Prevalence of high-risk HPV types in women with cervical cancer in Eastern Uganda

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***Abstract*—**Infection of the cervix with human papillomavirus (HPV) is the main cause of intraepithelial neoplasm (CIN) and cervical carcinoma. Accurate identification and typing of these viruses might detect patients with different risk of cervical malignancy. Eighty-three cervical swabs were obtained from the regional referral hospitals of Mbale, Tororo, Butalejja, and Soroti between 2017 and 2019. DNA-Sorb-A DNA extraction kits were used, followed by sensitive HPV Real-Time PCR for genotyping high-risk HPV types. High-risk HPV genotypes were identified using HPV High-Risk Screen Real-TM Quant 2x kit, an in vitro Real-Time amplification test for the quantitative identification of 12 high-risk human papillomavirus (genotypes16,18,31,33,35,39,45,51,52, 56,58,59). This procedure utilizes probes targeting regions of the HPV group A9 (16, 31, 33, 35, 52, 58), HPV group A7 (18, 39, 45, 59), HPV group A5-A6 (51, 56), as well as the human β-globin gene as an internal control to screen for isolated DNA. Out of the 83 endocervical uterine swabs, 51 tested positive for HPV DNA. Different HPV genotypes were detectable in 49 incidences: HPV 16, 18, 31, 33, 45 and 52. They manifested as single infections in 46/51 patients (90.2%) and in 3/51 patients (5.9%) as multiple infections. HPV genotypes 16 or 18 contributed to 56.9% (29/51) of the single-infections in the study. The findings of this research affirm the involvement of HPV 16 and 18 in cervical intraepithelial neoplasm and cervical carcinoma pathogenesis in low-income setups. The findings show that the presently available HPV 16 and 18 vaccines might help to deter the significant portion of cervical cancer malignancies in Uganda

***Keywords* — Human papillomavirus; Cervical Intraepithelial Neoplasia; High-grade squamous intraepithelial lesion; Low-grade squamous intraepithelial lesion; Oral contraceptives; and Carcinoma in situ.**

1 INTRODUCTION

The interaction between human papillomavirus (HPV) and cervical cancer is now well documented [1–3]. To date, over 200 HPV genotypes are recognized, and many of them, including HPV 16, 18, 31 and 33, are part of the High Risk (HR) HPV category as they enhance carcinogenesis. Human papillomavirus types 16 and 18 are now well established primary causative viruses of cervical carcinoma and its pre-malignant abnormalities amongst carcinogenic HPV types [4,5]. These two viral categories have been reported in the majority of instances of invasive cervical cancer in 22 countries around the globe [6]. HPV 16 appears to be predominant in squamous cell carcinomas, while HPV 18 also remains dominant in adenocarcinomas [7–9]. Many analyses have revealed that there are regional differences in the distribution of HPV genotypes [10,11]. However, regional differences in the incidence of HPV types might affect the effectiveness of the presently available HPV vaccine. VLPs for HPV 16 and 18 are currently eligible for HPV vaccinations [12–14] besides HPV 11 and 6 [15–17]. Even though the incidence of carcinogenic HPV genotypes is a significant huge concern as an indicator of endocervical premalignancies and cervical carcinoma, the detection of carcinogenic HPV genotypes has analytical validity. Consequently, there is a significant attention in the identification and genotyping of these viral types. We performed an analysis to classify HPV genotypes from endocervical swabs using HPV High-Risk Screen

Real-TM Quant 2x kit (Sacace Biotechnologies, Como, Italy) to quantitatively identify 12 high-risk human papillomaviruses (genotypes 16,18,31,33, 35,39,45,51,52,56,58,59) in endocervical specimens in a single reaction in our recent study.

2 METHODS

This research was performed in designated national regional hospitals in Uganda. The healthcare facilities comprised of; Butalejja, Tororo, Mbale, and Soroti hospitals (Figure 1) below.

