Human papillomavirus prevalence among unvaccinated young female college students in Botswana: A cross-sectional study

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Background. Human papillomavirus (HPV) is a sexually transmitted infection and a causative agent of cervical cancer. It is common in adolescent girls and young women, and the majority of infections are transient and asymptomatic. In Botswana, there are currently no data on the HPV prevalence against which the impact of prophylactic HPV vaccines can be measured.
Objectives. To establish a baseline HPV prevalence in an unvaccinated cohort of young women.
Methods. Women aged ≥18 years were recruited from the University of Botswana between September 2016 and May 2020. Demographic and behavioural characteristics of participants were collected. Subsequently, cervicovaginal swabs were obtained and tested for HPV using polymerase chain reaction-restriction fragment length polymorphism. We determined the prevalent HPV types, and evaluated the risk factors associated with HPV positivity.
Results. A total of 978 young women were recruited. Overall, there were 589 (60.2%) participants with HPV infection and 12 (1.2%) with HIV. The median (interquartile range) age of the study participants was 19 (18 - 20) years. Multivariate logistic regression analysis showed that significant factors associated with HPV positivity were sexual activity (adjusted odds ratio (aOR) 2.06; 95% confidence interval (CI) 1.49 - 2.63; $p<0.001$), number of sex partners ≥3 (aOR 2.10; 95% CI 1.39 - 3.18; $p<0.001$), and smoking (aOR 2.00; 95% CI 1.26 - 3.20; $p=0.004$).
Conclusion. Our results demonstrate for the first time the prevalence of HPV in unvaccinated young women in Botswana. We found a high prevalence of HPV infection, with statistical differences with different risk factors. This finding supports the need for HPV vaccination strategies for females prior to sexual debut to reduce the future burden of cervical cancer in Botswana.

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Human papillomavirus (HPV) is the most common sexually transmitted pathogen. Its peak prevalence is observed in adolescent girls and young women soon after sexual debut,[1] and prevalence decreases with increasing age.[2] Most individuals will be infected with at least one HPV genotype during their lifetime.[3] The majority of HPV infections clear spontaneously within a couple of months.[4] However, viral persistence of cervical infection for over a year is strongly associated with an increased risk of cervical cancer.[5] Globally, the prevalence of HPV varies across populations, and higher HPV prevalence has been found in less developed regions.[5] In Africa, HPV prevalence ranges from 7% to 60%, without consideration of cytological findings.[6]

Bruni *et al.*[7] reported an HPV prevalence of 24% in women aged >25 years with normal cytology in sub-Saharan Africa. HPV prophylactic vaccines have been found to be highly effective in preventing HPV infection and pre-invasive and invasive cervical cancer.[8] The vaccine is routinely recommended to prepubertal girls and boys prior to sexual debut, as its efficacy is highest in HPV-naive individuals.[8] Currently, there are three prophylactic HPV vaccines rolled out internationally: bivalent, quadrivalent and nonavalent vaccines.[8,9] These three vaccines prevent 70 - 90% of the HPV-related cancers.[9] The bivalent vaccine is directed against HPV-16 and HPV-18, the quadrivalent vaccine protects against HPV-6, HPV-11, HPV-16 and HPV-18, while the nonavalent vaccine targets HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58.[10] The government of Botswana is committed to the prevention of cervical cancer through the HPV vaccination programme and expansion of the national cervical cancer screening programme.[11,12] The Botswana national HPV immunisation programme was initiated in 2015, using the

quadrivalent HPV vaccine as a two-dose schedule (0, 6 - 12 months schedule) through a class-based cohort for in-school girls. During the first year of implementation, girls aged 9 - 11 years (standards 5 - 7) were vaccinated, and over 90% coverage was achieved in the target group.[13,14] Subsequently, the programme followed a single class cohort (standard 5/age 9).

Studies have been carried out in Botswana to assess the prevalence of HPV genotypes in women with pre-invasive and invasive cervical cancer, with or without HIV infection.[15-17] However, there is no documentation of HPV prevalence in young women. Understanding the HPV prevalence is critical for evaluating public health initiatives such as the integration of HPV vaccination into cervical cancer prevention programmes. To address the knowledge gap, we present the HPV prevalence results from unvaccinated female students at the University of Botswana and possible related biological and behavioural risk factors.

Methods

Study population and sample collection

This prospective study was conducted in Gaborone, Botswana, from September 2016 to May 2020 on a convenience sample of unvaccinated undergraduate female students at the University of Botswana aged ≥18 years. Potential participants received information on HPV and study procedures. Study participants needed to show documentation of HIV status within 12 months (as per the Botswana national testing guidelines) to be eligible for the study. HIV counselling and testing were offered at the study site by trained research assistants. Young women who reported being pregnant were excluded from the study. Participants who consented to the study responded to a closed-ended questionnaire that inquired about their demographics, sexual practices, contraceptive use and smoking habits. The participants underwent a gynaecological examination by a nurse, and two cervicovaginal swabs were collected and stored in a phosphate-buffered saline solution at –80°C until processing.

Study procedures

DNA extraction

Total DNA from the cervicovaginal swab samples was extracted using the NucliSENS easyMAG (bioMérieux, France) automated platform. Briefly, 100 µL of the liquid sample was vortexed for 30 seconds and added to 100 µL of the lysis buffer and then incubated overnight at room temperature. Successively, 200 µL of lysed solution was added into the NucliSENS easyMAG disposable vessels. After 10 minutes' incubation, 120 µL of magnetic silica was added to each sample and DNA extraction was completed. DNA was eluted in 70 µL of NucliSENS easyMAG buffer 3. Sterile water aliquots were processed in parallel with specimens as negative controls for possible contamination. For quality assessment, DNA concentration in the extracts was determined using the NanoDrop 2000 (Thermo Scientific, USA), and subsequently 2 µL of DNA was used as a template for polymerase chain reaction (PCR).

HPV detection

Nested PCR assay was performed in two consecutive amplification reactions. For the outer PCR, an established protocol was adopted with slight modifications.[18,19] PCR was run in a total volume of 20 µL using consensus primers SB01/SB02 (inqaba biotech, SA) with $MgCl_2$ concentration adjusted to 2.5 mM. Outer PCR was performed on a Bio-Rad DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad, USA) with the following programme: 4 minutes at 95°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 56°C and 45 seconds at 72°C, with a final extension step at 72°C for 8 minutes.[19]

The second round of the PCR amplification (nested reaction) was carried out with consensus primers MY09/MY11 (inqaba biotech, SA).[20] The reaction was carried out in a total volume of 20 µL using 1 µL of outer-PCR amplicon, and the $MgCl_2$ concentration was adjusted to 2.5 mM. Thermal cycling conditions were adopted from Tawe *et al.* [19] and Chen *et al.*,[20] with some modifications: 10 minutes at 95°C, 32 cycles of 15 seconds at 95°C, 30 seconds at 52°C and 30 seconds at 72°C, with a final extension step at 72°C for 5 minutes. Positive and negative controls were included in all experiments and were run in parallel with the samples. Amplified reaction products underwent electrophoresis in 2.5% agarose gel. Positive and negative results were evaluated based on the presence of fragments of the expected band size (~450 base pair (bp)) as per the illustration in Chen *et al.*[20] HPV results were considered invalid if both controls were negative and no HPV was detected. All HPV-positive samples were typed by restriction fragment length polymorphism analysis according to Chen *et al.*[20] using restriction enzyme *HpyCH4V*. This allowed obtaining HPV genotypes information for nested PCR-positive samples. In particular, the technique adopted allows the easy detection of only HPV-16 and HPV-18. All the other HPV-positive, non-HPV-16 or HPV-18 infections were classified as *other* HPV genotypes, including low-risk and high-risk HPV genotypes into the same category. In parallel with HPV detection, the β-globin gene was also amplified to monitor sample adequacy, extraction and amplification using the primers PC04 (5′-CAACTTCATCCACGTTCACC-3′) and GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′)[21] (inqaba biotech, SA) amplifying a 268 bp fragment.

Statistical analysis

This analysis was restricted to participants with valid HPV detection results. Demographic variables included age (18, 19, 20, 21, ≥22 years) and marital status (single or married). Sexual and behavioural characteristics included being sexually experienced, lifetime number of sex partners (0, 1 - 2, ≥3), age of sexual debut (<18, ≥18 years), other sexually transmitted infection (yes/no), and smoking (yes/no). HPV genotypes were categorised as HPV-16, HPV-18, HPV-16 and *other*-HPV, HPV-18 and *other*-HPV, HPV-16/18 and *other*-HPV, and *other*-HPV.

Descriptive statistics were used to describe the demographic and behavioural characteristics of the study participants using medians (interquartile range (IQR)) for continuous variables (age and age of sexual debut), and frequencies with the corresponding percentages for categorical variables. The χ^2 or Fisher's exact tests were used to identify statistically significant differences in categorical variables stratified by HPV status ($p<0.05$). We determined the odds of HPV positivity with logistic regression analysis and reported both the unadjusted and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) and corresponding *p*-values. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 27 (IBM, USA).

Ethical considerations

The study was approved by the Institutional Review Board (IRB) at the University of Botswana (ref. no. UBR/RES/IRB1584), the Health Research Development Committee at the Botswana Ministry of Health and Wellness (ref. no. HPDME 13/18/1) and the University of Pennsylvania IRB (ref. no. 822837). Direct consent was obtained from the study participants, and they signed assent forms.

Results

Demographic details

A total of 997 participants were enrolled in the study, of whom 978 were included in the analysis. Twelve participants had β-globin-negative results, and 7 participants had no cervicovaginal swabs. The median (IQR) age of the study participants was 19 (18 - 20) years (Table 1). The majority of the study participants were sexually experienced (66.2%). About 1 in 20 of the study participants reported having had at least one episode of sexually transmitted infection (STI) other than HPV in their lifetime. Only 12 (1.2%) of the participants had HIV infection at baseline.

HPV status by demographics and behavioural characteristics

When stratified by sexual activity, HPV was detected in 435 (73.9%) of the sexually experienced group (n=647) and 154 (26.1%) of the sexually inexperienced group (n=331), and this was statistically significant (p<0.001) (Table 2). HPV infection was also frequently observed in participants with multiple sex partners (p<0.001), those who smoked (p<0.001), and those reporting STIs (p=0.004).

Table 1. Demographic and behavioural characteristics of study participants (*N*=978)

Age (years)	
Median (IQR)	19 (18 - 20)
18, *n* (%)	251 (25.7)
19, *n* (%)	366 (37.4)
20, *n* (%)	239 (24.4)
21, *n* (%)	73 (7.5)
≥22, *n* (%)	49 (5.0)
Marital status, *n* (%)	
Married	6 (0.6)
Single	972 (99.4)
HIV status, *n* (%)	
Positive	12 (1.2)
Negative	966 (98.8)
HPV status, *n* (%)	
Positive	589 (60.2)
Negative	389 (39.8)
Sexually experienced, *n* (%)	
Yes	647 (66.2)
No	331 (33.8)
Age of sexual debut (years)*	
Median (IQR)	19 (18 - 20)
<18, *n* (%)	102 (15.8)
≥18, *n* (%)	545 (84.2)
Lifetime number of sex partners, *n* (%)	
0	331 (33.8)
1 - 2	479 (49.0)
≥3	168 (17.2)
Other STI, *n* (%)	
Yes	53 (5.4)
No	925 (94.6)
Smoking, *n* (%)	
Yes	114 (11.7)
No	864 (88.3)

IQR = interquartile range; HPV = human papillomavirus; STI = sexually trnamitted infection.
*Among sexually experienced participants.

