and analysis.

4.2 Data Analysis

Descriptive analyses were expressed in terms of means and medians for continuous variables. The main analysis examined cross-sectional prevalence ratios and differences of HPV types, number of HPV strains, high risk HPV infection, demographic characteristics, and CD4 count, comparing patients with and without the combined exposure of severe dysplasia and ICC. HPV genotypes were separated into categories of high risk and low risk, based on the oncogenic potential as listed above in a description of the INNO-LiPA assay. Crude and covariate-adjusted prevalence odds ratios were calculated using logistic regression analysis, and were performed separately for each HPV-type. In addition, modification of HPV-type prevalence ratios by CD4 count was analyzed. All data analysis was performed using Stata 12.1 (College Station, TX).
5. Results

237 participants were recruited from 3 sites: Eldoret, Turbo, and Mosoriot. Of the total group, 205 women had mild cervical dysplasia and 32 women had severe dysplasia or invasive cervical cancer (ICC). Of the participants in the severe dysplasia/ICC category, 15 had severe dysplasia and 17 had ICC. Table 1 provides a comparison between the two patient categories across all data points collected. Apart from the prevalence of HPV 56 and HPV 74, history of syphilis, and pregnancy number, there was not a statistically significant difference between the patient groups.
Table 1: Prevalence of HPV genotypes and demographic characteristics between patient categories

