ORIGINAL ARTICLE

GENOTYPING THE HUMAN PAPILLOMAVIRUS AND ASSOCIATED RISK FACTORS IN MONO- AND CO-INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS AMONG WOMEN ATTENDING KIU-TH

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ABSTRACT

The study set out to identify the circulating "high-risk HPV (Hr-HPV) genotype" and examine the risk factors linked to HPV/HIV co-infection and HPV single infection among women receiving medical attention at the Kampala International University Teaching Hospital (KIU-TH). A total of 114 women attending the KIU-TH Clinton Health Accesses Initiative (CHAI) and gynecology units were enrolled. Questionnaires were administered and information regarding the "risk factors associated with Hr-HPV infection" was collected. Subjects were examined for cervical cytological features with use of "visual inspection with acetic acid" (VIA) and Pap smears, and HPV genotype were assessed using the Xpert gene. The analysis of data was done using SPSS v29. The study hypotheses were tested at the common level of $P \leq 0.05$. In this study, 37(32%) patients were positive for VIA, 28(24%) were Pap smear-positive, and 3(3%) and 2(2%) were atypical "squamous cells" of undetermined significant (ASCUS) positive for VIA and Pap smears, respectively. Furthermore, a study revealed that 20% of Hr-HPV was from co-infections and 10% from mono-infections. The circulating Hr-HPV genotype were HPV (17.2%), HPV16(9%), and HPV 18/45(7%). Results showed significant relationships ($P < 0.05$) between condom use, marital status and having many partners as "risk factors" and Hr-HPV infection. In contrast, age, education, vaccination, and educational level were not. This study revealed several factors linked to HPV infection and calls for the integration of cervical cancer screening using the combination of both VIA and Pap smear or gene Xpert, as well as creating awareness and implementation of vaccination programs within Bushenyi District and the country at large.

Keywords: Cervical cancer, Cytology, High-risk HPV, HIV, Genotypes, Risk factors

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INTRODUCTION

The circular, non-enveloped, "double-stranded DNA virus", also called the "human papillomavirus" (HPV) is categorized based on its ability to cause cervical cancer (CC). (1). HPV is the primary cause of CC in women worldwide (2). Most of the CC patients are infected with "high-risk" HPVs (Hr-HPV), notably HPV16 and HPV18, both of which include the oncoproteins E6 and E7 (3). Cervical cancer is a slow-growing cancer that develops in the tissues of the cervix and invades nearby organs, including the vagina and uterus (4). The fourth among the most prevalent cancer types globally among women is CC, accounting for over 570,000 new cases and 311,000 fatalities in 2018 (5). It may be avoided by receiving an HPV vaccination and scheduling regular cervical cancer screenings, which assists to identify and manage precancerous lesions (6).

Uganda is second in Eastern Africa and among the top ten countries worldwide for CC incidence (28.8/100,000 per year), with 6413 new cases and 4301 deaths per year (7)(8). In 2020, approximately 35.7% of new cases of CC were reported among Ugandan women (9), and almost 11 million women in Uganda may develop CC one day, which is caused by oncogenic HPV (10) (11). Age, sexual intercourse, many sex partners, infection with other sexually transmitted diseases, condom use, long-term contraceptive use, education level, genetic susceptibility, and early sexual debut are all risk factors (12). Furthermore, HIV infection elevates HPV infection development to CC. An alarming increase in HPV-related cancers has been found in HIV-positive women. High-risk HPV (Hr-HPV) infection necessitates decreased T cell activation, which is produced by HIV infection (13). HIV is an infection transmitted through sexual activity that is most prevalent in women aged 15 to 64, with an incidence rate of 7.6% among them collectively in Uganda (Population-based HIV-impact assessment, 2016–2017) (14). Because HIV-positive women are very susceptible to CC, the Centers for Disease Control and Prevention (CDC) and the Uganda National HIV Treatment Programme urge yearly screening in this demographic (15). Nonetheless, due to a lack of funding, no particular CC screening program has been created for women living with HIV/AIDS, and CC screening services have not been fully integrated into HIV care services. As a result, CC screening services for HPV-positive women are on and off in some places, especially in rural settlements (16).

