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PREVALENCE OF HIGH RISK HPV IN HIV+ AND HIV- WOMEN WITH CERVICAL DYSPLASIA AT THE MOI TEACHING AND REFERRAL HOSPITAL
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ABSTRACT

Background: **Cervical cancer, caused by Human Papillomavirus, is the second commonest cancer among women. HIV+ women are at a higher risk of acquiring HPV, developing pre-cervical cancer lesions (dysplasia) and cervical cancer. Early diagnosis is key to prevention of cervical cancer but reduced sensitivities and specificities of available screening methods pose challenges. The role of HPV in VIA related dysplasia has not been extensively interrogated. We sought to understand HPV infection in the context of HIV status and its relationship to VIA dysplasia**

Objectives: **To compare prevalence of high risk HPV in HIV positive and HIV negative women with and without cervical dysplasia.**

Study Design: **A cross sectional study design.**

Setting: **AMPATH/MTRH cervical cancer and prevention program clinic located at the Moi Teaching and Referral Hospital provides cervical cancer screening services for women.**

Participants: **Women attending cervical cancer screening clinics**

Results: **A total of 88 women were enrolled into the study. HR HPV prevalence was 59.1% among HIV+ and 43.2% among HIV- women. Women below 25 years had higher HRHPV prevalence. HPV prevalence was higher in women with higher parity. Higher HRHPV prevalence in younger women attributed to early sexual debut. The higher prevalence of HRHPV in HIV+ women was as a consequence of depressed immunity and greater exposure to risk factors**

Conclusion: **HIV+ women are more infected with HRHPV than their HIV- counterparts. Immune system related factors which affect the interpretation of screening tests like VIA require further investigation especially in immune compromised individuals.**

INTRODUCTION

Human papillomavirus (HPV) infection causes cervical pre-cancerous and cancerous lesions. Worldwide 2.5 billion women are at risk of developing cervical cancer and 15.3% of them eventually develop cervical cancer. The burden of cervical cancer is a substantial estimate 527,624 new cases and 265,653 deaths annually. 86% of this burden occurs in developing countries. Cervical cancer ranks third worldwide among cancers in women aged 15-24years 1.

The International Agency for Research on Cancer (IARC) has classified HPV into high risk (HR)/ oncogenic types e.g. 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and low-risk (LR) types e.g. 6, 11, 42, 43, 44, 54, 61, 70, 72, 81 2. There are over 100 types of HPV identified so far 3,4, however great interest is on the high risk (HR) oncogenic types which are frequently isolated from cancers. HPV types 16 and 18 are responsible for over 70% of all cervical cancer cases 1.

In Kenya 39.6% of women harbour cervical HPV infection at any point in time putting 12.92 million women aged 15 years and older at risk of developing cervical cancer. In the year 2013, 4802 Kenyan women were diagnosed with cervical cancer; 2451 died from the disease making it the most common cancer among women 1.

HPV mostly infects women within the reproductive age bracket. The age distribution of cervical HPV infection often peaks in the age group of less than 25 years but in other areas the peaks occur in more than one age group, the first peak occurring at younger ages (just after sexual debut), a lower prevalence plateau at middle ages, and another smaller peak at older ages above 45 years4,5. Increasing age (every decade) doubles the risk of infection with high risk HPV types 6 and is correlated with increased risk of cervical cancer 7,8.

Several factors including immune suppression can predispose one to cervical cancer. HR HPV types are more common among cervical cancer patients and HIV+ respondents. A Ugandan hospital based study found HPV prevalence of 59.5% in HIV+ and 39.1% in HIV- women. Majority of these HPV infections were high risk, 88.9% in HIV- and 100% in HIV+ 9. Similarly in Kenya, a study conducted among female sex workers (FSW) found overall HR HPV prevalence of 55.6%; 73.3% in HIV+ women compared to 45.5% in HIV– women 10. Multiple HPV infection occurs more commonly in HIV infection, and low multiple HPV infection has been observed in low HIV prevalence 8. Increased cervical cytological abnormalities have also been observed in HIV+ women 9.