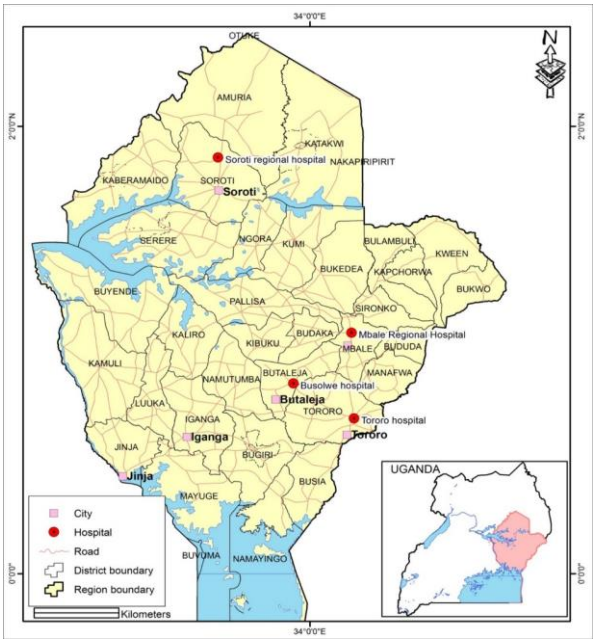

Figure.1. Map of Uganda showing study sites (Uganda Bureau of Statistics. Copyright © 2018

Study design and study participants

This was a longitudinal study in which participants were followed until they were diagnosed with high-grade intraepithelial neoplasia (CIN) and cancer [18]. After meeting the inclusion criterion, a total of 1,077 women aged 15-55 years who were accessing healthcare services at the cancer diagnostic health facilities of Mbale, Tororo, Butalejja, and Soroti referral hospitals were invited to participate in the screening. Follow up schedules were in months. Between June 2017 and August 2017, 416/1,077 (38.6 %) women had abnormal cytology on pap smear examinations, with 394/1,077 (36.6 %) having High-grade squamous intraepithelial lesions (HSIL) and 22/1077 (2.0 %) having Low-grade squamous intraepithelial lesions (LSIL). All patients detected with abnormal cytology were immediately referred for colposcopy and histological examination. Between September 2017 and December 2017, their histological examination revealed that all 394 (36.6%) of the women with HSIL had developed either CIN2 or CIN3, while 22 (2.0%) had developed CIN1. Six patients (6/180) with CIN2 advanced to CIN3 between January and June 2018. Between June 2018 and January 2019, 83 patients with CIN3 were detected with invasive cancer, which was classified as follows: 58 patients had squamous cell carcinoma, 24 patients had adenocarcinoma, and only 1 patient had adenosquamous carcinoma (Figure.2 below). Since the aim of this research was to determine HPV prevalence in women with cervical cancer, only 83 patients with confirmed cervical cancer status have been considered and addressed in this study. We also considered other risk factors for cervical cancer development besides HPV, since there was an observable progression from CIN2/CIN3 to invasive cancer during the course of the study.

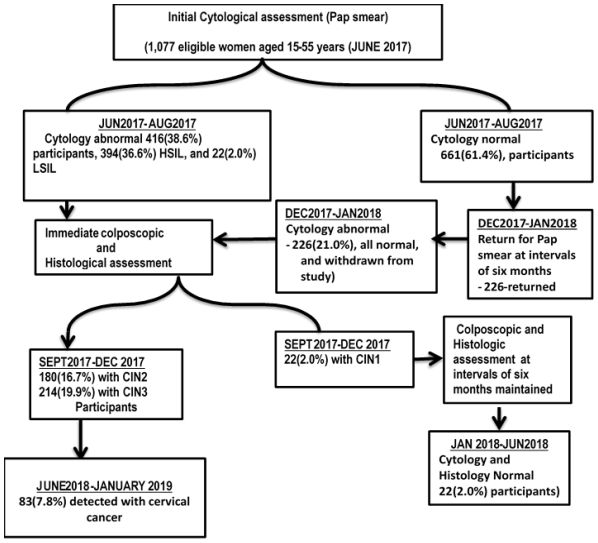

Figure 2: Flow chart showing study stages during the enrolment to completion between 2017-2019