The most commonly used test for CC screening in Uganda is VIA because it is more affordable, less time-consuming, and can be used to detect pre-invasive lesions. It has limited sensitivity (60–70%) and specificity (50%) (17). Despite this limitation, VIA is probably preferable to no screening. In some areas, clinics perform CC screening using samples collected from the patient and test cytologically (Pap smear) and/or molecularly (testing for Hr-HPV). Owing to the requirements for infrastructure, training, and high expenses, these tests are slow or lacking in some places. Therefore, it is crucial that we move past VIA and that more accurate and focused biomarkers of early disease and development are found, especially those that may be particularly similar to indicators in HIV-positive women (18).

To date, the circulating HPV genotype responsible for CC in the Bushenyi-Ishaka municipality remains unknown. According to KIU-TH management records, 35 of 53 women tested positive for cervical cancer in 2019, accounting for 66%, which is somewhat higher than the Ministry of Health's yearly rate of 54.4% (19). Following these findings, there may be a high HPV prevalence in the Bushenyi-Ishaka municipality, which could result in a significant proportion of women with HPV who could also be HIV-positive, which may pose a substantial public health risk for the area. Thus, the study aimed to identify HPV genotype in women with HPV/HIV co-infection, and HPV mono-infection, and regarding the dangerous signs of the transmission of HPV among women attending KIU-TH.

MATERIALS AND METHODS

Ethical Approval

The Research Ethical Review Committee of KIU (KIU-REC) approved this study and the approval number is KIU-2021-15. The criteria of the study were explained to willing participants, who were also advised that their withdrawal from the study at any time would not have any influence on their regular medical care. The participants provided signed consent after being fully informed. All participants received assurances regarding the confidentiality of sample usage and the distribution of results, which were not disclosed to anybody without their consent. Before and after receiving both HPV and HIV testing, participants were counseled by a qualified practitioner. Those who tested positive were referred to medical experts for further guidance. Every

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procedure was carried out in compliance with the rules and regulations that KIU-REC approved.

Study Design and Setting.

This descriptive case-control research included women who attended the CHAI and gynecology departments at KIU-TH in Ishaka. The researcher contacted only women aged 18 - 50 years, who consented to participate with single or mixed infections of HPV/HIV, not pregnant, with no previous diagnosis of cervical cancer and had not used UTI antibiotics in the last two days. Whereas all women who did not meet the inclusion criteria, such as less than 18 years and more than 50 years, those on menstruation, without HPV infection and has not consented, were not excluded.

Sample Size Determination

Research conducted at "Naguru Teenage Information and Health Centre" found that the prevalence of HPV and HIV respectively stood at 87.8% and 73.2% (20). As a result, this study's sample size utilized the formula of difference in proportion, as indicated below:

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

Where,

For, Zβ = 0.84 80% power, Zα = 1.96 at a significance level of 0.05 , r = 1 for equal number of cases and controls, and P1 = 0.88 for HPV prevalence among women with. P2 = 0.73 (HPV prevalence in women who are HIV-negative).

Average proportion, $\bar{p} = \frac{p1+p2}{2} =$

Average proportion = 0.88+0.73/2=0.805

+ 10% attrition rate (104/10) = 114

Therefore, minimum sample size = 114

Data Collection Tool

Questionnaires administered for the interview were comprised mostly of "yes or no" questions. The questionnaires were designed to capture some specific information on risk factors like age, intercourse, intercourse frequency, number of sexual partners, marital status, use of contraceptives, use of condoms, and educational level associated with HPV infection and medical history was also captured.

The instrument was validated, reviewed by experts and finally 114 participants were selected for this study, data from the questionnaires were analyzed using logistic regression.