<table>
<thead>
<tr>
<th></th>
<th>Mild dysplasia</th>
<th>Severe dysplasia/cancer</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV detected</td>
<td>177 (86%)</td>
<td>30 (94%)</td>
<td>0.241</td>
</tr>
<tr>
<td>High risk HPV</td>
<td>1/2 (84%)</td>
<td>29 (91%)</td>
<td>0.324</td>
</tr>
<tr>
<td>HPV 6</td>
<td>18 (9%)</td>
<td>2 (6%)</td>
<td>0.632</td>
</tr>
<tr>
<td>HPV 11</td>
<td>5 (2%)</td>
<td>2 (6%)</td>
<td>0.236</td>
</tr>
<tr>
<td>HPV 16</td>
<td>33 (16%)</td>
<td>8 (25%)</td>
<td>0.216</td>
</tr>
<tr>
<td>HPV 18</td>
<td>27 (13%)</td>
<td>3 (9%)</td>
<td>0.548</td>
</tr>
<tr>
<td>HPV 26</td>
<td>5 (2%)</td>
<td>2 (6%)</td>
<td>0.236</td>
</tr>
<tr>
<td>HPV 31</td>
<td>17 (8%)</td>
<td>3 (9%)</td>
<td>0.838</td>
</tr>
<tr>
<td>HPV 33</td>
<td>37 (18%)</td>
<td>4 (13%)</td>
<td>0.44</td>
</tr>
<tr>
<td>HPV 35</td>
<td>18 (9%)</td>
<td>2 (6%)</td>
<td>0.632</td>
</tr>
<tr>
<td>HPV 39</td>
<td>44 (21%)</td>
<td>4 (13%)</td>
<td>0.241</td>
</tr>
<tr>
<td>HPV 43</td>
<td>2 (1%)</td>
<td>0 (0%)</td>
<td>0.544</td>
</tr>
<tr>
<td>HPV 44</td>
<td>9 (4%)</td>
<td>2 (6%)</td>
<td>0.647</td>
</tr>
<tr>
<td>HPV 45</td>
<td>10 (5%)</td>
<td>2 (6%)</td>
<td>0.742</td>
</tr>
<tr>
<td>HPV 51</td>
<td>20 (10%)</td>
<td>1 (3%)</td>
<td>0.22</td>
</tr>
<tr>
<td>HPV 52</td>
<td>68 (43%)</td>
<td>15 (47%)</td>
<td>0.675</td>
</tr>
<tr>
<td>HPV 53</td>
<td>13 (6%)</td>
<td>1 (3%)</td>
<td>0.473</td>
</tr>
<tr>
<td>HPV 54</td>
<td>43 (21%)</td>
<td>6 (19%)</td>
<td>0.772</td>
</tr>
<tr>
<td>HPV 56</td>
<td>34 (17%)</td>
<td>1 (3%)</td>
<td>0.046</td>
</tr>
<tr>
<td>HPV 58</td>
<td>13 (6%)</td>
<td>3 (9%)</td>
<td>0.525</td>
</tr>
<tr>
<td>HPV 66</td>
<td>18 (9%)</td>
<td>0 (0%)</td>
<td>0.081</td>
</tr>
<tr>
<td>HPV 68</td>
<td>12 (6%)</td>
<td>1 (3%)</td>
<td>0.528</td>
</tr>
<tr>
<td>HPV 69</td>
<td>25 (12%)</td>
<td>5 (16%)</td>
<td>0.587</td>
</tr>
<tr>
<td>HPV 70</td>
<td>8 (4%)</td>
<td>0 (0%)</td>
<td>0.256</td>
</tr>
<tr>
<td>HPV 71</td>
<td>25 (12%)</td>
<td>5 (16%)</td>
<td>0.587</td>
</tr>
<tr>
<td>HPV 73</td>
<td>2 (1%)</td>
<td>1 (3%)</td>
<td>0.314</td>
</tr>
<tr>
<td>HPV /4</td>
<td>42 (20%)</td>
<td>1 (3%)</td>
<td>0.018</td>
</tr>
<tr>
<td>HPV 82</td>
<td>7 (3%)</td>
<td>1 (3%)</td>
<td>0.933</td>
</tr>
<tr>
<td>Single</td>
<td>27 (13%)</td>
<td>2 (6%)</td>
<td>0.384</td>
</tr>
<tr>
<td>Married</td>
<td>97 (47%)</td>
<td>12 (38%)</td>
<td>0.384</td>
</tr>
<tr>
<td>Widowed</td>
<td>42 (20%)</td>
<td>11 (34%)</td>
<td>0.304</td>
</tr>
<tr>
<td>Divorced</td>
<td>28 (14%)</td>
<td>5 (16%)</td>
<td>0.384</td>
</tr>
<tr>
<td>Partner, unmarried</td>
<td>11 (5%)</td>
<td>2 (6%)</td>
<td>0.384</td>
</tr>
<tr>
<td>History of chlamydia</td>
<td>24 (12%)</td>
<td>1 (3%)</td>
<td>0.142</td>
</tr>
<tr>
<td>History of gonorrhea</td>
<td>10 (5%)</td>
<td>3 (9%)</td>
<td>0.299</td>
</tr>
<tr>
<td>History of syphilis</td>
<td>11 (5%)</td>
<td>6 (19%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Partner violence</td>
<td>/4 (36%)</td>
<td>15 (47%)</td>
<td>0.242</td>
</tr>
<tr>
<td>Previous pregnancy</td>
<td>203 (99%)</td>
<td>32 (100%)</td>
<td>0.575</td>
</tr>
<tr>
<td>&lt;5 pregnancies</td>
<td>171 (83%)</td>
<td>21 (66%)</td>
<td>0.017</td>
</tr>
<tr>
<td>&gt;5 pregnancies</td>
<td>34 (17%)</td>
<td>11 (34%)</td>
<td>0.017</td>
</tr>
<tr>
<td>History of anemia</td>
<td>46 (22%)</td>
<td>12 (38%)</td>
<td>0.065</td>
</tr>
<tr>
<td>History of tuberculosis</td>
<td>44 (21%)</td>
<td>8 (25%)</td>
<td>0.653</td>
</tr>
<tr>
<td>History of vaginal bleeding</td>
<td>40 (20%)</td>
<td>11 (34%)</td>
<td>0.057</td>
</tr>
<tr>
<td>HIV &lt;12 months</td>
<td>36 (18%)</td>
<td>6 (19%)</td>
<td>0.87</td>
</tr>
<tr>
<td>HIV &gt;12 months</td>
<td>169 (82%)</td>
<td>26 (81%)</td>
<td>0.87</td>
</tr>
<tr>
<td>ARV therapy</td>
<td>157 (77%)</td>
<td>27 (84%)</td>
<td>0.325</td>
</tr>
<tr>
<td>ARV therapy &lt;12 months</td>
<td>48 (31%)</td>
<td>8 (30%)</td>
<td>0.922</td>
</tr>
<tr>
<td>ARV therapy &gt;12 months</td>
<td>109 (69%)</td>
<td>19 (70%)</td>
<td>0.922</td>
</tr>
</tbody>
</table>
5.1 HPV Genotype Prevalence

HPV 52 was by far the most common genotype detected in both patient groups, followed by HPV 54 and HPV 39. HPV 16 had a prevalence of 17%, and HPV 18 13%.

Figure 6: Population HPV genotype prevalence

5.1.1 HPV Prevalence by Patient Category

There was not a statistically significant difference in the prevalence odds of infection with any particular HPV genotype between the patient categories.
5.1.2 High risk HPV infection and cervical lesion severity

There was not a statistically significant difference in the prevalence of infection with high risk HPV types between those with mild dysplasia (84%), and severe dysplasia/ICC (91%).
5.2 Number of HPV strains

Co-infection with multiple strains of HPV was noted in 63% of all participants. The maximum number of strains was 13, mean 2.85, median 2, with a standard deviation of 2.65.