Gynecological examination and collection of clinical materials

Following a consultation with study participants, informed written consent was provided and a short oral questionnaire was distributed. Two nursing care sisters who had been educated in cervix DVI

and Papanicolaou (Pap) conducted a gynecological test on spot. In this study, 83 cervical scrapes from women with cervical cancer were collected by 360-degree rotation around the transition region using endocervical uterine swab. The end of the endocervical swab were broken into 15 mL of retention tubes holding 5 mL of PBS (pH 7.2) and then stored for future molecular examination. Endocervical specimens were initially preserved at 4°C for approximately 6 hours and then transferred in a freezer at-20°C for subsequent molecular studies.

Isolation of HPV DNA

DNA extraction was conducted using the DNA-Sorb-A DNA extraction kit (Sacace Biotechnologies, Como, Italy)[19]. Higher endocervical swab was vigorously emulsified in 200µl nuclease-free water by vortexing, and 100µl of the emulsified sample was added to the labeled 1.5ml microfuge tube and then to 300µl of Lysis Solution. The sorbent solution was vigorously vortexed and 20µl of sorbent was added to each tube containing the supernatant. Then vortexed for 5-7 seconds, and all tubes were incubated at room temperature for 3 minutes, and this process was repeated one more time.

The tubes were centrifuged for 30 seconds at 10000g. The supernatant was then carefully removed and discarded from each tube without disturbing the pellet. The tips were changed between the tubes. 500 µl of wash solution was added to each tube, vigorously vortexed and centrifuged for 30 seconds at 10,000g. Then the supernatant was separated and removed from each tube. The above procedure was repeated and the open cap tubes were incubated at 65°C for 5-10 min to dry the excess wash buffer. The DNA-containing pellet was resuspended in 100 µl of DNA-eluent and incubated at 65°C for 5 min and continuously vortexed. The tubes were then centrifuged for 1 min at 12000g and the resulting supernatant contained DNA. This was used immediately or stored at -20 degrees Celsius prior to usage.

PCR amplifications

The Real Time PCR was performed using the HPV High Risk Screen Real-TM Quant 2x kit (Sacace Biotechnologies, Como, Italy), an in vitro Real-Time amplification test for the quantitative identification of 12 high-risk human papillomavirus (genotypes16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) in cervical specimens. Target region of amplification for high-risk HPV was E1 and E2 regions. 10ul extracted DNA was mixed with 15ul of PCR reaction Mix composed of 7ul of PCR-mix-1- FRT and 8ul of PCR-buffer-FRT/TaqF DNA polymerase. PCR-mix-1 tube contained primers directed against regions of HPV A7, A9 groups (HPV types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59), HPV A5 group (HPV type 51), HPV A6 group (HPV type 56) and b-globin gene used as Internal Control. Reagents were mixed by tapping the tubes and transferred the tubes in the PCR machine (Rotor Gene Q, Qiagen, Germany) along with positive control DNA (10ul provided in the kit) tube and processed negative control tube. The primer sequences were not disclosed by the kit producer and PCR protocol was programmed according to kit manufacturer's instruction. In brief, the PCR system used was upholding: 1 cycle of 95-C for 15 minutes; 5 cycles of 95-C for 5 seconds, 60-C for 20 seconds, 72-C for 15 seconds; and 40 cycles of 95-C for 5 seconds, 60-C for 30 seconds, and 72-C for 15 seconds. Threshold was set manually and analysis was done according to the kit manufacturers instruction. The sample was deemed positive if the fluorescence signal (Ct ≤ 33) was present in at least one of the two tubes in the Joe (yellow) channel. If amplification was observed only in the Fam (green) channel, the test was deemed negative.