Acetic acid visual inspection (VIA) and Pap smear

All research participants undergo cytological examination by

$$n = 2\frac{(0.805)(1-0.805)(0.84+1.96)^2}{(0.88-0.73)^2} = 104$$

VIA and Pap smears. The VIA method involved applying 5% acetic acid to the cervix. The data were recorded after one minute with use of a halogen lamp. The appearance of an aceto-white area in the transformation zone was taken positively, while the formation of a white spot on the cervix was not seen as negative or suspicious of invasive malignancy. For the pap smear, a tiny portion of the cervical swab was put using ethanol in 30 minutes before it was stained with hematoxylin and eosin dye and rinsed with 1% dilute hydrochloric acid (HCl) and water. The slides were examined under a microscope at ×100.

HPV Genotyping

The HPV DNA genotyping process was done utilizing the Gene Xpert and Xpert HPV Assay kits (GXHPV-CE-10). The samples were prepared by first treating them with 3 mL of concentrated glacial acetic acid (GAA) and lysing the cells by incubating them for 2 hours at room temperature.

The Xpert HPV Assay is a multiplex real-time PCR test that can simultaneously detect 13 high-risk HPV types, as well as one additional possible high-risk type (HPV66). The assay also includes three controls: an internal probe check control (PCC), a specimen adequacy control, and a human reference gene (HMBS).

The 14 targeted HPV variants were identified using five different fluorescence channels. The test results were displayed on the screen as either "positive" or "negative" for HPV16 and HPV18/45 specifically, while other HPV types of high-risk detected were reported collectively.

Statistical Analysis

The analysis of data was done through SPSS to compute descriptive statistics for cervical cytological lesions and HPV genotype distributions and the chi-square test was used to test the null hypothesis at the common significance of 0.05. The risk factors association with high-risk HPV (Hr-HPV) were analyzed using logistic regression.

RESULTS

VIA versus Pap Smears from Patients Attending KIU-TH

Table 1 shows the VIA and Pap results. The VIA-positive

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patients were 37(32%), while the Pap smear-positive patients were 28(24%). Three (3%) and 2(2%) ASCUS results were obtained for VIA and Pap smears, respectively.

VIA versus HIV from Patients Attending KIU-TH

Table 2 shows the results of VIA and HIV tests. 37 (32%) of the patients tested positive for both VIA and HIV, while 30 (26%) tested positive for VIA alone. In addition, 7 (6%) tested positive for VIA alone.

Pap Smear versus HIV from Patients Attending KIU-TH

Table 3 shows the results of Pap smears and HIV tests. 28 (24%) of the patients tested positive for Pap smear, while 21 (18%) tested positive for both Pap smear and HIV (Aa). In comparison, 7 (6%) patients tested positive for only Pap smears (aB). Furthermore, 2 (2%) patients tested positive for HIV alone using ASCUS (Ac). No patients tested positive for ASCUS alone, and all were HIV-negative (cB).

HPV genotype versus HIV from Patients Attending KIU-TH

According to **Figure 1,** the most prevalent circulating Hr-HPV genotype were different Hr-HPVs (31,33,35, 39,51,52,56,58,59,66). There is a 17% prevalence of HPV/HIV co-infections. HPV16 has 6%, HPV 18/45 has 3%, and HPV 16 and HPV18/45 have 3% and 1%, respectively.

Correlation-ship between HPV/HIV and Associated Factors

Tables 4 and 5 depict the link between various risk factors and Hr-HPV among women with HPV/HIV co-infection and mono-infection with KIU-TH.

The bivariate logistic regression analyses represented in **table 4** showed that multiple sex partners (aOR=10.43, Cl:4.06-26.85, *P*<0.001), marital status; single (aOR=7.2, Cl:2.59-20.04, *P*<0.001), divorced (aOR=6.14, Cl:1.98-91.06, *P*=0.002), use of condoms (aOR=6.11, Cl:2.45-14.98, *P*<0.001), use of contraceptives (aOR= 0.58, Cl=0.25-1.33, *P*=0.197), and secondary education (aOR=0.46, Cl=0.14-1.54, *P*=0.211) were significantly associated with Hr-HPV infection, whereas age, sexual intercourse frequency, and vaccination were not.