Cervical cancer prevention and treatment depends heavily on mass screening. Screening tests detect early changes in the cervical epithelium. The performance of these tests varies with reports of low sensitivities or specificities, especially because the interpretation some of these tests are very subjective and depend on personal factors for example experience 11,12. See and treat screening approach is designed to reduce screening costs and losses to follow up which are common among other cervical cancer screening methods. The use of visual inspection with Lugol's iodine or acetic acid (VILI/ VIA) to identify precancerous lesions is an alternative cervical cancer screening approach that has rapidly gained interest because of its simplicity, safety and acceptability especially when performed by trained personnel 11. The pathophysiological basis of VIA is that acetic acid causes a reversible coagulation or precipitation of cellular proteins resulting in a momentary change of colour of the squamous epithelium from pink to white 13.

We compared HPV infection among HIV+ and HIV-women in relation to their dysplasia status as indicated by a VIA test. Several methods of screening for cervical

cancer are in use today including VIA, and their main objective is to determine dysplasia status of an individual. However the role of HPV in VIA related dysplasia has not been extensively interrogated. We sought to further understand HPV infection in the context of HIV status and the relationship of this to VIA dysplasia.

MATERIALS AND METHODS

A cross sectional study conducted on women attending cervical cancer screening clinic at Moi Teaching and Referral Hospital/Academic Model Providing Access to Health care (MRTH/ AMPATH) . All women above 18 years attending cervical cancer screening clinics were included in the study. Women with a current or recent pregnancy (<3 months) and those with active cervical or vaginal infection or on menses were excluded. Ethical consent was sought from MOI UNIVERSITY/MTRH Institutional Review and Ethics Committee (IREC). Informed consent was administered.

Stratified sampling was used to select 88 respondents meeting the participation criteria. Upon determination of HIV status an equal number of HIV positive and HIV negative respondents was selected.

Cervicovaginal lavage (CVL) was collected by inserting 5ml normal saline into the external cervical os and irrigating the endocervical region for approximately 1 minute using a sterile disposable plastic pasteur pipette. The CVL pooled in the posterior fornix of the vagina, was withdrawn using the same pipette, transferred into a sterile 15millilitres ml) conical tube and transported to the laboratory at 4ºC within 2 hours and processed by centrifugation at 1000 x gravity (g) for 10 minutes at 4ºC. The supernatant was aliquoted and stored at -80ºC until cytokine analysis. The remaining cell pellet was re-suspended in normal saline and frozen at -80ºC until HPV processing.

Visual Inspection with acetic acid (VIA) was conducted by two qualified nurses trained on VIA examination as per World Health Organization (WHO) and International Agency for Research on Cancer (IARC) guidelines. In brief 5% acetic acid soaked in a cotton swab was gently placed on the cervix after wiping off secretions. After 1 minute the swab was removed and the cervix was observed for any whitish lesions, particularly in the transformation zone close to the squamo-columnar junction, or dense, non-removable aceto-white areas in the columnar epithelium. A positive VIA outcome was defined as a sharp, distinct, well-defined, dense (opaque, dull, or, oyster white) aceto-white area with or without raised margins that border the squamo-columnar junction in the transformation zone. Positivity was also defined as a strikingly dense aceto-white area in the columnar epithelium or condyloma and leukoplakia occurring close to the squamo-columnar junction turning intensely white. Colour change was observed 1 minute after the application of 5% acetic acid solution since the aceto-white lesions appear and then disappear rapidly 13.

High Risk (HR) HPV DNA was detected using Digene® Hybrid capture 2 (HC2) method. This is a nucleic acid hybridization technique with signal amplification using microplate chemiluminescence that quantitatively detects 13 HR HPV DNA. Manufacturer's instructions were followed.

Demographic and clinical data for each respondent was collected by employing a semi-structured questionnaire. Data was entered using EpiData® software version 2.1. SPSS® version16 was used for statistical operations. Normality assumption was checked using histograms. Parametric tests such as mean were reported for normally distributed variables while nonparametric tests such as median reported for variables

that did not follow the normal distribution. The T test was used to compare means of normally distributed variables while for skewed data Wilcoxon Mann–Whitney U-test was used to compare mean ranks. Chi-square was used to check for relationships between categorical outcomes. P-values <0.05 were considered significant.