Data management and statistical analysis

Analysis was undertaken using SPSS 23.0 version (SPSS Inc., Chicago, IL, USA). Data were initially assigned to a descriptive statistic analysis using a chi-square scale. Followed by multiple logistic regression analysis to test for the relationship between HPV infection rate and socio-demographic characteristics. The frequency of HPV genotypes in the sample population was described as frequency and proportions $P < 0.05$ was found to be statistically important. Interaction and uncertainty were measured and $p \leq 0.05$ scores were deemed to be statistically relevant relationships

3 RESULT

Table I showed HPV/DNA positivity results by cancer type in the studied participants. Of the 83 endocervical swabs, 58 were collected from women with squamous cell carcinoma, 24 from women with adenocarcinomas, and 1 from women

with adenosquamous carcinoma. Of these, 51 were HPV positive while this could not be identified in 32 women. Both negative samples tested negative for the presence of human β-globin gene indicating low-quality sample. There were no significant variations seen between histopathologic forms and the actual HPV infections ($\chi^2 = 3.54$, $p = 0.3$). HPV 16 and HPV 18 genotypes demonstrated a stable distribution during the study period, taking into consideration histopathologic types (Table I).

Table I: Identification of HPV/DNA in 83 endocervical specimens by cancer type

Cancer type	HPVDNA negative[a]	HPVDNA positive[b]	Overall total
Swabs from patients with squamous carcinoma	21	37	58
Swabs from patients with adenocarcinoma	10	14	24
Swabs from patients with adenosquamous	1	0	1
Total	32	51	83

[a]HPV HPV DNA not identified. These tests were negative for the human β-globin gene and were thus not suitable for HPV genotyping.
[b]Entirely positive human β-globin gene samples

Table II shows histologic distributions of HPV infections among study participants**.** Different HPV genotypes were detectable in 49 cervical swabs, but in two specific HPV types could not be genotyped (Type Z). Six distinct forms of HPV genotypes were detected:(HPV16, 18, 31, 33, 45 and 52). These manifested in 46 cervical swabs as single infections as well as in two scenarios as double HPV infections. Examination of the range of HPV genotypes within HPV positive samples revealed that HPV 16 and 18 were the most prevalent genotypes preceded in the decreasing order by HPV 33, 52, 31 and 45. In single type HPV infections, type 16 existed in 19 patients, type 18 in 10 patients, type 33 and 52 in 6 patients, type 31 in three patients, and type 45 in two patients.

Table II: Distribution of HPV genotypes in 51 women with invasive cervical cancer

HPV types	Patients with Squamous carcinomas	Patients with Adenocarcinomas	Overall total
	Infections with single HPV types		**48 (94.1%)**
	N (%)	N (%)	N (%)
HPV 16	17 (45.9)	2 (14.3)	19(37.3)
HPV 18	3 (8.1)	7 (50.0)	10 (19.6) a
HPV 31	3 (8.1)	0	3 (5.9)
HPV 33	5 (14.0)	1(7.1)	6 (11.8)
HPV 45	1 (2.7)	1 (7.1)	2 (3.9)
HPV 52	4 (10.8)	2(14.4)	6 (11.8)
Type Z	1 (2.7)	1 (7.1)	2 (3.9)
	HPV infection with multiple types		**3 (5.8%)**
HPV 16/18	2 (5.0)	0	2 (3.9)
HPV 18/33	1 (2.7)	0	1 (1.9)
Total	**37**	**14**	**51a**

The occurrence of genotypes 16/18 and 18/33 were considered as double HPV infections. When the study findings were limited to histopathologic HPV types, HPV 16 was the most prevalent in squamous cell carcinomas (45.9%), while type 18 was predominant in samples

from adenocarcinoma (50 %) patients. In samples from patients with squamous cell carcinomas, 12 women were found to have HPV type 16, 8 with genotype 18, five with type 33, three with type 31, four with type 52, three with type 31 and one with single HPV 45 infection. In samples from patients with adenocarcinoma, 7 patients had HPV type 16, two had type 18, and type 52 respectively, and one single infection occurred with type 33, and type 45 in each case (Table II).

Table III: Bivariate analysis using a logistic regression analysis for cervical cancer related factors associated with high-risk human papillomavirus (HPV) in women living in Eastern Uganda

Variables	Number of participants n (%)	Squa.cell carcinoma n (%)	Adenocarcinoma n (%)	Negative n (%)	Unadjusted Odds Ratio	(95% CI)	p-values
Age group (years)							
19-24	9(100.0)	1(11.1)	0(0.0)	8(88.9)	0.779	(0.272-2.233)	.642
25-34	25(100.0)	11(44.0)	7(28.0)	7(28.0)	0.412	(0.142-1.193)	.102
35-44	36(100.0)	16(34.4)	6(16.7)	14(38.9)	0.454	(0.136-2.084)	.542
45-5	13(100.0)	7(53.8)	3(23.1)	3(23.1)	1		
Marital Status							
Single	22(100.0)	4(18.2)	7(31.8)	11(50.0)	1.017	(0.255-4.055)	.981
Divorced	13(100.0)	11(84.6)	1(7.7)	1(7.7)	0.461	(0.085-2.516)	.371
Widow	35(100.0)	14(40.0)	6(17.1)	15(42.9)	7.877	(2.37326.144)	<.001*
Married	**13**(100.0)	6(46.2)	2(15.4)	5(38.4)	**1**		
Husband circumcised							
Yes	**32**(100.0)	12 (37.5)	6(18.8)	14(43.7)	20.222	(6.449-63.416)	<.0001*
No	**51**(100.0)	23(45.1)	10(19.6)	18(35.3)	**1**		
Contraceptive usage							
Yes	48(100.0)	24(50.0)	12(25.0)	12(25.0)	5.045	(1.943-13.100)	<.0001*
No	**35**(100.0)	11(31.4)	4(11.4)	20(57.2)	**1**		
History of STI							
Yes	46(100.0)	23(50.0)	10(21.7)	13(28.3)	0.730	(0.296-1.803)	.496
No	37(100.0)	12(32.4)	6(16.2)	19(51.4)	1		
HIV status							
Yes	49(100.0)	25(51.0)	9(18.4)	15(30.6)	0.375	(0.142-0.990)	.048*
No	34(100.0)	10(29.4)	7(20.6)	17(50.0)	1		

In logistic regression analysis, p≤0.05 is significant statistically, CI=confidence interval, p=probability

Table III shows Bivariate analysis for cervical cancer associated risk-factors associated with high-risk human papillomavirus (HPV) in women living in Eastern Uganda**.** When bivariate analysis for predictor variables associated with infection of the cervix was performed, the logistic regression results were as follows: age (OR = 0.412; 95% CI: 0.142-0.1.193; p < 0.05), marital status (OR = 7.877; 95% CI: 2.373-26.144; p < 0.05),husband circumcised (OR = 20.222; 95% CI: 6.449-63.416; p < 0.05), contraceptive usage (OR = 5.045; 95% CI: 1.943-13.100; p < 0.05), and HIV infection status (OR = 0.375; 95% CI: 0.142-0.990; p < 0.05), Significant statistical interactions (p < 0.05) with human papillomavirus (HPV) infections were detected (Table III).

Table IV shows Multivariate analysis for cervical cancer related risk-factors using logistic regression analysis with a stepwise forward approach. When the significant univariate predictor variables for coinfections of the cervix uteri with human papillomavirus (HPV) were subjected to multivariate analysis using logistic regression analysis, they had the following logistic regression values: circumcision (OR = 17.124; 95% CI: 5.257-55.780; $p < 0.05$), contraceptive usage (OR = 3.634; 95% CI: 1.122-11.777; $p < 0.05$) and HIV status (OR = 0.256; 95% CI: 0.069-0.945; $p < 0.05$) were found to have statistically significant relationships ($p < 0.05$) with acquisition of HPV infections (Table 4). However, age groups, education level, marriage condition, as well as history of STI were observed to have no major correlation with the acquisition of HPV infections.

Table IV: Multivariate analysis for cervical cancer related factors using logistic regression analysis with a stepwise forward approach

Factors in association cervical cancer	Adjusted odds ratio	95% CI	p-value
Circumcision	17.124	(5.257-55.780)	.0001
Contraceptive usage	3.634	(1.122-11.777)	.031
HIV Status	0.256	(0.069-0.945)	.041

In logistic regression analysis, p≤0.05 is significant statistically, CI=confidence interval, p=probability.

Discussion

This research was the very first large-scale hospital-based HPV-type examination amongst patients with cervical cancer aged 15 to 55 years in Eastern Uganda. This research identified the incidence of HPV types and factors correlated with cervical cancer development and their severity in patients receiving healthcare services at selected health facilities in Eastern Uganda. Apart from HPV infection, age of study participants, marital status, patient history of STIs, contraception usage, and HIV status were all health issues linked to advancement of precancerous lesions to cervical cancer.

In the current research, HPV/DNA was observed in 51 of 83 females with cervical cancer, giving a total HPV prevalence of 61.4% of the specimens. Our study compared well with other researchers conducted [20], Uganda (61.3%)[1], Kenya 64% [21], Ethiopia (67.1%) [22] and Zambia (69.9%) [9]. Higher HPV prevalence rates were reported in studies previously conducted in Uganda (71.8%)[23],Iran (79.59%)[24], Botswana (87.5%) [25],USA (91.3%) [26], Thailand (94.8%)[27], Malawi (97.0%) [28], and South Africa (92.1%)[29]. Nevertheless, comparatively low HPV prevalent rates have also been reported in Uganda (54.0%)[30], Khuzestan Iran (43.3%) [31] and Mexico (46.15%) [32]. These discrepancies recorded in HPV prevalence rates for cervical cancer may indeed be due to a variety of reasons, including regional variations [33–36], quality and quantity of biological specimens [35,37,38], methods of DNA extraction and sensitivity [24], and specificity of HPV detection methods [35,37,38].

In this study, a majority of infections were linked to single HPV types, with multiple HPV types occurring in two cases. HPV type 16 as well as type 18 were responsible for 56.9% of the infections, while HPV 33 and 52 predominated the majority of the remaining infections. In study participants with squamous cell malignancies, 54.0% of the cases were due to single HPV 16 and 18 infections while 64.3% of the cases were detected in patients with adenocarcinomas. Of special concern was the elevated percentage of squamous cell carcinomas (14.0%) and (10.8%) attributed to HPV 33 and 52 respectively. All infections in this study were variants of high-risk HPV.

Our study compared well with researches conducted [39] in Nigeria, in which the incident HPV infections was 69.8% in patients with cervical carcinoma, while the detected carcinogenic HPV16 and HPV18 occurred in 39.6% and 19.8% of the cases concurrently and 59.4% as combined infections. Similarly, in a study conducted [40] in south Italy, the incidence of HPV infections was reported to be at 57.9% and 94.1% among West African migrants, and among Italian migrants with normal and abnormal cytology ,the prevalence was reported at19.4% and 88.5% respectively, while carcinogenic HPV types, accounted for 73.4% of all infections, slightly higher than in our study. Keeping in line with our current study, was an American study [41] in which 22 females (46.8%) tested positive for HPV 16, 12 (25.5%) tested positive for HPV 18, and in 13 women

(27.7%) tested positive for 12 other high-risk HPV types.

In our current study, HPV type16 and type18 were reported as the leading etiological agents of uterine cancer and the combined incidence of both the two main HPV types 16 and type18 was 56.9%. HPV 16 was identified in 45.9 % of patients with squamous cell carcinomas, and HPV18 was reported in (50 %) of patients with adenocarcinomas. Keeping in line with our findings, was the study [6] in which HPV 16 appeared in 51.2 % and 28 % of patients with squamous carcinoma and adenocarcinoma singly, whereas HPV 18 occurred in 56 % of patients with adenocarcinoma and 12.1% in squamous-carcinoma, with a slightly lower incidence (45.9 %) of HPV 16 in patients with squamous cell carcinoma but a lower incidence (14.3 %) in patients with adenocarcinoma carcinoma. We also did not detect HPV type 31 in adenocarcinomas, implying that this type could be less frequently linked with an increased risk of adenocarcinomas which is in compliance with previous reports. Furthermore, HPV16 prevalence in squamous cell carcinoma in a meta-analysis of 85 studies [34] including 10,058 women with cervical cancer, varied from 46% in Asia to 63% in North America [34,42]. HPV18 was the second most frequent, with a prevalence of 10–14 % in squamous cell carcinoma patients. The percentage of patients with adenocarcinoma varied from 4% in Africa to 32% in North America. HrHPV type 18 was most prevalent in adenocarcinoma patients 37% to 41%, while HPV 16 and 45 were most prevalent in 26–36% and 5–7 % of the samples, respectively. When compared to other continents, Africans [40,43,44] have a higher prevalence of HPV infection than Europeans, with 26.3 % in Nigeria, 47.9 % in Guinea, 41% in South Africa, and 38.8–42.3 % in Kenya. The probable higher incidence of HPV amongst women in sub-Saharan African nations is attributed to the rising human immunodeficiency virus infection (HIV)[30] early age of sexual debut [23]and multiple sexual partners [34].