In multivariate logistic regression, multiple sex partners (aOR=8.93, Cl; 2.45-32.58, *P*=0.001), marital status (aOR=6.53, Cl=3.32-10.34, *P*=0.001), or divorced women (aOR=4.97 Cl; 2.04-9.62, P=0.007) condom use (aOR=3.67, Cl;3.7-16.48, P=<0.001) were significantly associated with Hr-HPV infection as represented in Table 5.

DISCUSSION

The results from this study showed 32% and 24% positivity for the VIA and Pap smears, respectively. These results align with those of previous research projects carried out by (21), in Iran where 27.5% were VIA positive and 20.9% were Pap smear positive. Regardless of the differences in the geographical locations of Uganda and Iran, the results follow the same trend. This could be a result of the sensitivity and specificity variations between the two tests. Most studies reported that VIA has higher sensitivity than Pap smear while Pap smear has higher specificity than VIA (22), (23) (24), and (25).

Of the 32% VIA positive, 26% were VIA and HIV positive, 1% were ASCUS and HIV negative, and 2% were ASCUS and HIV positive. The findings are consistent with two other studies, one conducted in Kampala, Uganda by Namale, G et al., 2021 (26), who also reported the prevalence of VIA among HIV-positive patients, and another conducted by TASO HIV Clinic at Mulago facility, Kampala (27), also reported 44% of VIA and HIV positive patients. Hence, UNAIDS, 2018 (28) reported that the prevalence of HIV-positive women within the age–15-49 was at 5.4%. The reason for the similarity between these two studies and ours could be the HIV prevalence among women in Uganda, as reported by the Uganda Population HIV Impact Assessment (UPHIA).

Another finding from this study was 24% Pap smear positive, 18% Pap smear and HIV positive, 2% ASCUS, and HIV positive. These findings agree with another study conducted in India by Gupta, K. et al., 2019 (29), who reported a 12% Pap smear among HIV-positive patients and 6% among HIV-negative patients. This similarity could also be attributed to the prevalence of HIV infections. HIV-positive women have a higher risk of developing cytological cervical abnormalities as a result of the rapid progression or reactivation of Hr-HPV infection. Various studies have reported that HIV-positive women have an impaired immune response against Hr-HPV as they are 2-5 times more likely to develop cervical intraepithelial abnormalities and neoplasia (30) and (31).

This study's total Hr-HPV was 30%, 20% HPV/HIV co-infections, and 10% HPV mono-infection, in collaboration with three additional research. One study conducted in Kampala by the Naguru Teenage Information and Health Centre found HPV/HIV co-infection and HPV mono-

infection at 87.8% and 73.2%, respectively (32). Another study in Tanzania (33) found that HPV/HIV co-infection and HPV mono-infection were 46.7% and 17%, respectively. Another study in Bushenyi and Sheema Districts by (34) found HPV/HIV co-infection and HPV mono-infection rates of 17% and 7%, respectively. Several studies have reported the prevalence of Hr-HPV among HIV-positive women, and the key reason could be impaired immune response to HPV and reactivation of Hr-HPV infection by HIV.

The most prevalent genotypes in this investigation were other HR-HPVs (31,33,35,39,51,52,56,58,59,66), with a prevalence of 17.2%, accounting for 56.6% of all HR-HPV cases. HPV16, with a prevalence of 9%, accounts for 30% of all Hr-HPV-positive cases, 6% of HPV/HIV co-infections, and 3% of HPV mono-infections. HPV 18/45 had a prevalence of 7% and accounted for 23.3% of all Hr-HPV-positive cases, 6% of HPV/HIV co-infections, and 1% of HPV mono-infections. These findings contradict those of Ginindza, T. G. et al., 2019 (35) in Switzerland, where another high-risk HPV accounted for 12.4%, HPV 16 13.8% had HPV 18/45. Several Hr-HPV genotypes have been reported in various studies, (36) (37) and the key reason for this variation is differences across different geographical locations. In addition, our study population included HIV-positive women who have been reported to have an impaired immune response against Hr-HPV. Hence, this could be the reason for the expression of other Hr-HPV genotypes in our study, as well as HPV16 and HPV18, as seen in Figure 1. However, our findings are consistent with two earlier studies of HIV-positive women conducted in Tehran, Iran. The overall HPV prevalence was 10.3%, with other HR-HPV accounting for 8.2%, HPV16 3%, and HPV18-45 0.7% (38). Another study in Mbarara, Uganda (39) found an overall HPV prevalence of 16.4%, with additional Hr-HPV strains accounting for 2.9%, HPV16 (3%), and HPV18-45 (1.9%). Sheema and Mbarara Districts border the region and hence similar observations could be expected, with no significant wide variations.