RESULTS

Socio-demographic characteristics

A total of 88 women, 44 with HIV and 44 without HIV were studied. The socio demographic characteristics for the study participants are shown in Table 1 below. The Overall and category specific (HIV+ and HIV-) participants' age, age at first marriage, and age at first sexual encounter followed a normal distribution pattern while the distribution of parity `was skewed. The overall mean age of participants was 37.41 years (Standard Deviation [SD] 9.297); the mean age at first marriage was 18.8 years (SD3) while the mean age at first sexual intercourse was 16.59 years (SD 2.629). The median parity was 5 children (inter quartile range [IQR] 0-20 children].

Table 1
Socio Demographic Characteristics

CHARACTERISTIC	TOTAL PARTICIPANTS	HIV POSITIVE[1]	HIV NEGATIVE[2]	P-VALUE[3]
Age (in years)	37.41 (SD 9.297)	38.6 (SD 9.4)	36.3 (SD 9.2)	0.244
Age at 1st marriage	18.8 (SD 3.0)	18.8 (SD 3.3)	18.7 (SD 2.7)	0.878
Age at 1st sexual encounter	16.59 (SD 2.629)	16.6 (SD 2.0)	16.6 (SD 3.1)	0.872
Active Sex Partners				
None	18 (20.5%)	13 (29.5%)	5 (11.4%)	0.036
One	69 (78.4%)	30 (68.2%)	39 (88.6%)	
More Than One	1 (1.1%)	1 (2.3%)	0 (0%)	
Parity [median, Interquartile Range (IQR)]	5 (0, 20)	4 (0, 11)	5 (1, 20)	0.115
Marital status				
Single	5 (5.7%)	2 (4.5%)	3 (6.8%)	
Married	54 (61.4%)	22 (50%)	32 (72.7%)	0.045
Widow/separated/Divorced	29 (33.0%)	20 (45.5%)	9 (20.5%)	
Kind of marriage				
Monogamous	57 (68.7%)	22 (52.4%)	35 (85.4%)	0.001
Polygamous	26 (31.3)	20 (47.6%)	6 (14.6%)	

[3] = significance between [1] and [2]

There were only a few significant differences in demographic characteristics between the HIV positive and HIV negative groups. Among the HIV positive women, 22 (50%) were currently married. Their mean age at first marriage and age at first sexual intercourse was 18.8 (SD 3.3) and 16.6 (SD2.0) years respectively. Thirty two (72.7%) of the HIV negative women were married. Their mean age, age at marriage and age at first sexual intercourse was 36.3 (SD 9.2), 18.7 (SD 2.7) and 16.6 (SD 3.1) years respectively.

Significant differences between the HIV+ and HIV- group were only observed in the marital status where more of the HIV negative group were married compared to the HIV positive group, most of whom were widowed /divorced/separated (45.5%) (p=0.045). Significant difference was also observed in the number of active sexual partners where more HIV negative group (88.6%) reported to have one sexual partner compared to their HIV positive counterparts (68.2%) (p=0.036). Majority of the HIV negative women (85.4%) were also found to be or have been in monogamous marriages compared to their HIV+ counterparts (52.4%) (p=0.001).

High Risk HPV Prevalence

The overall HRHPV prevalence among participants was 45/88 (51.1%). HIV + women had higher prevalence 26/44 (59.1%) of high risk (HR) HPV than HIV- women 19/44 (43.2%). The highest prevalence of HR HPV was among HIV+ VIA- group 7/11 (63.6%) followed by HIV- VIA+ group 11/19 (57.9%). The prevalence was least in the HIV- VIA- group (32%) as indicated in Figure I. The prevalence of HPV was highest in the 18-24 age group 5/5 (100%) followed by the 25-34 age group 18/33 (54.54%) as shown in Table 2.