HPV partial genotypes

The most prevalent HPV category was that of *other*-HPV (44.0%). The prevalence of HPV-16 and *other*-HPV, or HPV-18 and *other*-HPV, was 8.0% and 3.0%, respectively, and that of combined HPV-16/18 and *other*-HPV was 4.0%. Single HPV-16 or HPV-18 was detected in 0.9% and 0.3% of the participants, respectively. No combined HPV-16/18 was identified.

Factors associated with HPV distribution

In bivariate analyses, the prevalence of any HPV was associated with being a smoker, being sexually experienced, having a higher number of sex partners, additional STI infections, and age 20 and 21 years (Table 3). In multivariable analyses, being sexually experienced, a higher number of sex partners and smoking remained significantly associated with any HPV prevalence (Table 3). The OR of any HPV prevalence in sexually experienced participants was 2.06 (95% CI 1.54 - 2.74) compared with those who were sexually inexperienced. Among the sexually experienced, the OR of any HPV prevalence was 2.10 in those reporting ≥3 sex partners compared with those reporting 1 - 2 partners (95% CI 1.49 - 2.63). Participants who smoked were twice as likely to have any HPV (OR 2.00; 95% CI 1.26 - 3.20) compared with the non-smokers.

Discussion

To our knowledge, this is the first study enrolling unvaccinated young women to provide new information on HPV prevalence taking into account the possible related biological and behavioural risk factors in Botswana. Although the overall HPV prevalence (60.2%) detected in the present study was high, it is comparable to other studies in sub-Saharan Africa.[22,23] Our findings were similar to those reported in South Africa (61.0%)[22] and Mozambique (63.6%).[23] Conversely, the prevalence was higher than that reported in another study carried out in Mozambique (28.6%)[24] among college students of comparable age. The reason for the prevalence variation could be different HPV detection assay methods, varying genotyping target sequence, amplicon size and sensitivity adopted in the present study and the study by Bule *et al.*[24]

We observed a significant association between sexual activity and the prevalence of HPV infections. Of self-declaring sexually experienced young women aged 18 - 20 years, 73.9% had any HPV infection. This is similar to several studies that have reported sexual activity as a risk factor for HPV infection.[1,25] Surprisingly, the prevalence of HPV among the sexually inexperienced was higher than expected (26.1%). A study in Tanzania showed a low HPV infection rate of 8.4% in sexually inexperienced participants.[25] Another study from India reported only 6% HPV infection in young sexually inexperienced girls aged 17 - 25 years.[26] The appreciable difference in our cohort may be due to lack of disclosure of previous sexual activity or no penetrative sexual behaviours. Previous studies in adolescents in different regions of the world consistently illustrate under-reporting of sexual behaviours.[27-29] In addition, vertical HPV transmission and other recognised non-sexual routes have been hypothesised in other studies.[30-33] Our findings support evidence that HPV infection can occur even before the first sexual intercourse. However, irrespective of reporting errors, detection of HPV in specimens collected from young women who report no previous vaginal sex remains important to understand the dynamic of HPV infection.

In our study population, smoking and number of sex partners were risk factors associated with increased risk for acquisition of HPV infection. Smoking has been shown to perpetuate premalignant

Table 2. HPV status by demographic and behavioural characteristics

	HPV positive (*n*=589), *n* (%)	HPV negative (*n*=389), *n* (%)	*p*-value*
Age (years)			0.067
18	134 (22.8)	117 (30.1)	
19	223 (37.9)	143 (36.8)	
20	149 (25.3)	90 (23.1)	
21	51 (8.7)	22 (5.7)	
≥22	32 (5.4)	17 (4.4)	
HIV status			0.557
Positive	6 (1.0)	6 (1.5)	
Negative	583 (99.0)	383 (98.5)	
Sexually experienced			<0.001
Yes	435 (73.9)	212 (54.5)	
No	154 (26.1)	177 (45.5)	
Age of sexual debut (years)†			0.140
<18	75 (17.2)	27 (12.7)	
≥18	360 (82.8)	185 (87.3)	
Lifetime number of sex partners			<0.001
0	154 (26.1)	177 (45.5)	
1 - 2	303 (51.5)	176 (45.2)	
≥3	132 (22.4)	36 (9.3)	
Other STI			0.004
Yes	42 (7.1)	11 (2.8)	
No	547 (92.9)	378 (97.2)	
Smoking			<0.001
Yes	88 (14.9)	26 (6.7)	
No	501 (85.1)	363 (93.3)	

HPV = human papillomavirus; STI = sexually transmitted infection.
*χ^2 or Fisher's exact tests.
†Among sexually experienced participants.

Table 3. Logistic regression analysis of factors associated with HPV positivity

	Bivariate			Multivariate		
	OR	95% CI	*p*-value	aOR	95% CI	*p*-value
Age (years)						
18	Ref.	-	-	Ref.	-	-
19	1.36	0.98 - 1.88	0.063	1.23	0.88 - 1.71	0.234
20	1.45	1.01 - 2.07	0.045	1.16	0.80 - 1.70	0.433
21	2.02	1.16 - 3.54	0.013	1.43	0.80 - 2.55	0.224
>22	1.64	0.87 - 3.11	0.127	1.21	0.63 - 2.34	0.567
Sexually experienced						
No	Ref.	-	-	Ref.	-	-
Yes	2.36	1.80 - 3.09	<0.001	2.06	1.54 - 2.74	<0.001
Age of sexual debut (years)*						
<18	Ref.	-	-	Ref.	-	-
≥18	0.70	0.44 - 1.13	0.141	0.78	0.48 - 1.27	0.320
Lifetime number of sex partners						
0	Ref.	-	-	-	-	-
1 - 2	1.98	1.49 - 2.63	<0.001	Ref.	-	-
≥3	4.21	2.75 - 6.46	<0.001	2.10	1.39 - 3.18	<0.001
Other STI						
No	Ref.	-	-	Ref.	-	-
Yes	2.64	1.34 - 5.19	0.005	1.59	0.83 - 3.06	0.161
Smoking						
No	Ref.	-	-	Ref.	-	-
Yes	2.45	1.55 - 3.88	<0.001	2.00	1.26 - 3.20	0.004

HPV = human papillomavirus; OR = odds ratio; CI = confidence interval; aOR = adjusted odds ratio; Ref. = reference values for statistical comparison; STI = sexually transmitted infection.
*Among sexually experienced participants.

lesions in the cervix, as tobacco smoke contains well-known carcinogens that could have a direct transformation effect on cervical tissues and/or cause immunosuppression, allowing HPV infection to persist and progress to cancer.[34,35] We also found significant associations between the overall risk of HPV infection and having multiple sex partners. These findings are concordant with reported results for adolescents and adult women in large population studies.[36,37] Several studies have indicated the significant association between STI and the increased risk of HPV acquisition.[38,39] However, this was not observed in the present study, which could have been due to the inherent inaccuracy in self-reported sexual risks such as STI symptoms.

Study limitations

This study had some limitations. It was carried out at one site, the University of Botswana, and used convenience sampling, which makes the results less generalisable to other young females in other parts of the country. However, the study population can still be considered diverse, as students come from all over the country to attend this major Botswana university. Participants self-reported their sexual history, which could have limited the accuracy and validity of the study data. Furthermore, the study only identified HPV-16 and HPV-18. Any other HPV-positive genotypes were classified as *other*, and this did not differentiate between *other* high-risk HPV and *other* low-risk HPV genotypes. We could not conclude whether HIV drives the distribution of the HPV among young women, because the HIV prevalence in this cohort was low. Despite these limitations, these data provide an important baseline with which to compare future changes in overall and type-specific HPV-16 and 18 prevalence after vaccination.

Conclusion

We demonstrate for the first time a significantly high prevalence of HPV infection in a diverse sample of unvaccinated young women in Botswana. These findings support current recommendations of vaccination before sexual debut in order to prevent HPV acquisition to reduce HPV-related morbidity. However, further studies are warranted to monitor HPV prevalence and distribution among HPV-vaccinated and unvaccinated adolescents and young women and men to monitor the impact of HPV vaccination in Botswana.

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Conflicts of interest. None.

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