Regarding the number of sexual partners, our findings are consistent with a study conducted in Brazil, where multiple sex partners showed a significant association with HPV-positive patients (40). Similarly, our findings agree with Nascimento, M.et al., 2018 (41), who demonstrated that three Hr-HPV-positive patients with a history of more sexual partners progressed to CC, as well as Kehinde, O. et al., 2018 (42). A cross-sectional study conducted in Nigeria found a statistically significant connection between an increased number of lifetime sexual partners and Hr-HPV infection. The rationale for this link could be that having multiple sexual partners increases the risk of STIs, including Hr-HPV infection, as shown in Table 5. The proportion of participants with many partners (73.68%) was significantly larger ($P < 0.001$) than those without multiple relationships (26.32%). Contrary to our findings, Ginindza, T. G. et al., 2017 (43) observed no linear relationship between Hr-HPV infection and the number of sexual partners a person had in their lifetime. This discrepancy may be due to the fact in their study, participants were stratified according to urban/rural, occupations, and other covariates, such as age, the total number of sexual partners in life, career, parity, and marital status.

In terms of marital status, our findings were consistent with another conducted in South Africa where single/divorced women were 1.4 times more likely to be infected with Hr-HPV (44), and another in Switzerland by Ginindza, T. G. et al., (2017) (45) who also reported a linear relationship between Hr-HPV and marital status that married women had a decreased risk of being infected with Hr-HPV. This could be because married women stick to one sexual partner, which reduces their chances of being infected with Hr-HPV. Regarding the use of condoms, our findings agreed with another study in England conducted by Winer, R. L. et al., (2006) (46) and another in China (47), who also reported that women who used condoms with their partner had a 5-time lesser chance of being infected with Hr-HPV. The reason could be that condoms are one of the major preventions of STIs, and infection with Hr-HPV is not an exception. Risk factors, such as age, intercourse frequency, and vaccination, were not substantially linked to Hr-HPV infection; although in other studies, they were strongly associated with Hr-HPV infection. For example, several studies have reported a linear association between age and Hr-HPV infection among younger populations and declined at later ages (48), (49), and (50) was also the case in our study as <20 (2.94%), 21-30 (35.29%), 31-40 (47.07%) and 41-50 (14.71%); however, these were not statistically significant, as was sexual intercourse frequency. The reason could be that since the majority of our study population was married and within the risky age range, the fact remains that even if they had

intercourse more than 10 times a week with the same partner, it reduced their risk of being infected with STIs of which Hr-HPV is one of them. In addition, regardless of whether one is vaccinated, sticking to one partner could reduce the risk of Hr-HPV infection. Hence, our dataset from this study population could be the reason why these three factors were not statistically significant.

CONCLUSION

In conclusion, this study identified the prevalent high-risk HPV (Hr-HPV) genotypes circulating in the population, as well as the risk factors associated with HPV infection. These findings highlight the need to integrate cervical cancer (CC) screening and vaccination programs, particularly for HIV-positive women. Additionally, the study suggests that a combination of visual inspection with acetic acid (VIA) and Pap smear, or the Xpert gene-based test, could be utilized for the diagnosis of cervical cancer and HPV infection.