Remarkably, unexpected finding in the study was that 2 endocervical samples labelled HPV Z tested HPVDNA positive, but distinct HPV genotypes could not be identified. This inevitably led to the idea that some obscure HPV types might exist, or that some specific HPV types may have a broader range of probes, so we look forward to being able to genotype those samples in future studies. Earlier studies [1,20] found that in 10.2% and 9.6% of HPV-positive cases, specific HPV types could not be identified, and this deficiency was related to the narrow range of HPV-specific probes.

Infections with multiple HPVtypes were observed in only three positive HPV cases (5.8%) in our present analysis. This was relatively comparable to some earlier research in which the incidence ranged from 3.9% in Nigeria [29], 3.9% in Thailand [45], 4.1% in Brazil [46],6.1% in Nepal [47],7.49% in China [48],7.9% in Morocco [49], and 12.9% in women with squamous tumors in Peru [50]. Conversely, somewhat higher incidence has also been revealed by other studies, 32% in Costa Rica. [51], 32.8% in Kenya [21],36.6% in Portugal [52],36.9% in Turkey [53], 54.1% in Uganda [20],and 61.4% in Nigeria [39]. These variations may be attributed to the inherent occurrence of multiple diseases in different geographical regions [35,41] or variations in the specificity of the methodologies applied in identification of single and multiple HPV types in the study [35,37,38]. The inclusion of multiple HPV infections in cancer of the cervix uteri progression remains uncertain. Studies [51] reported that the likelihood of cervical carcinoma linked to HPV 16 infection is indeed equivalent or higher than that of infection with multiple HPV 16 and other HPV types. In another study [54] it was found out that cervical infections with multiple HPV types were correlated with an elevated risk of cervical carcinoma. While the majority of incidents in the present study was comparatively limited (5.8%), a great concern still emerges on whether to include all other HPV types in the subsequent HPV vaccine design. It is because various infections with other different HPV genotypes also account for 5% to 10% of cervical carcinogenesis [55].

Currently, three HPV vaccines that are commercially available include the bivalent (2vHPV; Cervarix, GSK, Rixensart, Belgium) and quadrivalent (4vHPV; Gardasil/Silgard, Merck, Kenilworth, NJ, USA) vaccines and a nonavalent (9vHPV; Gardasil9, Merck). Every vaccines aims at HPV 16/18, while 4vHPV vaccine aims at HPV 6/11 and 9vHPV shot includes 5 carcinogenic types 31/33/45/52/58 [15–17]. Surprisingly, these vaccines do not offer full protection from other categories of HPV genotypes that are still not in the current vaccine schedule [15,54]. Probably, the inclusion of all other HPV genotypes in the

future HPV vaccines will offer 100% protection in the pathogenesis of HPV related cancers. The newly launched nonavalent HPV vaccine was awarded commercial approval throughout the USA and Europe in mid-2015[39]. This vaccine is rated as a secure and reliable shot that would likely minimize the possibility of HPV incidence and HPV-related carcinogenesis. This might also shield non - vaccinated persons via herd immunity [39,56].

Factors associated with cervical cancer development besides HPV were linked to circumcision, contraceptive usage and underlying HIV status of the patients. Our current research found that in women with cervical cancer, the risk of a false negative HPV-test is higher when the husband is not circumcised (OR = 20.222; 95% CI: 6,449-63,416; p<0,05). These results were consistent with other research that showed that circumcision in men correlated with the protection from multiple sexually transmitted infections [57,58]. In addition, reports from the International Agency for Cancer Research (IARC) revealed that circumcised men both had a lower chance of HPV infection compared to uncircumcised males, as well as lower rate of HPV infection among their spouses and a reduced likelihood of cervical carcinogenesis[59]. The preventive role was much more evident in females whose husbands engaged in risky or aggressive sexual activity. Additional validation of the protective role stems from two previous randomized clinical trials in Uganda which showed that circumcision of young teenagers in rural Uganda had significantly reduced the prevalence of HPV by 35% [60,61]. There is a significant relationship between circumcision and risky sexual behavior. Conversely, given this close correlation, circumcised males are far less prone to be HPV-positive than uncircumcised males, which could mean that the positive effects of circumcision exceed the expected danger of risky sexual behavior[61].

In general, experiments[62] have shown that long-term use of hormonal contraceptives is linked to an elevated incidence to cancer of the cervix uteri and its progenitors, although this has not been noticeable in certain research findings [63]. Our recent research also showed that women on hormonal contraception were substantially at a high risk of developing cervical cancer (OR = 3.634; 95% CI: 1.122-11.777; p<0.05).

The research of Mosciski et al., further revealed that HIV infections might elevate the risk of cervical carcinoma pathogenesis through inflammatory response or micro-abrasion of cervical epithelium due to sexual activity, that might enable immediate gateway to the cervical epithelial cells . HIV is likely to increase the carcinogenesis of an already established HPV infection by an inflammatory response in the basal epithelium [65].This was in line with our current study, which showed a substantial statistical relationship (OR = 0.256; 95% CI: 0.069-0.945; p<0,05)between HIV positivity and false HPV negative results among the study participants. HIV disease is a significant predictor for cervical epithelial neoplasm [58,66,67] and invasive cervical carcinoma [67], even though some earlier reports showed no correlation[68]. Sufferers of HIV appear to develop complications with a wider range of HPV groups besides HPV 16, and are more likely to have multiple HPV infections at the same time.

Study limitations

There were quite a variety of advantages in our research. The system used is sensitive and had a high performance compared to other approaches using general primers in HPV genotyping [69]. Furthermore, drastic measures were put in place to prevent contaminants as well as strong standard operation procedures (SOP's) were enforced during the analysis. A few of the drawbacks in our analysis was indeed the very few samples tested. Because samples originated from various regional hospitals, the timeframe of DNA extraction and HPV genotyping were not standardized and may have led to certain samples being unfavorable for HPV genotyping. Among our laboratory analyses, no distinct distribution of HPV genotypes of the most common forms, nor a greater number of multiple infections were noticed.

Ethics approval and participation accord

Ethical clearance was granted by the combined Institutional Research and Ethics Committee (IREC) on Human Research (Approval No. 06/01-17) and the Uganda National Council for Science and Technology (Approval No. HS2246). The intent and credibility of the research was clarified before obtaining a signed agreement to participate from the study participants. For adults aged 18 years and above a written consent was obtained.

Also approved written consent for mature minors was obtained accordingly from the parents when the respondent was under18 years of age as recommended by the Ugandan national guidelines for research involving humans. This recommendation was ethically granted by Uganda National Council for Science and Technology which legally considers individuals aged 14-17 years as mature minors. Voluntary participation was emphasized and confidentiality of the information was maintained during the interviews and throughout the study by excluding personal identifiers from the data collection form.

Abbreviations

Pap, Papanicolaou; CCAs, cervical cytological abnormalities; CIN, Cervical Intraepithelial Neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; OCs, oral contraceptives; CIS, carcinoma in situ; OR: odds ratio; CI, confidence interval; TZ, transformation zone.

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The concept development, research methodology, implementation, collection of data, and evaluation, was solely carried by all the authors.

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Disclosure

The authors declare that they have no competing interest in this work.

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