![Frequency of the number of HPV strains detected per participant](image)

Figure 9: Frequency of the number of HPV strains detected per participant

5.2.1 Number of HPV strains and cervical lesion severity

There was not a statistically significant difference in the number of HPV strains detected between the two patient groups, as seen in the figure below.
Figure 10: The number of HPV strains stratified by patient category

5.2 CD4 count

CD4 counts ranged from a minimum of 0, to a maximum of 1335. The mean was 389, median 378, with a standard deviation of 212.

Figure 11: Population distribution of CD4 counts
5.2.1 Impact of CD4 Count on Cervical Lesion Severity

Based on the distribution of CD4 counts in the population, participants were stratified by CD4 count into categories as follows: <300, 300-600, >600. As seen in Figure 12, there was not a statistically difference in the CD4 count values between the two patient categories.

![Figure 12: Prevalence of cervical lesion severity by CD4 count strata](image)

5.2.2 Impact of CD4 count on infection with high risk HPV

The prevalence of infection with oncogenic HPV was consistent across all levels of CD4 counts.
5.2.3 Impact of CD4 count on the number of HPV strains

Lower CD4 counts were not associated with infection with a larger number of HPV strains.
5.3 Impact of Medical and Demographic Factors on Cervical Lesion Severity

Table 2 summarizes the modification of age, history of syphilis, more than 5 pregnancies, anemia, and vaginal bleeding on the odds of having severe dysplasia or cervical cancer compared to mild dysplasia.

Table 2: Influence of demographic variables on the prevalence odds between mild dysplasia and severe dysplasia/ICC

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Mild dysplasia N=205</th>
<th>Severe dysplasia/ICC N=32</th>
<th>Odds ratio</th>
<th>P value</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; 38</td>
<td>42%</td>
<td>69%</td>
<td>2.6</td>
<td>0.005</td>
<td>1.30-5.30</td>
</tr>
<tr>
<td>History of syphilis &gt;5 pregnancies</td>
<td>5%</td>
<td>19%</td>
<td>4.1</td>
<td>0.01</td>
<td>1.39-11.9</td>
</tr>
<tr>
<td>History of anemia</td>
<td>17%</td>
<td>34%</td>
<td>2.66</td>
<td>0.018</td>
<td>1.18-5.95</td>
</tr>
<tr>
<td>History of vaginal bleeding outside menses</td>
<td>22%</td>
<td>38%</td>
<td>2.07</td>
<td>0.05</td>
<td>1.04-4.56</td>
</tr>
</tbody>
</table>

5.3.1 Age

Study participants were subdivided into two groups, those less than age 38 and those greater than age 38, based on the normal distribution of age. (Figure 15).
In the subgroup of participants who were over age 38, the risk of SD/ICC was about 14% higher for those infected with more than one strain of HPV, than those infected with only one strain (RD 0.135; p 0.0373).

In the subgroup of participants who were over age 38, the risk of SD/ICC was about 13% higher for those infected with HPV 52, than those not infected with HPV 52 (RD 0.134; p 0.0467).

### 5.4 Intimate Partner Violence

Women reporting intimate partner violence (IPV) are at heightened risk of human papillomavirus (HPV) infection. The overall prevalence of IPV was 37.6% in our population. Among those women with severe dysplasia or invasive cervical cancer, the
prevalence of IPV was 46.9%, compared to an IPV prevalence of 38.8% in women with mild dysplasia.

**Figure 16: Prevalence of intimate partner violence by patient category**

In the subgroup of participants with a CD4 count greater than 300, the risk of SD/ICC was about 19% higher for those with a history of IPV, than those without IPV (RD 0.191; p 0.0063). (95% CI 0.063-0.320).

For women who tested positive for infection with HPV, the risk of SD/ICC was 7% higher for those with a history of IPV (RD 0.066; p 0.05). (95% CI 0.040-0.168)

The risk of SD/ICC was 8% more for participants with a history of IPV who tested positive for oncogenic strains of HPV (RD .075; p 0.02, 95% CI 0.028-0.178)
6. Conclusions

6.1 Genotype prevalence of HPV

We predicted a priori that the epidemiology of oncogenic HPV types would differ in our population of HIV infected women in Western Kenya from populations in North America. This was indeed discovered, as the most common HPV strain detected was HPV 52, followed by 39, 54, 74, 16 and 33. In North American, the most prevalent HPV strains are 16, 58, and 45, with 18, 16, 31, 33, and 45 being the strains most commonly detected in cervical cancer specimens.

In North America, HPV 16 is typically the most frequently isolated strain from cervical precancerous and cancerous lesions, with a varying prevalence around 30%. Our population had an overall prevalence of 17%, with a higher prevalence in those with severe dysplasia/ICC (25%) compared to those with mild dysplasia (16%), though this finding did not reach statistical significance.

There was no difference in the HPV genotypes between those with mild dysplasia and severe dysplasia or invasive cervical cancer. This finding suggests that perhaps duration of infection and host response to infection, rather than HPV genotype, are primarily at play in the oncogenic transformation of infected cervical tissue.

6.2 Number of HPV strains

63% of all participants were infected with more than one strain of HPV. There was no difference in the number of strains between those with severe dysplasia/ICC and those with mild dysplasia. It is possible that there is not a synergistic relationship between different HPV strains, and that it is primarily duration of infection that is responsible for oncogenic progression.
6.3 CD4 count

It was hypothesized that women with lower CD4 counts would be infected with a larger number of HPV strains, than those with higher CD4 counts. There was, however, no difference in the number of strains at varying levels of CD4 counts.

We also predicted that women with CD4 counts less than 300 would have a higher prevalence of oncogenic HPV genotypes than those with CD4 counts greater than 300. This was not the case in this population, as the prevalence of high risk HPV infection was not different when categorized by CD4 count.

While CD4 count likely plays a role in the progression of precancerous cervical lesions, it does not appear to be through an increased risk of high risk HPV or the number of HPV strains present.

6.4 Age

Duration of HPV carriage is known to be the primary factor in predicting cancerous progression. Women over the age of 38 years (the mean age of study participants) were 2.6 times more likely to have severe dysplasia/ICC than those less than 38 years. This finding is consistent with the natural history of HPV infection, which usually causes malignant transformation in the fourth and fifth decades of life.

6.5 Syphilis

The odds of severe dysplasia/ICC were four times higher in participants with a history of syphilis. This finding is consistent with previous data that indicate those with a history of one sexually transmitted disease are more likely to be infected with another. It is also possible that the genital lesions that result from syphilis facilitate HPV infection and make the infection more difficult to clear.
6.6 Pregnancy history

Grandmultiparity was a positive predictor of severe dysplasia and invasive cervical cancer in this cohort. The odds of severe dysplasia/ICC were 2.7 times higher in women with a history of five or more pregnancies, compared to women with less than five pregnancies. It is unclear whether the history of pregnancy itself, or the episodes of unprotected intercourse that result in pregnancy contributed to this finding.

6.7 Anemia and vaginal bleeding outside of menses

Women with severe dysplasia and invasive cervical cancer were twice as likely to have both a history of anemia and a history of vaginal bleeding outside of menses, compared to women with only mild dysplasia. One of the most pervasive symptoms of undiagnosed cervical cancer in this population is profuse vaginal bleeding. It is possible that this finding is indicative of a history of vaginal bleeding related to precancerous or cancerous cervical lesions.

6.9 Intimate partner violence

The 37.6% overall prevalence of IPV among women in this study is similar to the countrywide IPV prevalence of 39%, reported in the Kenya Demographic and Health Survey Data from 2009. A higher prevalence of IPV among women with severe dysplasia/ICC, and among subgroups with a CD4 count > 300, HPV infection, and oncogenic HPV infection indicates that IPV may be independently associated with higher-grade cervical lesions. Our data indicate that it may be beneficial to incorporate screening for IPV into the AMPATH cervical cancer-screening program and that further research in this area is warranted.
6.10 Study limitations

The biggest weakness of this study was the limited sample size, which was constrained by cost of full HPV genotyping. It is possible that more differences in HPV number and type of infection would have been noted had a larger population been sampled. This is particularly true for the subgroup of participants with severe dysplasia/ICC, who comprised only about one fifth of the total sample.

Another weakness was due to the fact that all data, apart from HPV genotypes and CD4 counts, were obtained by patient report. It is likely that inaccuracies could be present in these data, as recollection is based on participant memory.

6.11 The Role of HPV Genotyping in the Primary Prevention of Cervical Cancer

Studying HPV genotype distribution in HIV-infected Kenyan women provides an opportunity to assess the impact of HIV and region on HPV genotype distributions. This has the potential to have serious implications regarding the efficacy of the primary prevention of cervical cancer through vaccination against HPV acquisition. Current vaccines target oncogenic HPV types 16 and 18, which are believed to be the etiologic genotypes in at least 70% of cervical cancers. The findings of this study indicating that HPV 52 has a significantly higher prevalence that HPV 16 or 18 suggest that vaccination may not provide as much protection from cervical cancer as in other populations. It is not yet known whether vaccination solely against the HPV 16 and 18 provides cross-protection against infection with other oncogenic types. This may have important implications for the design of HPV vaccines that target the types of HPV associated with disease in HIV-infected women in Kenya.
6.12 The Use of HPV Genotyping in the Secondary Prevention of Cervical Cancer

Emerging epidemiologic studies suggest that screen-and-treat strategies using HPV genotyping might be suitable, targeted after the peak age of new and typically self-limited infections. In fact, the International Agency for Research on Cancer now recommends that HPV-DNA testing can be used for primary screening, as an alternative to cytology, in resource-poor settings.

A ‘see-and-treat’ strategy incorporating visual inspection with acetic acid with immediate cryotherapy for eligible women has been initiated in the AMPATH cervical cancer-screening clinic. When comparing an abnormal VIA result to the gold standard of cervical biopsy, the sensitivity of VIA to predict severe dysplasia was 69.6%, specificity was 51%, positive predictive value 38.6%, and negative predictive value 79.1%. Based on these data, it would be beneficial to establish a more robust screening process that would improve the potential of identifying cervical dysplasia and ICC. Correlating oncogenic HPV types confirmed at the time of VIA would allow screen-and-treat modalities to be tailored to the treatment of clinically significant lesions.

One of the primary goals of this study was to pilot the incorporation of HPV genotyping into the screen-and-treat model in Western Kenya, as well as to build local capacity for molecular diagnostics at Moi Teaching and Referral Hospital. This has the potential to significantly improve the quality of cervical cancer screening performed among HIV positive women in this low resource setting.
Appendix A

ALL PATIENTS FROM WHOM CERVICAL SWAB SPECIMEN IS OBTAINED

GENERAL INFORMATION

1) Maternal date of birth __ __/___ __/__ ___

2) Maternal age at exam ________ years

3) Patient category:
   □₁ Cancer screening patient
   □₂ Known cancer/severe dysplasia patient

4) If in category 2 above:
   □₁ severe cervical dysplasia □₂ invasive cervical cancer

5) Age at first intercourse ________ years

6) Length of time HIV diagnosis:
   □₁ 0-6 months □₂ 6 months – 12 months
   □₃ > 12 months □₉₉ Unknown / NA

7) Relationship status:
   □₀ single □₁ married □₂ widowed □₃ divorced
   □₄ partner, unmarried □₉₉ Unknown / NA

8) Patient currently on antiretroviral therapy
   □₀ No □₁ Yes □₉₉ Unknown / NA

9) Length of time on antiretroviral therapy
   □₀ < 6 mo □₁ 6-12 mo □₃ >12 mo □₉₉ Unknown / NA

10) First visit to AMPATH cervical cancer screening clinic:
    □₀ No □₁ Yes □₉₉ Unknown / NA

STD HISTORY:
11) History of chlamydia
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

12) History of gonorrhea
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

13) History of syphilis
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

14) History of other sexually transmitted disease
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

**SOCIAL HISTORY**

15) Current tobacco use:
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

16) Current alcohol use:
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

17) History of intimate partner violence:
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

**OBSTETRICAL HISTORY**

18) History of previous pregnancy:
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

19) Number of previous pregnancies:
   - [ ] none
   - [ ] 1-5
   - [ ] more than 5
   - [ ] Unknown / NA

20) Prior term delivery by C/S or Vaginal (>37 weeks):
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

21) Prior preterm delivery at <37 weeks (spontaneous or medically indicated):
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

22) History of stillbirth:
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

23) History of early (< or = 10 wks) pregnancy loss:
   - [ ] No
   - [ ] Yes
   - [ ] Unknown / NA

24) History of preeclampsia:
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Unknown / NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**MEDICAL HISTORY**

25) History of anemia:  
0 No 1 Yes 99 Unknown / NA

26) History of tuberculosis:  
0 No 1 Yes 99 Unknown / NA

27) History of high blood pressure:  
0 No 1 Yes 99 Unknown / NA

28) History of heavy vaginal bleeding not during menses:  
0 No 1 Yes 99 Unknown / NA

29) History of other cancer:  
0 No 1 Yes 99 Unknown / NA

**SURGICAL HISTORY**

30) History of prior abdominal surgery:  
0 No 1 Yes 99 Unknown / NA

31) Prior non-abdominal surgery:  
0 No 1 Yes 99 Unknown / NA
References