Figure 1
HPV Prevalence

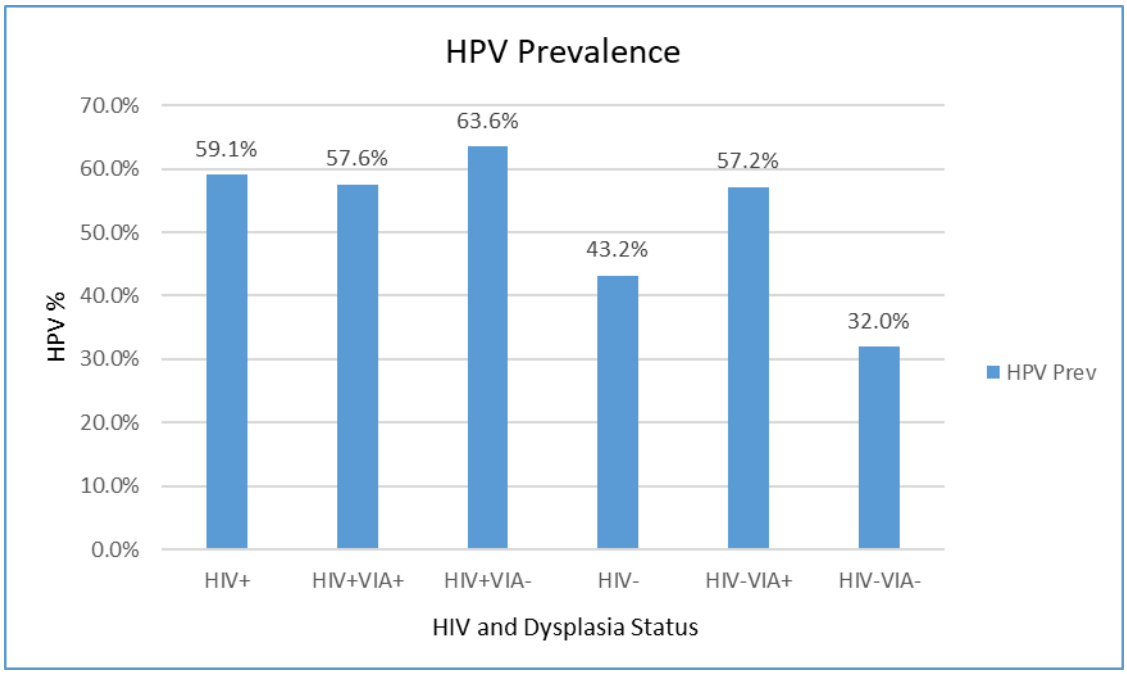

Table 2
HPV Prevalence across Age

Class (years)	HPV Prev x/n (%)
18-24	5/5 (100)
25-34	18/33 (54.5)
35-44	11/28 (39.3)
>45	11/22 (50)
Total	**45/88 (51.1)**

Table 3
HPV Prevalence and Number of Sexual Partners

No. Partners	HPV Prev. x/n (%)
None	10/18 (55.6)
One	34/69 (49.3)
>1	1/1 (100)
Total	45/88 (51.1)

HPV infection and Sexual Exposure and Parity

HR HPV prevalence was higher in women reporting to having no sexual partner 10 (55.6%) and highest in those more than one partner 1 (100%) (Table 3). It was also high in women with 4-6 children 13 (46.4%) and less than 3 children 25 (62.5%). Majority of HIV+ women with 4-6 children had HPV infection 9 (75%) (Table 4).

Table 4
HPV Prevalence and Parity

Parity	HPV Prevalence n/total (%)	HPV Prevalence in HIV+ n/total (%)
<3	25/40 (62.5)	14/23 (60.9)
4-6	13/28 (46.4)	9/12 (75)
>6	7/35 (35)	3/9 (33.3)
Total	45/88 (51.1)	26/44 (59.1)

DISCUSSION

The mean age of 37.41 years for participants is a good indicator of the average age at which women perceive themselves as being at risk of cervical cancer, given that this is the age of majority for women who sought cervical screening services. The population displays peaks of HPV infection; the 18-24 years and ages of 25-34 years age groups. This can be attributed to the greater sexual activity occurring at these age. In addition this is the usual age of giving birth which can be accompanied by cervico-vaginal trauma that may serve as entry points for HPV. Previous studies have found similar peaks in the ages of 18-24 years4,5. The higher burden of High Risk (HR) HPV found among the 18-24 years age group of women is a probable manifestation of onset of sexual intercourse by the ages of 16-18 years reported by the women. Similar results have been observed by other authors4,5. Some authors have established the age of sexual debut as a predictor of infection with HR HPV6. The higher HPV infection amongst women with high parity is possibly a manifestation of the increased exposure to the virus. The association of higher parity with increased HPV prevalence has been observed by some authors but not by others10,14,15.

The higher HRHPV prevalence in HIV+ women compared to HIV- women is consistent with many other studies which found higher prevalence of HPV in HIV populations. In Zambia, Ngandwe and colleagues found a two-fold risk of HRHPV among HIV+ than HIV-; the HR HPV prevalence among HIV+ was 70% compared to 35% in HIV- women 6. In Kenya 73% of HIV+ FSW had HR HPV compared to 45% in HIV- counterparts10. The higher HPV prevalence could be attributed to a greater susceptibility of HIV populations to HPV infection, persistence and reactivation of latent HPV as a result of a suppressed immune system. This is evidenced by the ability of HIV proteins to interfere with components of the immune system (like antigen presenting cells) and also promote up regulation of HPV proteins resulting in increased HPV prevalence16. In addition, studies have shown that HPV prevalence is still comparably higher in HIV positive women with better immunity (higher CD4 levels and low HIV viral loads) than their HIV negative counterparts. Furthermore HPV prevalence has been demonstrated to correlate to an increase in HIV viral load in women with CD4 levels above 200 cells per mm3 and HPV clearance found to be lower in HIV positive women compared to the HIV negative women17,18. The relationship between immunity and HPV has also been shown by studies which have linked HIV immunosuppression as a predictor of cervical dysplasia19.

An increase in the number of sexual partners increases the risk of HPV infection17. The number of active sexual partners implies recent sexual encounters and can therefore be an indicator of one's risk to sexually transmitted infections including HPV. In this study, as in other studies17, HPV prevalence was higher in those with more than one active sexual partners. In addition a larger proportion of HIV infected women were more exposed to multiple risk factors of HPV infection including active multiple sexual partners and more were in (or had a history of) polygamous marriages. This partially accounted for their higher HPV prevalence. The 55.6% infection among those who claimed not to have any sexual partner could be as a result of persistent HPV infection or reactivation, especially among HIV positive women. New HPV infections have been reported before among sexually inactive women. These incident HPV infections were attributed to a reactivation latent infections. The majority of these women were HIV positive. However, there is also a possibility of infection through other means like auto-inoculation (for example from anus to cervix/vagina) 4,17.

Overall, women with dysplasia (VIA+) had a high HPV prevalence explainable by the pathological role of HPV in dysplasia development. The lower prevalence of HPV among HIV+ women with dysplasia (VIA+) (57.6%) than their VIA- counterparts (63.6%) is a reflection of a greater discordance between HPV and VIA reporting especially among the HIV+ women, as previously noted by Gravitt and colleagues 12 This may be as a result of reduced sensitivity of VIA but it can also be as a consequence of the compromised immune system. VIA interpretation is based on the visualization of colour change on the cervix after application of vinegar, and this colour change is due to a reversible coagulation of proteins by Vinegar13. Inflammation, a function of the immune system, occurs during HPV infection and dysplasia formation and it is accompanied by production of proteinous substances (like cytokines) 16, 20, 21. Therefore when the immune system is affected (as in immune compromised persons) VIA outcome may be affected.

CONCLUSION

HIV+ women are more infected with HRHPV than their HIV- counterparts, and their susceptibility may be influenced by several socio-demographic and immune system related factors. There is increased effort in promoting cervical cancer screening in these high risk groups. However we report an increased discordance between HPV and VIA results especially in HIV+ women. We recommend that further investigations targeting the role of the Immune system related factors and how they affect the interpretation of subjective tests like VIA be done especially in immune-compromised individuals.

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