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TABLES AND FIGURE

Table 1: Visual Inspection with Acid (VIA) and Pap Smear in Cervical Cancer Screening

		Pap Smear			
		Positive[A]	Negative[B]	DISCUSS	**Total**
VIA	Positive	22(19%)[Aa]	15(13%)[aB]	0(0%)Ca	37(32%)
	Negative	6(4%)[Ab]	68(62%)[bB]	0(0%)Cb	74(65%)
	Undetermined	0(0%)Ac	1(1%)cB	2(2%)Cc	3(3%)
	Total	28 (24%)	83 (75%)	2(2%)	114 (100%)

ASCUS = Atypical Squamous Cell of Undetermined Significant (undetermined). Aa represents pap smear and VIA positive patients, Ab pap smear positive and VIA negative, Ac pap smear positive and undetermined VIA, aB VIA positive and pap smear-negative, bB VIA and pap smear-negative, cB pap smear-negative and undetermined VIA, Ca ASCUS (undetermined) and VIA positive, Cb ASCUS and VIA negative and Cc ASCUS (undetermined) and undetermined VIA.

Table 2: Macroscopic Cervical Features Among Women With HPV/HIV Co-Infection Among Women Attending KIU-TH.

		HIV STATUS		
		Positive[A]	Negative[B]	**Total**
VIA	Positive	30(26%)[Aa]	7(6%)[aB]	37(32%)
	Negative[b]	44(38%)[Ab]	30(26%)[bB]	74(65%)
	Undetermined	2(2%)Ac	1(1%)cB	3(3%)
	Total	76(24%)	38 (75%)	114 (100%)

Aa represents HIV and VIA positive patients, Ab HIV positive and VIA negative patients, Ac HIV positive and undetermined VIA patients, aB VIA positive and HIV negative patients, bB both VIA and HIV negative patients and cB HIV negative and undetermined VIA patients.

Table 3: Microscopic cervical features among women with HPV/HIV co-infection and mono-infection attending KIU-TH

		HIV STATUS		
		Positive[A]	Negative[B]	**Total**
Pap Smear	Positive	21(18%)[Aa]	7(6%)[aB]	28(24%)
	Negative[b]	53(46%)[Ab]	31(27%)[bB]	84(74%)
	ASCUS[c]	2(2%)Ac	0(0%)cB	2(2%)
	Total	76(24%)	38 (75%)	114 (100%)

ASCUS = Atypical Cell of Undetermined Significant. Aa represents HIV and pap smear-positive patients, Ab HIV positive and pap smear-negative patients, Ac HIV positive and ASCUS, aB pap smear positive and HIV negative patients, bB both pap smear and HIV-negative patients and cB represents HIV negative and ASCUS.

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Table 4: Bivariate Analysis of Risk Factors Associated with Hr-HPV Among Women with HPV/HIV Co-Infection and Mono-Infection Attending KIU-TH

Risk Factors	Number (N=114)	Hr-HPV status%		COR (95%Cl)	P value
		Negative	Positive		
Age					
<20	2	1(1.25)	1(2.94)	REF	
21-30	50	38(47.50)	12(35.29)	0.32(0.02-5.44)	0.427
31-40	48	32(40)	16(47.06)	0.5(0.03-8.52)	0.632
41-50	14	9(11.25)	5(14.71)	0.56(0.03-10.93)	0.699
Intercourse					
No	30	21(26.25)	9(26.47)	REF	
Yes	84	59(73.75)	25(73.53)	0.99(0.40-2.46)	0.980
Multiple partners					
No	69	61(76.25)	8(23.53)	REF	
Yes	45	19(23.75)	26(76.47)	10.43(4.06-26.85)	**<0.001***
Intercourse frequency					
None	13	8(10.00)	5(14.71)	REF	
Once a month	19	14(17.50)	5(14.71)	0.57(0.13-2.59)	0.469
1-3 times a week	51	38(47.50)	13(38.24)	0.55(0.15-1.97)	0.357
>3 times a week	31	20(25.00)	11(32.35)	0.88(0.23-3.3.35)	0.851
Marital status					
Married	62	54(67.50)	8(23.53)	REF	
Single	31	15(18.57)	16(47.06)	7.2(2.59-20.04)	**<0.001***
Divorced	21	11(13.75)	10(29.41)	6.14(1.98-91.06)	**0.002***
Vaccination					
No	99	68(85.00)	31(91.18)	0.55(0.14-0.08)	0.378
Yes	15	12(15.00)	3(8.82)	REF	
Use contraceptives					
No	37	23(28.75)	14(41.18)	REF	
Yes	77	57(51.25)	20(58.82)	0.58(0.25-1.33)	**0.197***
Use condom					
No	50	25(31.25)	25(73.53)	6.11(2.45-14.98)	**<0.001***
Yes	64	55(68.75)	9(26.47)	REF	
Educational level					
Tertiary	20	13(16.25)	7(20.59)	REF	
Secondary	40	32(40.00)	8(23.53)	0.46(0.14-1.54)	**0.211***
Primary	38	23(28.75)	15(44.12)	1.21(0.39-3.73)	0.730
None	16	12(15.00)	4(11.76)	0.62(0.14-2.66)	0.519

Variables with * superscripts were statistically significant at P <0.05, COR=crude old ratio, Cl=Confidence Interval, and REF=Reference Group.

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Table 5: Multivariate Analysis of Risk Factors Associated with Hr-HPV Among Women with HPV/HIV Co-Infection and Mono-Infection Attending KIU-TH

Risk Factors	Hr-HPV status		COR (95%Cl)	P value	AOR (95%CL)	P value
	Negative	Positive				
Multiple sex partners						
No	61(76.25)	8(23.53)	REF		REF	
Yes	19(23.75)	26(76.47)	10.43(4.06-26.85)	**<0.001***	8.93(2.45-32.58)	**0.001***
Marital status						
Married	54(67.50)	8(23.53)	REF		REF	
Single	15(18.57)	16(47.06)	7.2(2.59-20.04)	**<0.001***	6.53(3.32-10.34)	**0.001***
Divorced	11(13.75)	10(29.41)	6.14(1.98-91.06)	**0.002***	4.97(2.04-9.62)	**0.007***
Use contraceptives						
No	23(28.75)	14(41.18)	REF		REF	
Yes	57(51.25)	20(58.82)	0.58(0.25-1.33)	**0.197***	0.77(0.21-2.84)	0.698
Use condom						
No	25(31.25)	25(73.53)	6.11(2.45-14.98)	**<0.001***	3.67(3.7-16.48)	**<0.001***
Yes	55(68.75)	9(26.47)	REF		REF	
Educational level						
Tertiary	13(16.25)	7(20.59)	REF		REF	
Secondary	32(40.00)	8(23.53)	0.46(0.14-1.54)	**0.211***	0.56(0.11-2.91)	0.489
Primary	23(28.75)	15(44.12)	1.21(0.39-3.73)	0.730	0.40(0.08-2.02)	0.267
None	12(15.00)	4(11.76)	0.62(0.14-2.66)	0.519	0.92(0.14-5.97)	0.930

Variable with two * superscripts in the same row was significant in both bivariate and multivariate analyses: COR, crude old ratio; Cl=Confidence Interval, AOR=Adjusted Old Ratio, and REF=Reference Group.

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RESEARCH ETHICS COMMITTEE (REC)

To: Swase Terkimbi

06/12/2021

Kampala International University-Western campus
0773555465

Type: Initial Review

Re: KIU-2021-15: Genotyping and Cyto-immunophenotyping of Human Papillomavirus and Human Immunodeficiency Virus Co-infection and Human Papillomavirus Mono-infection Among Women Attending Kampala International University Teaching Hospital, ,

I am pleased to inform you that at the **39** convened meeting on **07/07/2021**, the KIU REC, committee meeting, etc voted to approve the above referenced application.
Approval of the research is for the period of **06/12/2021** to **06/12/2022.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **06/12/2022** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by KIU REC: