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Human papillomavirus prevalence, genotype distribution and HPV16 variants among young women and men in Maputo city, Mozambique

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Abstract

Objectives

Human papillomavirus (HPV) is a well-known cause of cervical cancer, the 2nd most frequent cancer in female African populations. This study determined the prevalence of HPV infection and the genotype distribution in young adults aged 18-24, in Maputo city, Mozambique, a country with the 2nd highest incidence rate of cervical cancer in the world.

Methods

This cohort study was conducted between 2009 and 2010 at a youth clinic in Maputo Central Hospital. Cervical and urethral samples were obtained from 236 women and 176 men. Demographic and behavioural data were collected using structured questionnaires. HPV genotyping was performed for 35 different high, probably or possibly high and low-risk HPV types using the Clart® Human Papillomavirus 2. Major HPV16 variants were determined by pyrosequencing.

Results

HPV prevalence was 168/412 (40.8%; 95%CI: 36.0-45.5) and was significantly higher in women than in men (63.6% vs 10.2%). HPV52 was the most frequent type found in women, followed by HPV35, -16, -53, -58, -6, and -51. In men, HPV51 ranked the highest, followed by HPV6, -11, -52, -59, and -70. HIV infection and sexual debut before 18 years of age were associated with multiple HPV infections (OR 3.03; 95%CI: 1.49-6.25 and OR 6.03; 95%CI: 1.73-21.02, respectively). African 2 was the most frequently identified HPV16 variant lineage (68.4%), followed by North-American/Asian-American (15.8%), European-Asian (10.5%) and African 1 (5.6%). The 9-valent HPV vaccine would cover 36.8% of the high-risk genotypes circulating in women in this study, compared to 26.3% and 15.8% coverage by the bivalent and quadrivalent vaccines, respectively.

Conclusion

This study confirmed the high burden of HPV infections in young women. The HPV prevalence was associated with high-risk sexual behaviour. Sexual education and STI prevention interventions should be intensified in Maputo city, Mozambique. The delivery of an HPV vaccine should be prioritized, particularly for young females.

Strengths and limitations

- This is the first study to describe the Human Papillomavirus (HPV) prevalence and genotype distribution in young adults aged 18-24 in Mozambique as well as to describe the HPV16 variant lineages circulating in this population
- This study provides insights on the HPV types circulating in young populations, in Maputo city, which is an important public health information particularly for discussions on HPV vaccine introduction in the country
- The lower HPV prevalence in the male population could have been related to the site of sample collection, therefore additional studies in male population, using different anatomic collection sites should be considered.

Key messages

- There is a high burden of HPV infection in young women, in Maputo city, Mozambique
- High-risk sexual behavior was demonstrated to be associated with high prevalence of HPV infection
- There is a need to intensify sexual education and STI prevention for young adults
- The delivery of an HPV vaccine should be prioritized, particularly in young females

Introduction

Globally, cervical cancer (CC) is the 4th most common cancer in women and the 2nd most common cancer in female African populations[1]. More than 3/4th of cases occur in less developed areas. Eastern Africa is the most affected region with an age-standardized incidence rate of 42.7%. Mozambique has the 2nd highest incidence of CC after Malawi[2].

Human papilloma virus (HPV) infection has been implicated in virtually all CC cases. To date, 201 different HPV types have been identified[3]. More than 40 of these types can infect the anogenital tract and have been classified as low-risk (lrHPV6,-11,-40,-42,-43,-44,-54,-61,-62,-71,-72,-81,-83,-84, and -89), probably or possibly high-risk (pHR-HPV26,-30,-34,-53,-66,-67,-68,-69,-70,-73,-82,-85, and -97) and high-risk HPV types (hr-HPV16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58, and -59) depending on their ability to lead to malignant progression[4-6].

Worldwide, HPV16 has been responsible for more than half (54.4%) of the CC cases followed by HPV18 (16.5%), HPV58 (5.1%), HPV33 (4.7%), HPV45 (4.4%), HPV31 (3.6%), HVP52 (3.4%) and HPV35 (1.9%)[7]. However, only a small proportion of women infected with these HPV types develop the disease. This suggests that intra-type variations are associated with viral persistence and the development of cervical neoplasia[8]. Four major HPV16 variant lineages have been identified thus far according to their geographic distribution as follows: (a) the European-Asian (EAS), (b) the African 1 (AFR1), (c) the African 2 (AFR2), and (d) the North-American (NA) and Asian-American (AA; NA/AA). In sub-Saharan Africa, AFR1a and AFR2 variants are the most frequently found[9].

Currently, the following three HPV vaccines are available: Gardasil® (which confers protection against HPV6,-11,-16, and -18 and cross-protection against HPV31); Cervarix® (which prevents against HPV16 and -18 and provides high and moderate cross-protection against HPV31,-45 and -33, respectively); and Gardasil®9 (which protects against HPV6,-

11,-16,-18,-31,-33,-45,-52, and -58). Gardasil®9 is a second generation 9-valent vaccine that can theoretically prevent 87% of all hrHPV infections in Africa[10]. Nonetheless, the HPV distribution varies between African regions, and the identification of country-specific profiles and their adequacy to the currently available vaccines still remains of great importance when deciding on vaccine introduction.

In the present study, we aimed to (1) determine the prevalence and distribution of HPV infections in sexually active young adults; (2) describe the frequency of HPV16 variants; and (3) determine the suitability of the current HPV vaccines in the context of the Mozambican epidemic.

Subjects and Methods

Ethics statement

Ethical approval was granted by the National Health Bioethics Committee of Mozambique (Ref. 148/CNBS of May 8, 2009 and Ref. 18/CNBS/11). Study investigators followed the GCP-ICH guidelines. Subjects signed an informed consent form prior to any study activities.

Study design and population

This was a cross-sectional ancillary study of a human immunodeficiency virus (HIV) incidence study conducted at a youth clinic in Maputo city, Mozambique, from August 2009 to October 2011[11]. In the parent study, briefly, 1,380 males and females aged 18–24 years were screened for HIV, syphilis and hepatitis B virus. HIV-seronegative subjects were enrolled and followed for one year with quarterly visits to the clinic for assessment of HIV serostatus. Baseline demographic and behavioural data were collected using structured questionnaires. In the present study, samples from male and female subjects were collected at

one time point, at screening or one of the follow-up visits. Cervical samples were collected in female participants via speculoscopy, using a Rovers® Viba-Brush (Rovers Medical Devices B.V., Oss., The Netherlands). Urethral samples were collected from male participants by gently inserting a cotton swab approximately 2–4 cm in the urethral meatus and rotating it in one direction. Both the brushes and swabs were immerged in 5 mL of SurePath cell-preservation solution (TriPath Imaging, Burlington, NC, USA), transported to the study laboratory within the same day of collection and stored at +4 ºC to +8ºC for three months and then at -80ºC.

Laboratory testing

DNA was extracted from an initial sample volume of 1.5 mL using a QIAamp DNA Mini Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions and eluted to a final volume of 200 µl.

HPV genotyping was performed using the Clart® Human Papillomavirus 2 (Genomica, Madrid, Spain), a low-density microarray platform based on PCR amplification of a 450 bp fragment within an HPVL1 highly conserved region from 35 different HPV types (HPV6,-11,-16,-18,-26,-31,-33,-35,-39,-40,-42,-43,-44,-45,-51,-52,-53,-54,-56,-58,-59,-61,-62,-66,-68,-70,-71,-72,-73,-81,-82,-83,-84,-85, and -89) and a human gene control (CTFR). Individual genotyping results were analysed on the Clinical Array Reader (Genomica, Madrid, Spain). Adequacy of samples was assessed by amplification of the CTFR. Samples with undetectable DNA were rerun and the second result was considered final.

HPV16 variant determination was performed by analysing 7 positions; nucleotides 109, 131, 132, 143, 145, 178 and 350, in the E6 gene (reference sequence NC_001526) using PCR followed by pyrosequencing as described elsewhere[12]. Sequencing was performed with a

PSQ 96 MA system (Qiagen, Hilden, Germany) using PyroMark Gold Q96 reagents (Qiagen, Hilden, Germany). PSQ 96 MA Software was used to analyse the results.

HIV testing was previously described in the parent study[11].

Statistical analysis

Demographic and risk behavioural data were entered into a MySQL database version 5.1 (MySQL AB, 2008) and HPV laboratory results in a Microsoft Office Excel 2010 spreadsheet (Microsoft, Redmond, WA). For processing and analysis, data were imported into Stata version 14 (StataCorp. 2015. Stata: Release 14. Statistical Software. College Station, TX: StataCorp LP). Descriptive statistics were employed as follows: frequencies for categorical variables, and means and standard deviations (SD) for quantitative variables. The confidence intervals (CI) and p-values of the proportions are from exact estimation.

Here, we report the prevalence of HPV infections per socio-demographic and behavioural characteristics. Given that multiple HPV genotypes were identified per participant, we first grouped the participants as follows: a) subjects without an HPV infection; b) subjects with one HPV infection (mono-infection); and c) subjects with at least two HPV infections (multiple infections), and we then analysed the association with potential risk factors through a multinomial logistic regression (MNLR). The MNLR per level of the factor under consideration provided two odds-ratios (one for mono-infected versus not-infected subjects and another for the subjects with multiple-infections versus not-infected subjects). We report both the unadjusted and adjusted odds-ratios and their respective 95%CI. For adjustment, we included factors whose p-values through the global likelihood ratio test were below 0.2 in the univariate analysis; gender and age were mandatory factors to include in the model. The significance level was set at 5%.

Results

In total, 489 participants (263 females and 226 males) were enrolled, but samples from 77 (15.7%) subjects had undetectable DNA levels and were excluded from the data analysis. Of those samples, 27 (35.1%) were from females and 50 (64.9%) from males.

Baseline demographic and behavioural characteristics

Table 1A shows the baseline characteristics of the study population. Of the 412 subjects with valid HPV results, 236 (57.3%) were females, and 176 (42.7%) were males. The mean age of study participants was 21.1 years (SD: 1.71) with males being slightly older than females (21.4 vs 20.8). Almost all participants were students (94.4%) and all had some formal education, with half having completed a primary or secondary level. Nearly all participants were single (98.8%). The median age at sexual debut was 17 years (interquartile range: 15–18). The majority of subjects (89.3%) had more than one sexual partner in life with 24% reporting two or more sexual partners in the last 6 months. Around one-fourth of the study population reported having at least one episode of sexually transmitted infection (STI) in life and only 66% reported having used a condom at the time of their last sexual intercourse. HIV infection was diagnosed in 21 (5.1%) participants, of which 17 (81.0%) were females.

Table 1A - Baseline socio-demographic and behavioral characteristics: OR from Univariate Multinomial Logistic Regression

	Total		HPV negative		HPV mono-infection			HPV multiple-infections			Total HPV		Mono-infection vs Negative			Multiple-infections vs Negative			p
	N	%	N	%	N	%	Prevalence (%)	N	%	Prevalence (%)	N	Prevalence (%)	OR	CI 95%	p	OR	CI 95%	p	
Total	412		244		75		18.2%	93		22.6%	168	40.8%							
Gender																			
Male	176	42.7%	158	64.8%	13	17.3%	7.4%	5	5.4%	2.8%	18	10.2%	1.00	-	< 0.001	1.00	-	< 0.001	< 0.001
Female	236	57.3%	86	35.2%	62	82.7%	26.3%	88	94.6%	37.3%	150	63.6%	8.76	4.56 - 16.83		32.33	12.65 - 82.66		
Age (years)																			
Mean (SD)	21.1 (1.71)		21.3 (1.72)		20.7(1.62)		18.1%*	20.7 (1.64)		22.4%*		40.5%*	0.80†	0.69 - 0.94	0.006	0.80†	0.69 - 0.92	0.002	0.001
Marital Status																			
Single	407	98.8%	242	99.2%	74	98.7%	18.2%	91	97.8%	22.4%	165	40.5%	1.00	-	0.690	1.00	-	0.332	0.632
Married/Cohabitating	5	1.2%	2	0.8%	1	1.3%	20.0%	2	2.2%	40.0%	3	60.0%	1.64	0.15 - 18.29		2.66	0.37 - 19.16		
Education																			
Primary and Secondary	213	51.7%	117	48.0%	43	57.3%	20.2%	53	57.0%	24.9%	96	45.1%	1.00	-		1.00	-		
Technical training	109	26.5%	61	25.0%	22	29.3%	20.2%	26	28.0%	23.9%	48	44.0%	0.98	0.54 - 1.79	0.951	0.94	0.54 - 1.65	0.832	0.040
University grade	90	21.8%	66	27.0%	10	13.3%	11.1%	14	15.1%	15.6%	24	26.7%	0.41	0.19 - 0.87	0.021	0.47	0.24 - 0.91	0.025	
Occupation																			
Student	388	94.4%	229	93.9%	70	93.3%	18.0%	89	95.7%	22.9%	159	41.0%	1.00	-		1.00	-		
Employed	23	5.6%	14	5.7%	5	6.7%	21.7%	4	4.3%	17.4%	9	39.1%	1.17	0.41 - 3.36	0.773	0.74	0.24 - 2.29	0.596	0.784
Missing	1	0.2%	1	0.4%	0	0.0%	0.0%	0	0.0%	0.0%	0	0.0%							
Religion																			
Christian	375	91.0%	224	91.8%	68	90.7%	18.1%	83	89.2%	22.1%	151	40.3%	1.00	-	0.757	1.00	-	0.463	0.764
Other	37	9.0%	20	8.2%	7	9.3%	18.9%	10	10.8%	27.0%	17	45.9%	1.15	0.47 - 2.84		1.35	0.61 - 3.00		

Age at sexual debut																		
Less than 18	285	69.2%	156	63.9%	52	69.3%	18.2%	77	82.8%	27.0%	129	45.3%	1.00	-	0.490	1.00	-	0.001	0.003
18 or more	122	29.6%	84	34.4%	23	30.7%	18.9%	15	16.1%	12.3%	38	31.1%	0.82	0.47 - 1.44		0.36	0.20 - 0.67		
Missing	5	1.2%	4	1.6%	0	0.0%	0.0%	1	1.1%	20.0%	1	20.0%							
Number of sex partners in life																		
0 - 1	44	10.7%	22	9.0%	10	13.3%	22.7%	12	12.9%	27.3%	22	50.0%	1.00	-	0.279	1.00	-	0.292	0.424
> 1	368	89.3%	222	91.0%	65	86.7%	17.7%	81	87.1%	22.0%	146	39.7%	0.64	0.29 - 1.43		0.67	0.32 - 1.41		
Number of sex partners in the last 6 months																		
0 - 1	313	76.0%	180	73.8%	61	81.3%	19.5%	72	77.4%	23.0%	133	42.5%	1.00	-	0.185	1.00	-	0.491	0.369
> 1	99	24.0%	64	26.2%	14	18.7%	14.1%	21	22.6%	21.2%	35	35.4%	0.65	0.34 - 1.23		0.82	0.47 - 1.44		
Condom use in the last sexual intercourse																		
No	139	33.7%	74	30.3%	32	42.7%	23.0%	33	35.5%	23.7%	65	46.8%	1.00	-	0.051	1.00	-	0.376	0.143
Yes	272	66.0%	169	69.3%	43	57.3%	15.8%	60	64.5%	22.1%	103	37.9%	0.59	0.35 - 1.00		0.80	0.48 - 1.32		
Missing	1	0.2%	1	0.4%	0	0.0%	0.0%	0	0.0%	0.0%	0	0.0%							
Had a STI in life																		
No	305	74.0%	195	79.9%	48	64.0%	15.7%	62	66.7%	20.3%	110	36.1%	1.00	-	0.004	1.00	-	0.008	0.003
Yes	105	25.5%	47	19.3%	27	36.0%	25.7%	31	33.3%	29.5%	58	55.2%	2.33	1.32 - 4.12		2.07	1.21 - 3.55		
Missing	2	0.5%	2	0.8%	0	0.0%	0.0%	0	0.0%	0.0%	0	0.0%							
HIV infection																		
Positive	21	5.1%	7	2.9%	1	1.3%	4.8%	13	14.0%	61.9%	14	66.7%	0.46	0.06 - 3.79	0.468	5.50	2.12 - 14.27	< 0.001	< 0.001
Negative	391	94.9%	237	97.1%	74	98.7%	18.9%	80	86.0%	20.5%	154	39.4%	1.00	-		1.00	-		

* Prevalence at overall mean age (21.1 years)

† Associated effect of 1 year increase

HPV prevalence and genotype distributions

The distribution of HPV infections is presented in Figure 1 and 2 and Table 2. The overall HPV prevalence was 40.8% (168 infected subjects; 95%CI: 36.0–45.5) and was significantly higher in women than in men [150/236 (63.6%) vs 18/176 (10.2%), p<0.001]. Almost half, 75/168 (44.6%), of infected subjects had a single HPV infection, 48/168 (28.6%) had two concurrent infections and 45/168 (26.8%) had three or more HPV co-infections. The HPV prevalence among HIV infected subjects (14/21, 66.7%) was 69% higher than in the HIV negative subjects (154/391, 39.4%; p=0.013).

Overall, HPV52 was the most frequent type found (9.1%), followed by HPV35,-6,-16,-53,-58, and -51. Altogether, these HPV types accounted for 49.9% of the infections in women and men. In female participants, the most common genotypes were similar to the overall distribution (HPV52,-35,-16,-53,-58,-6, and -51) and accounted for half (50.3%) of the infections in women (Figure 2A). In contrast, male participants´ HPV infections were less diverse with HPV51 being the most frequent type found, followed by HPV6,-11,-52,-59, and -70 in equal proportions. These six types were responsible for 52% of the infections in men (Figure 2B). High-risk and pHR-HPV types were present in 124/150 (82.7%, 95%CI: 75.6-88.4) and 14/18 (77.8%, 95%CI: 52.4-93.6) HPV infected women and men, respectively (p=0.533).

High-risk HPV genotypes were identified in 11/21 (52.4%) of HIV-infected subjects. Three hrHPVs (HPV35,-52, and -58) accounted for 60% of all hrHPV infections in this population. There were no differences in the number of subjects infected with hrHPVs and pHR-HPVs in the HIV infected (p=0.552) and uninfected populations (p=1.000). Contrarily, lrHPVs were more frequently observed in the HIV-infected subjects (p=0.022).

HPV16 variant testing

All 24 HPV16 positive samples were pyrosequenced to determine HPV16 variants and were classified according to Cornet et al. [9]. Five samples had invalid results and were not included in the analysis. Of the 19 samples with valid results, 18 were from females, and one was from a male participant. In total, the following four HPV16 variant branches were identified: a) the AFR2 (13/19, 68.4%); b) the NA/AA (3/19, 15.8%); c) the EAS (2/19, 10.5%) and d) the AFR1 (1/19, 5.3%).

Risk factors for HPV infection

Women were significantly more at risk of having an HPV infection than men (p<0.001). Although the odds of being infected with HPV reduced by 80% for each year of age, in the multivariate analysis this was not significant (Table 1B). Participants with higher educational degree had less HPV infections (p=0.04). HIV infection and sexual debut before the age of 18 were associated with multiple HPV infections in the univariate and multivariate analysis (p=0.003 and p=0.008, respectively). Subjects reporting at least one episode of STI in life had higher odds of being infected with HPV. Condom use in the last sexual intercourse was not significantly associated with protection against HPV infection (p=0.143).

Table 1B. Baseline socio-demographic and behavioral characteristics: adjusted OR from the Multinomial Logistic Regression

	Mono-infection vs Negative			Multiple-infections vs Negative			p
	OR	CI 95%	p	OR	CI 95%	p	
Total Observations	406						
Gender							
Male	1.00	-	< 0.001	1.00	-	< 0.001	< 0.001
Female	7.23	3.64 - 14.33		28.38	10.78 - 74.70		
Age (years)							
Mean (SD)	0.85†	0.71 - 1.02	0.080	0.87†	0.72 - 1.04	0.117	0.134
Education							
Primary and Secondary	1.00	-		1.00	-		
Technical training	1.42	0.71 - 2.83	0.324	1.77	0.87 - 3.61	0.117	0.246
University grade	0.59	0.26 - 1.33	0.202	0.84	0.38 - 1.85	0.661	
Age at sexual debut							
Less than 18	1.00	-	0.774	1.00	-	0.002	0.008
18 or more	0.91	0.48 - 1.72		0.33	0.16 - 0.67		
Condom use in the last sexual intercourse							
No	1.00	-	0.492	1.00	-	0.467	0.423
Yes	0.81	0.45 - 1.48		1.25	0.68 - 2.31		
Had a STI in life							
No	1.00	-	0.260	1.00	-	0.620	0.530
Yes	1.44	0.76 - 2.72		1.18	0.62 - 2.24		
HIV infection							
Positive	0.44	0.05 - 4.04	0.471	6.03	1.73 - 21.02	0.005	0.003
Negative	1.00	-		1.00	-		

† Associated effect of 1 year increase

Vaccine-associated HPV genotypes

Table 2 shows the distribution of HPV genotypes in women and men, and their correspondence to the genotypes present in the current HPV vaccines. Genotypes associated with vaccine cross-protection were included in the analysis and considered as covered by the vaccine. The total number of circulating HPV genotypes matching the vaccine genotypes were 5/33 (15.2%; Gardasil®: HPV6,-11,-16,-18, and -31), 5/33 (15.2%; Cervarix®: HPV16,-18,-31,-33, and -45) and 9/33 (27.3%; Gardasil®9: HPV6,-11,-16,-18,-31,-33,-45,-52, and -58), for the female population, and 4/18 (22.2%; Gardasil®: HPV6,-11,-16, and -31), 2/18 (11.1%; Cervarix®: HPV6 and -31) and 6/18 (33.3%; Gardasil®9: HPV6,-11,-16,-31,-52, and -58) for the male population. The three vaccines can cover 3/19 (15.8%; Gardasil®: HPV16,-18, and -31), 5/19 (26.3%; Cervarix®: HPV16,-18,-31,-33, and -45) and 7/19 (36.8%; Gardasil®9: HPV16,-18,-31,-33,-45,-52, and -58) hrHPVs circulating in women and 2/12 (16.7%; Gardasil®: HPV16 and -31), 2/12 (16.7%; Cervarix®: HPV16 and -31) and 4/12 (33.3%; Gardasil®9: HPV16,-31,-52, and -58) in men.

Table 2. HPV genotypes distribution and vaccine associated genotypes

	Total	Gender		HIV Serostatus		HPV genotype coverage		
		Male	Female	Negative	Positive	Gardasil®	Cervarix®	Gardasil®9
Total Subjects	412	176	236	391	21			
Number of HPV infections	351	25	326	305	46			
Subjects with at least 1 HPV infection[a]	168 (40.8)	18 (10.2)	150 (63.6)	154 (39.4)	14 (66.7)			
Subjects with High-Risk HPV infections[b]	115 (27.9)	10 (5.7)	105 (44.5)	104 (26.6)	11 (52.4)			
HPV 52	32 (7.8)	2 (1.1)	30 (12.7)	28 (7.2)	4 (19.0)			++++
HPV 35	27 (6.6)	1 (0.6)	26 (11.0)	22 (5.6)	5 (23.8)			
HPV 16	24 (5.8)	1 (0.6)	23 (9.7)	22 (5.6)	2 (9.5)	++++	++++	++++
HPV 58	24 (5.8)	1 (0.6)	23 (9.7)	21 (5.4)	3 (14.3)			++++
HPV 51	20 (4.9)	3 (1.7)	17 (7.2)	19 (4.9)	1 (4.8)			
HPV 59	13 (3.2)	2 (1.1)	11 (4.7)	13 (3.3)	0 (0.0)			
HPV 18	11 (2.7)	0 (0.0)	11 (4.7)	10 (2.6)	1 (4.8)	++++	++++	++++
HPV 31	6 (1.5)	1 (0.6)	5 (2.1)	5 (1.3)	1 (4.8)	++++[ρ]	++++[§]	++++
HPV 33	4 (1.0)	0 (0.0)	4 (1.7)	2 (0.5)	2 (9.5)		++++[ρ]	++++
HPV 39	4 (1.0)	0 (0.0)	4 (1.7)	4 (1.0)	0 (0.0)			
HPV 56	4 (1.0)	1 (0.6)	3 (1.3)	4 (1.0)	0 (0.0)			
HPV 45	2 (0.5)	0 (0.0)	2 (0.8)	1 (0.3)	1 (4.8)		++++[§]	++++
Total number of infections[c]	112 (31.9)	9 (36.0)	103 (31.6)	151 (49.5)	20 (43.5)			
Subjects with Probably or Possibly High-Risk HPV infections[b]	54 (13.1)	5 (2.8)	49 (20.8)	50 (12.8)	4 (19.0)			
HPV 53	24 (5.8)	1 (0.6)	23 (9.7)	22 (5.6)	2 (9.5)			
HPV 70	12 (2.9)	2 (1.1)	10 (4.2)	11 (2.8)	1 (4.8)			

HPV 66	10 (2.4)	1 (0.6)	9 (3.8)	9 (2.3)	1 (4.8)		
HPV 82	6 (1.5)	1 (0.6)	5 (2.1)	6 (1.5)	0 (0.0)		
HPV 68	3 (0.7)	0 (0.0)	3 (1.3)	3 (0.8)	0 (0.0)		
HPV 73	2 (0.5)	0 (0.0)	2 (0.8)	2 (0.5)	0 (0.0)		
HPV 26	1 (0.2)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)		
HPV 85	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Total number of infections[c]	1 (0.3)	0 (0.0)	1 (0.3)	54 (17.7)	4 (8.7)		
Subjects with Low-Risk HPV infections[b]	89 (21.6)	7 (4.0)	82 (34.7)	79 (20.2)	12 (57.1)		
HPV 6	24 (5.8)	2 (1.1)	22 (9.3)	21 (5.4)	3 (14.3)	++++	++++
HPV 62	14 (3.4)	1 (0.6)	13 (5.5)	11 (2.8)	3 (14.3)		
HPV 61	13 (3.2)	0 (0.0)	13 (5.5)	11 (2.8)	2 (9.5)		
HPV 84	12 (2.9)	1 (0.6)	11 (4.7)	10 (2.6)	2 (9.5)		
HPV 42	11 (2.7)	0 (0.0)	11 (4.7)	10 (2.6)	1 (4.8)		
HPV 81	10 (2.4)	0 (0.0)	10 (4.2)	7 (1.8)	3 (14.3)		
HPV 11	8 (1.9)	2 (1.1)	6 (2.5)	6 (1.5)	2 (9.5)	++++	++++
HPV 83	8 (1.9)	1 (0.6)	7 (3.0)	7 (1.8)	1 (4.8)		
HPV 54	7 (1.7)	0 (0.0)	7 (3.0)	4 (1.0)	3 (14.3)		
HPV 40	6 (1.5)	0 (0.0)	6 (2.5)	5 (1.3)	1 (4.8)		
HPV 44	3 (0.7)	0 (0.0)	3 (1.3)	3 (0.8)	0 (0.0)		
HPV 71	2 (0.5)	0 (0.0)	2 (0.8)	2 (0.5)	0 (0.0)		
HPV 72	2 (0.5)	0 (0.0)	2 (0.8)	1 (0.3)	1 (4.8)		
HPV 43	1 (0.2)	1 (0.6)	0 (0.0)	1 (0.3)	0 (0.0)		
HPV 89	1 (0.2)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)		
Total number of infections[c]	122 (34.8)	8 (32.0)	114 (35.0)	100 (32.8)	22 (47.8)		

++++ Genotype covered by the vaccine

++++§ Genotype highly covered through vaccine cross-protection

++++p Genotype moderately covered through vaccine cross-protection

[a]The denominator is total subjects

[b]The denominator is total subjects

[c]The denominator is number of HPV infections

Discussion

This was a cross-sectional study reporting the prevalence of HPV infections and genotype distributions in young adults aged 18–24 in Maputo city, Mozambique. The HPV prevalence in the female population was 63.3% and was similar to previous reports of 75.9% in women aged 14–61 years in rural Southern Mozambique and other neighbouring countries[13-15]. The HPV prevalence in the male population was 10.2%, which was lower than previous reports for African countries and other regions[2]. Although no significant difference was observed in the number of hrHPV infections between women and men, a high susceptibility of women to HPV infections was demonstrated. This finding should be interpreted with caution since we have reported a lower prevalence of HPV infections in men, which is contrary to what has been shown in neighbouring countries and other regions.

Previous studies assessing different male anatomic collection sites have shown that HPV detection in urethral samples was lower when compared to other sampling sites in the male genitalia[16 17]. We found 50/226 (22.1%) urethral samples with undetectable DNA and 158/176 (89.8%) of valid samples tested negative. The first could be related to an inadequate sampling technique and the second to the selected anatomic collection site. Altogether, this could have contributed to the lower male HPV prevalence found in our study.

Worldwide, the prevalence of cervical HPV infections in healthy women has been demonstrated to be 11.7%. Nonetheless, in less developed regions, such as Eastern Africa, the regional prevalence is three times the global figures (33.6%)[18]. High-risk HPV16,-18,-52,-31,-58,-39,-51, and -56 have been found in women with normal cytology. In this study, we demonstrated that hrHPV genotype distribution in Mozambican healthy young women differs from the global figures with HPV52,-35,-16,-53,-58, and -51 being the most prevalent. Furthermore, HPV16 contributed to only 6.8% of female infections. Globally, HPV16 has been responsible for 22.5% of all infections in women, whereas in sub-Saharan Africa, it seems to have the lowest contribution (13.7%, 11.3%, and 11.1% for Southern, Eastern, and Western Africa, respectively)[18].

Several studies conducted in Mozambique have shown a strong association between HPV16,-33,-35,-45,-18,-31, and -58 with cervical neoplasia[19-22]. In the present study, we demonstrated a 42.9% match between the hrHPV types commonly circulating in the female population (HPV35,-16, and -58) and HPVs types commonly present in cervical neoplasia in Maputo, Mozambique.

We confirmed the association between early onset of sexual debut, history of past STI and HIV infection with multiple HPV infections[23 24]. These findings reiterate the need to intensify sexual behaviour education and STI prevention in young populations.

This study shows a high prevalence of HPV infections (66.7%) in the HIV-infected population. A previous report showed a prevalence of HPV infections of 56.6% in HIV-infected African women, which is concordant with our findings[25]. Other reports from South Africa have demonstrated an even higher HPV prevalence (75%) in the age group of 17–19 years[26]. We demonstrated that HPV infections and multiple HPV infections are more frequent in HIV-infected subjects. Similar

descriptions have been previously reported[26 27]. We have shown that HPV35,-52, and -58 were responsible for 60% of all hrHPV infections in this population. McDonald et al. have reported that hrHPV35,-58,-18,-45,-16, and -52 were the most common genotypes in HIV-infected women with normal cytology in neighbouring South Africa[26]. Lastly, other reports in Africa have shown that the most frequent genotypes circulating in HIV-infected women were HPV16,-58,-52,-31,-35, and -18[25]. These findings suggest 100% homology between the common hrHPV genotypes in Mozambique vs South Africa and other African regions. We have also described a high prevalence of lrHPV infections in the HIV-infected subjects. These findings were also demonstrated elsewhere[28].

In the present study, we showed that AFR2 variants are responsible for two-thirds of HPV16 infections followed by NA/AA variants. We have also demonstrated a lower proportion of AFR1 and EAS infections. Tu et al. have previously reported a high frequency of AFR2 variants in sub-Saharan African women with normal and abnormal cytology[29] and Cornet et al. have confirmed these findings[9]. Nonetheless, a high proportion of the European variants has also been described[29]. Previous studies have shown an increased likelihood of having high grade cervical lesions when AFR2 and AA lineages were present[30], but the opposite has also been described [29].

Since 2014, Mozambique has been engaged in the HPV vaccine demonstration project which aimed at accessing the country´s preparedness for the introduction of an HPV vaccine in the national vaccination programme. Information regarding HPV types circulating in young populations is crucial for selection of a suitable vaccine. Currently available HPV vaccines, Gardasil®, Cervarix®, and Gardasil®9, can prevent up to seven hrHPV infections. Moderate to high cross-protection against HPV types phylogenetically related to HPV16 and -18 have also been reported. Overall, Gardasil®9 has the highest genotype coverage in both women and men whereas Gardasil® has the lowest in women and Cervarix® in men. Gardasil®9 can provide protection against HPV52, the most common genotype found in this study.

Conclusion

This study confirmed the high burden of HPV infections in young women in the Maputo city, Mozambique. The HPV prevalence was demonstrated to be associated with high-risk sexual

behaviour, thus confirming the need to intensify sexual education and STI prevention interventions. The delivery of an HPV vaccine should be prioritized, particularly in young females. Additional studies involving other anatomic collection sites in men to confirm the comparatively low prevalence of HPV are needed.

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Data sharing and Contributorship

Conceived and designed the study: Edna Viegas, Charlotta Nilsson, Kerstin Falk, Nafissa Osman, Ilesh Jani Soren Andersson

Performed the study and experiments: Edna Viegas, Nália Ismael, Mallin Kaliff, Gabriella Lillsunde-larsson, Nafissa Osman

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Competing Interests

The authors have declared that no competing interests exist.

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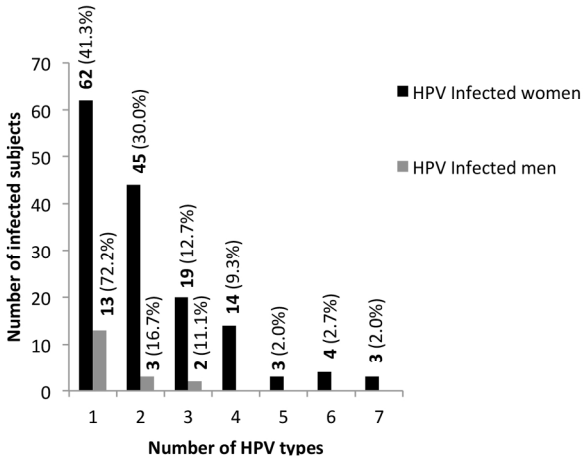
Figure Legend

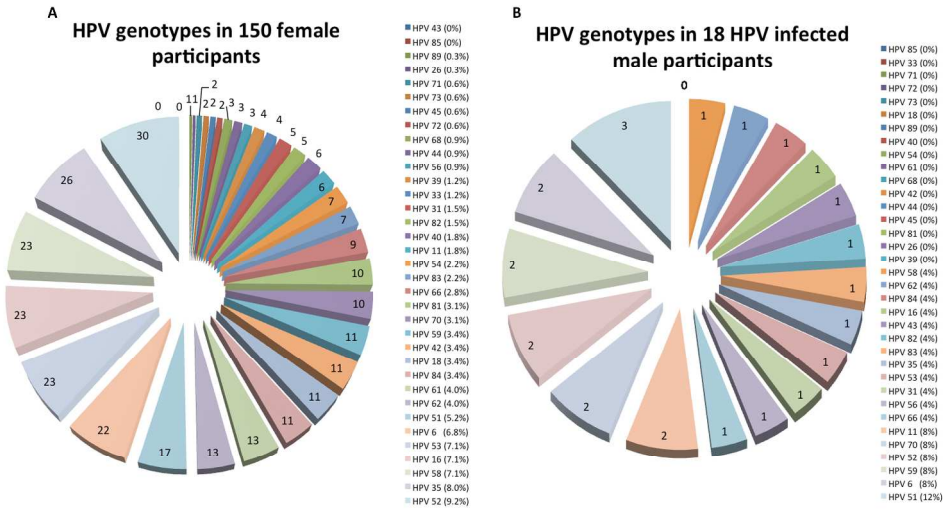
Figure 1. Distribution of HPV infections in female and male participants. The denominator is the total number of infected women and men, respectively.

Figure 2. HPV genotype distribution in female (A) and male (B) participants. For calculation of proportions, the denominator used was the number of HPV infected participants per gender.



Distribution of HPV infections in female and male participants. The denominator is the total number of infected women and men, respectively.

179x113mm (300 x 300 DPI)



HPV genotype distribution in female (A) and male (B) participants. For calculation of proportions, the denominator used was the number of HPV infected participants per gender.

273x159mm (300 x 300 DPI)

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Human papillomavirus prevalence and genotype distribution among young women and men in Maputo city, Mozambique

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Human papillomavirus prevalence and genotype distribution among young women and men in Maputo city, Mozambique

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Abstract

Objectives

Human papillomavirus (HPV) is a well-known cause of cervical cancer, the second most frequent cancer in female African populations. This study aimed at determining the prevalence of HPV infection and the genotype distribution in young adults aged 18-24, in Maputo city, Mozambique, and to assess the suitability of commercially available HPV vaccines.

Methods

This cross-sectional study was conducted between 2009 and 2011 at a youth clinic in Maputo Central Hospital. Cervical and urethral samples were obtained from 236 women and 176 men, respectively. Demographic and behavioural data were collected using structured questionnaires. HPV genotyping was performed for 35 different high, probably or possibly high and low-risk HPV types using the Clart® Human Papillomavirus 2.

Results

HPV prevalence was 168/412 (40.8%; 95%CI: 36.0-45.5) and was significantly higher in women than in men (63.6% vs 10.2%). HPV52 was the most frequent type found in women, followed by HPV35, -16, -53, -58, -6, and -51. In men, HPV51 ranked the highest, followed by HPV6, -11, -52, -59, and -70. HIV infection and sexual debut before 18 years of age were associated with multiple HPV infections (OR 3.03; 95%CI: 1.49-6.25 and OR 6.03; 95%CI: 1.73-21.02, respectively). Women had a significantly higher HPV infection prevalence than men ($p<0.001$). The 9-valent HPV vaccine would cover 36.8% of the high-risk genotypes circulating in women in this study, compared to 26.3% and 15.8% coverage by the bivalent and quadrivalent vaccines, respectively.

Conclusion

This study confirmed the high burden of HPV infections in young women, in Maputo city, Mozambique. The HPV prevalence was associated with high-risk sexual behaviour. Sexual education and STI prevention interventions should be intensified in Mozambique. Only a proportion of the high-risk HPV genotypes (37%) were covered by currently available vaccines.

Strengths and limitations

- This is the first study to describe the Human Papillomavirus (HPV) prevalence and genotype distribution in young adults (aged 18-24) in Mozambique.
- This study provides insights on the HPV types circulating in young populations, in Maputo city, which is an important public health information particularly for discussions on HPV vaccine introduction in the country.
- The absence of an older cohort (aged ≥ 25 years) for comparison of circulating HPV genotypes was a limitation of this study.
- The lower HPV prevalence in the male population may have been related to the site of sample collection, therefore additional studies in male population, in Mozambique, using different anatomic collection sites should be considered.

Key messages

- There is a high burden of HPV infection in young women, in Maputo city, Mozambique.
- High-risk sexual behavior was demonstrated to be associated with high prevalence of HPV infection.
- There is a need to intensify sexual education and STI prevention for young adults.

- Currently available HPV vaccines would only cover a proportion (37%) of the prevalent high-risk genotypes.

Introduction

Globally, cervical cancer (CC) is the fourth most common cancer in women and the second most common cancer in female African populations (1). More than 75% of cases occur in less developed areas. East Africa is the most affected region with an age-standardized incidence rate of 42.7 per 100.000 women. Mozambique has the second highest incidence of CC after Malawi (2).

Human papilloma virus (HPV) infection has been implicated in virtually all CC cases. To date, 201 different HPV types have been identified (3). More than 40 of these types can infect the anogenital tract and have been classified as low-risk (lrHPV6,-11,-40,-42,-43,-44,-54,-61,-62,-71,-72,-81,-83,-84, and -89), probably or possibly high-risk (pHR-HPV26,-30,-34,-53,-66,-67,-68,-69,-70,-73,-82,-85, and -97) and high-risk HPV types (hr-HPV16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58, and -59) depending on their ability to lead to malignant progression (4-6).

Worldwide, HPV16 has been responsible for more than half (54.4%) of the CC cases followed by HPV18 (16.5%), HPV58 (5.1%), HPV33 (4.7%), HPV45 (4.4%), HPV31 (3.6%), HVP52 (3.4%) and HPV35 (1.9%) (7). However, only a small proportion of infected women develop the disease. Persistent infection with hr-HPV types and integration of HPV-DNA in the host cells are usually required for malignant transformation of the cervix. The time between infection and initial pre-cancer lesions is around seven to ten years. Cervical cancer is more frequently diagnosed in middle-age women (40-64 years) thus suggesting that infections are taking place early in life. Globally, around 20% of all cervical cancers are

being diagnosed between the ages of 15-39 years (8). This suggests that a proportion of infections may be occurring even earlier.

Previous reports have shown a high prevalence of HPV infections in younger populations (9, 10) and reports in southern Africa demonstrated a high prevalence of HPV infections among women of 30 years or younger (11, 12). Limited information is available about the HPV epidemiology in Mozambique particularly in men and young populations in urban settings. Castellsagué et al have shown that the HPV prevalence is high (75%) in women aged 14-61 years in rural southern Mozambique (13). Cervical cancer is the most frequent cancer in Mozambican women (14). Others have described the contribution of HPV infection in the development of cervical cancers, in Mozambique (15-18).

By the end of 2016 HPV vaccination had only been introduced in 65 countries worldwide, seven being in the African continent. In Mozambique, HPV vaccine has yet not been introduced in the national expanded program on immunization. Currently 23 African countries, including Mozambique, are conducting or have recently finalized their HPV vaccine pilot projects (1).

Currently, the following three HPV vaccines are available: Gardasil® (which confers protection against HPV6,-11,-16, and -18 and cross-protection against HPV31); Cervarix® (which prevents against HPV16 and -18 and provides high and moderate cross-protection against HPV31,-45 and -33, respectively); and Gardasil®9 (which protects against HPV6,-11,-16,-18,-31,-33,-45,-52, and -58). Gardasil®9 is a second generation 9-valent vaccine that can theoretically prevent 87% of all hrHPV infections in Africa (19). Nonetheless, the HPV distribution varies between African regions, and the identification of country-specific profiles and their adequacy to the currently available vaccines remains of great importance when deciding on vaccine introduction.

In the present study, we aimed to (1) determine the prevalence and distribution of HPV infections in sexually active young adults; and (2) determine the suitability of the current HPV vaccines in the context of the Mozambican epidemic.

Subjects and Methods

This report adheres to the STROBE guidelines for the reporting of observational studies.

Study design and population

This was a cross-sectional ancillary study of a human immunodeficiency virus (HIV) incidence study conducted at a youth clinic in Maputo city, Mozambique, from August 2009 to October 2011 (20). In the parent study, briefly, 1,380 males and females aged 18–24 years were screened for HIV, syphilis and hepatitis B virus. HIV-seronegative subjects were enrolled and followed for one year with quarterly visits to the clinic for assessment of HIV serostatus. Baseline demographic and behavioural data were collected using structured questionnaires. In the present study, samples from male and female subjects were collected at one time point, at screening or at one of the follow-up visits. We aimed at sampling 500 individuals with equal gender distribution (250 females and 250 males) based on the possibility of recruiting male subjects at the youth clinic, which mainly caters to women. Cervical samples were collected in female participants via speculoscopy, using a Rovers® Viba-Brush (Rovers Medical Devices B.V., Oss., The Netherlands). Urethral samples were collected from male participants by gently inserting a cotton swab approximately 2–4 cm in the urethral meatus and rotating it in one direction. Both the brushes and swabs were immerged in 5 mL of SurePath cell-preservation solution (TriPath Imaging, Burlington, NC,

USA), transported to the study laboratory within the same day of collection and stored at +4 ºC to +8ºC for three months and then at -80ºC.

Laboratory testing

DNA was extracted from an initial sample volume of 1.5 mL using a QIAamp DNA Mini Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions and eluted to a final volume of 200 µl.

HPV genotyping was performed using the Clart® Human Papillomavirus 2 (Genomica, Madrid, Spain), a low-density microarray platform based on PCR amplification of a 450 bp fragment within an HPVL1 highly conserved region from 35 different HPV types (HPV6,-11,-16,-18,-26,-31,-33,-35,-39,-40,-42,-43,-44,-45,-51,-52,-53,-54,-56,-58,-59,-61,-62,-66,-68,-70,-71,-72,-73,-81,-82,-83,-84,-85, and -89) and a human gene control (CTFR). Individual genotyping results were analysed on the Clinical Array Reader (Genomica, Madrid, Spain). Adequacy of samples was assessed by amplification of the CTFR. Samples with undetectable DNA were rerun and the second result was considered final. The Clart® Human Papillomavirus 2 assay complies with the European Union (EU) safety, health or environmental requirements, with the EU legislation and with the European In-Vitro Diagnostic Devices Directive. This assay has been shown to have a similar performance as that of other well-established HPV screening assays, such as the FDA-approved Hybrid Capture 2 test (21, 22).

DNA extraction was performed at the Instituto Nacional de Saúde laboratories in Maputo, Mozambique. DNA Samples were then shipped to the Molecular Biology laboratories at the Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University Hospital, Örebro, Sweden, for HPV genotyping.

HIV testing was performed as previously described on site in Maputo (20).

Statistical analysis

Demographic and risk behavioural data were entered into a MySQL database version 5.1 (MySQL AB, 2008) and HPV laboratory results in a Microsoft Office Excel 2010 spreadsheet (Microsoft, Redmond, WA). For processing and analysis, data were imported into Stata version 14 (StataCorp. 2015. Stata: Release 14. Statistical Software. College Station, TX: StataCorp LP).

All analyses were stratified by gender. Descriptive statistics were employed as follows: frequencies for categorical variables, and means and standard deviations (SD) for quantitative variables. We used list wise deletion as the missing values were negligible. Here, we report the prevalence of HPV infections and HPV genotypes per gender and HIV status. Bivariate logistic analysis between sociodemographic and sexual behavioural characteristics and presence of any HPV was conducted. Age of individuals and age of sexual debut and characteristics whose p-value on bivariate logistic regression analysis was below 0.25 were included in the multivariable logistic regression. Additionally, to identify characteristics that would contribute to multiple HPV genotype infections a multinomial logistic regression (MNLR) was used. For this analysis, males and females are not stratified given the sample constraints. We first grouped the participants as follows: a) subjects without an HPV infection; b) subjects with one HPV infection (mono-infection); and c) subjects with at least two HPV infections (multiple infections). The MNLR per level of the factor under consideration provided two odds-ratios (one for mono-infected versus not-infected subjects and another for the subjects with multiple-infections versus not-infected subjects). We report both the unadjusted and adjusted odds-ratios and their respective 95% CI. For adjustment, we

included factors whose p-values through the global likelihood ratio test were below 0.25 in the MNLR bivariate analysis; gender and age were forced factors to include in the model. The significance level was set at 5%.

Ethics statement

Ethical approval was granted by the National Health Bioethics Committee of Mozambique (Ref. 148/CNBS of May 8, 2009 and Ref. 18/CNBS/11). Testing performed in Sweden was approved by the Regional Ethics Committee. Study investigators followed the GCP-ICH guidelines. Subjects signed an informed consent form prior to any study activities.

Results

In total, 489 participants (263 females and 226 males) were enrolled, but samples from 77 (15.7%) subjects had undetectable DNA levels and were excluded from the data analysis, being 27/263 (10.3%) from females and 50/226 (22.1%) from males.

Demographic and behavioural characteristics

Table 1 shows the socio demographic and sexual behavioural characteristics of study participants by sex. Of the 412 subjects with valid HPV results, 236 (57.3%) were females, and 176 (42.7%) were males. The mean age of study participants was 21.1 years (SD: 1.71) with males being slightly older than females (21.5 vs 20.8). Almost all participants were students (94.2%) and all had some formal education. Male subjects were more educated than females with more than half having secondary or higher educational degrees (56.8% vs 41.9%). Nearly all participants were single (98.8%). The median age at sexual debut was 17 years (interquartile range: 15–18) being 16 years (interquartile range: 14–18) for males and 17 years (interquartile range: 16–18) for females. The majority of subjects (89.3%) had more

than one sexual partner in life (97.2% of males and 83.5% of females) and 24% reported two or more sexual partners in the last 6 months (37.5% of males and 14% of females). Around one-fourth of the study population reported having at least one episode of sexually transmitted infection (STI) in life and this was seen more frequently in women than in men (34.3% vs 13.6%). Only 66.0% of subjects reported having used a condom at the time of their last sexual intercourse with females reporting a lower frequency compared to males (59.7% vs 74.4%). HIV infection was diagnosed in 21 (5.1%) participants, of which 17 (81.0%) were females.

Table 1 – Sociodemographic and sexual behavioural characteristics of study participants by sex.

	Total		Male		Female	
	N	%	N	%	N	%
Total	412	100.0	176	100.0	236	100.0
Age (years)						
Mean (SD)	21.1 (1.71)		21.5 (1.67)		20.8 (1.68)	
Marital Status						
Single	407	98.8	175	99.4	232	98.3
Married/Cohabitating	5	1.2	1	0.6	4	1.7
Education						
Secondary or less	213	51.7	76	43.2	137	58.1
Technical training	109	26.5	50	28.4	59	25.0
University grade	90	21.8	50	28.4	40	16.9
Occupation						
Student	388	94.2	163	92.6	225	95.3
Employed	23	5.6	12	6.8	11	4.7
Missing	1	0.2	1	0.6	0	0.0
Religion						
Christian	375	91.0	162	92.0	213	90.3
Other	37	9.0	14	8.0	23	9.7
Age at sexual debut						
Less than 18	285	69.2	115	65.3	170	72.0
18 or more	122	29.6	58	33.0	64	27.1
Missing	5	1.2	3	1.7	2	0.8
Number of sex partners in life						
0 - 1	44	10.7	5	2.8	39	16.5
> 1	368	89.3	171	97.2	197	83.5

Number of sex partners in the last 6 months						
0 - 1	313	76.0	110	62.5	203	86.0
> 1	99	24.0	66	37.5	33	14.0
Condom use in the last sexual intercourse						
No	139	33.7	45	25.6	94	39.8
Yes	272	66.0	131	74.4	141	59.7
Missing	1	0.2	0	0.0	1	0.4
Had a STI in life						
No	305	74.0	150	85.2	155	65.7
Yes	105	25.5	24	13.6	81	34.3
Missing	2	0.5	2	1.1	0	0.0
HIV infection						
Positive	21	5.1	4	2.3	17	7.2
Negative	391	94.9	172	97.7	219	92.8

HPV prevalence and genotype distributions

The overall HPV prevalence was 40.8% (168 infected subjects; 95% CI: 36.0–45.5) and was significantly higher in women than in men [150/236 (63.6%, 95% CI: 57.1-69.7) vs 18/176 (10.2%, 95% CI: 6.2-15.7), p<0.001]. The HPV prevalence among HIV infected subjects (14/21, 66.7%) was 69% higher than in the HIV negative subjects (154/391, 39.4%; p=0.013). Figure 1 and Table 2 show the distribution of HPV genotypes in male and female participants. Of the 412 enrolled participants, 115 (27.9%), 54 (13.1%) and 89 (21.6%) were infected with one or more high-risk, probably high-risk and low-risk HPV types, respectively. High-risk and pHR-HPV types were present in 124/150 (82.7%, 95% CI: 75.6-88.4) and 14/18 (77.8%, 95% CI: 52.4-93.6) of HPV-infected women and men, respectively (p=0.533). Overall, HPV52 was the most frequent type found (9.1%), followed by HPV35,-6,-16,-53,-58, and -51. Altogether, these HPV types accounted for 49.9% of the infections in women and men. In female participants, the most common genotypes were similar to the overall distribution (HPV52,-35,-16,-53,-58,-6, and -51) and accounted for half (50.3%) of the infections in women (Figure 1A). In contrast, male participants´ HPV infections were less diverse with HPV51 being the most frequent type found, followed by HPV6,-11,-52,-59, and

-70 in equal proportions. These six types were responsible for 52% of the infections in men (Figure 1B). High-risk HPV genotypes were identified in 11/21 (52.4%) of HIV-infected subjects. Three hrHPVs (HPV35, -52, and -58) accounted for 60% of all hrHPV infections in this population. There were no differences in the number of subjects infected with hrHPVs and pHR-HPVs in the HIV infected (p=0.552) and uninfected populations (p=1.000). In contrast, lrHPVs were more frequently observed in the HIV-infected subjects (p=0.022).

Table 2 – HPV prevalence and HPV genotype distribution by sex

	Total	Gender		HIV Serostatus		HPV genotype coverage		
		Male	Female	Negative	Positive	Gardasil®	Cervarix®	Gardasil®9
Total Subjects, N	412	176	236	391	21			
Number of HPV infections, N	351	25	326	305	46			
Subjects with at least 1 HPV infection[a], N (%)	168 (40.8)	18 (10.2)	150 (63.6)	154 (39.4)	14 (66.7)			
Subjects with High-Risk HPV infections[b], N (%)	115 (27.9)	10 (5.7)	105 (44.5)	104 (26.6)	11 (52.4)			
16	24 (5.8)	1 (0.6)	23 (9.7)	22 (5.6)	2 (9.5)	++++	++++	++++
18	11 (2.7)	0 (0.0)	11 (4.7)	10 (2.6)	1 (4.8)	++++	++++	++++
31	6 (1.5)	1 (0.6)	5 (2.1)	5 (1.3)	1 (4.8)	++++[ρ]	++++[§]	++++
33	4 (1.0)	0 (0.0)	4 (1.7)	2 (0.5)	2 (9.5)	++++[ρ]	++++[ρ]	++++
35	27 (6.6)	1 (0.6)	26 (11.0)	22 (5.6)	5 (23.8)			
39	4 (1.0)	0 (0.0)	4 (1.7)	4 (1.0)	0 (0.0)			
45	2 (0.5)	0 (0.0)	2 (0.8)	1 (0.3)	1 (4.8)		++++[§]	++++
51	20 (4.9)	3 (1.7)	17 (7.2)	19 (4.9)	1 (4.8)			
52	32 (7.8)	2 (1.1)	30 (12.7)	28 (7.2)	4 (19.0)			++++
56	4 (1.0)	1 (0.6)	3 (1.3)	4 (1.0)	0 (0.0)			
58	24 (5.8)	1 (0.6)	23 (9.7)	21 (5.4)	3 (14.3)			++++
59	13 (3.2)	2 (1.1)	11 (4.7)	13 (3.3)	0 (0.0)			
Total number of infections[c]	171 (48.7)	12 (48.0)	159 (48.8)	151 (49.5)	20 (43.5)			
Subjects with Probably or Possibly High-Risk HPV infections, N (%)	54 (13.1)	5 (2.8)	49 (20.8)	50 (12.8)	4 (19.0)			
26	1 (0.2)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)			
53	24 (5.8)	1 (0.6)	23 (9.7)	22 (5.6)	2 (9.5)			

66	10 (2.4)	1 (0.6)	9 (3.8)	9 (2.3)	1 (4.8)		
68	3 (0.7)	0 (0.0)	3 (1.3)	3 (0.8)	0 (0.0)		
70	12 (2.9)	2 (1.1)	10 (4.2)	11 (2.8)	1 (4.8)		
73	2 (0.5)	0 (0.0)	2 (0.8)	2 (0.5)	0 (0.0)		
82	6 (1.5)	1 (0.6)	5 (2.1)	6 (1.5)	0 (0.0)		
85	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Total number of infections[c]	58 (16.5)	5 (20.0)	53 (16.3)	54 (17.7)	4 (8.7)		
Subjects with Low-Risk HPV infections, N (%)	89 (21.6)	7 (4.0)	82 (34.7)	79 (20.2)	12 (57.1)		
6	24 (5.8)	2 (1.1)	22 (9.3)	21 (5.4)	3 (14.3)	++++	++++
11	8 (1.9)	2 (1.1)	6 (2.5)	6 (1.5)	2 (9.5)	++++	++++
40	6 (1.5)	0 (0.0)	6 (2.5)	5 (1.3)	1 (4.8)		
42	11 (2.7)	0 (0.0)	11 (4.7)	10 (2.6)	1 (4.8)		
43	1 (0.2)	1 (0.6)	0 (0.0)	1 (0.3)	0 (0.0)		
44	3 (0.7)	0 (0.0)	3 (1.3)	3 (0.8)	0 (0.0)		
54	7 (1.7)	0 (0.0)	7 (3.0)	4 (1.0)	3 (14.3)		
61	13 (3.2)	0 (0.0)	13 (5.5)	11 (2.8)	2 (9.5)		
62	14 (3.4)	1 (0.6)	13 (5.5)	11 (2.8)	3 (14.3)		
71	2 (0.5)	0 (0.0)	2 (0.8)	2 (0.5)	0 (0.0)		
72	2 (0.5)	0 (0.0)	2 (0.8)	1 (0.3)	1 (4.8)		
81	10 (2.4)	0 (0.0)	10 (4.2)	7 (1.8)	3 (14.3)		
83	8 (1.9)	1 (0.6)	7 (3.0)	7 (1.8)	1 (4.8)		
84	12 (2.9)	1 (0.6)	11 (4.7)	10 (2.6)	2 (9.5)		
89	1 (0.2)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)		
Total number of infections[c]	122 (34.8)	8 (32.0)	114 (35.0)	100 (32.8)	22 (47.8)		

++++ Genotype covered by the vaccine
++++§ Genotype highly covered through vaccine cross-protection
++++p Genotype moderately covered through vaccine cross-protection
[a]The denominator is total subjects
[b]The denominator is total subjects
[c]The denominator is number of HPV infections

Vaccine-matched HPV genotypes

Table 2 shows the distribution of HPV genotypes in women and men, and their correspondence to the genotypes present in the current HPV vaccines. Genotypes associated with vaccine cross-protection were included in the analysis and considered as covered by the vaccine. The total number of circulating HPV genotypes matching the vaccine genotypes were 5/33 (15.2%; Gardasil®: HPV6,-11,-16,-18, and -31), 5/33 (15.2%; Cervarix®: HPV16,-18,-31,-33, and -45) and 9/33 (27.3%; Gardasil®9: HPV6,-11,-16,-18,-31,-33,-45,-52, and -58), for the female population, and 4/18 (22.2%; Gardasil®: HPV6,-11,-16, and -31), 2/18 (11.1%; Cervarix®: HPV6 and -31) and 6/18 (33.3%; Gardasil®9: HPV6,-11,-16,-31,-52, and -58) for the male population. The three vaccines can cover 3/19 (15.8%; Gardasil®: HPV16,-18, and -31), 5/19 (26.3%; Cervarix®: HPV16,-18,-31,-33, and -45) and 7/19 (36.8%; Gardasil®9: HPV16,-18,-31,-33,-45,-52, and -58) hrHPVs circulating in women and 2/12 (16.7%; Gardasil®: HPV16 and -31), 2/12 (16.7%; Cervarix®: HPV16 and -31) and 4/12 (33.3%; Gardasil®9: HPV16,-31,-52, and -58) in men.

Factors associated with HPV infection

Overall, women had a significantly higher HPV infection prevalence than men ($p<0.001$). Although the odds of being infected with HPV reduced by 80% for each year of age, in the multivariate analysis this was not significant. Sexual debut before the age of 18 years, history of STI and infection with HIV were significantly associated with the presence of HPV infection ($p=0.008$; $p<0.001$; $p=0.013$, respectively) in the univariate analysis. When stratifying by gender (Tables 3A and 3B), the univariate analysis show that women who initiated sexual activity before the age of 18 were significantly more at risk of having a HPV infection ($p=0.041$) and a marginally significance association was seen in women who reported to have had more than 2 sexual partners in the last 6 months ($p=0.055$) but this was

not seen in the multivariate analysis. No significant associations were seen for male participants both in the univariate and multivariate analysis, but a marginally significant association was seen for a reported history of STI and HPV infection.

Table 3A – Factors associated with any HPV infection among male participants: unadjusted and adjusted

	Any HPV infection		Univariate			Multivariate		
	Negative (N)	Positive (N)	OR	CI 95%	p	OR	CI 95%	p
Total Observations	158	18				171		
Age (years)			0.90†	0.67 - 1.20	0.465	0.92†	0.66 - 1.27	0.605
Education					0.173			0.239
Primary and Secondary	66	10	1.00	-		1.00	-	
Technical training	44	6	0.90	0.31 - 2.65		1.02	0.31 - 3.34	
University grade	48	2	0.28	0.06 - 1.31		0.27	0.05 - 1.32	
Occupation					0.813			
Student	146	17	1.00	-				
Employed	11	1	0.78	0.09 - 6.43				
Religion					0.618			
Christian	146	16	1.00	-				
Other	12	2	1.52	0.31 - 7.41				
Age at sexual debut					0.268			0.191
Less than 18	101	14	1.00	-		1.00	-	
18 or more	54	4	0.53	0.17 - 1.70		0.43	0.12 - 1.52	
Number of sex partners in the last 6 months					0.254			
0 - 1	101	9	1.00	-				
> 1	57	9	1.77	0.67 - 4.72				
Condom use in the last sexual intercourse					0.822			
No	40	5	1.00	-				
Yes	118	13	0.88	0.30 - 2.63				
Had a STI in life					0.097			0.057
No	137	13	1.00	-		1.00	-	
Yes	19	5	2.77	0.89 - 8.65		3.21	0.97 - 10.64	

HIV infection					0.347
Positive	3	1	3.04	0.30 - 30.86	
Negative	155	17	1.00	-	

† Associated effect of 1 year increase

Table 3B – Factors associated with any HPV infection among female participants: unadjusted and adjusted

	Any HPV infection		Univariate			Multivariate		
	Negative (N)	Positive (N)	OR	CI 95%	p	OR	CI 95%	p
Total Observations	86	150				234		
Age (years)			0.87†	0.74 - 1.02	0.091	0.88†	0.75 - 1.04	0.139
Education					0.246			0.267
Primary and Secondary	51	86	1.00	-		1.00	-	
Technical training	17	42	1.47	0.76 - 2.84		1.57	0.80 - 3.11	
University grade	18	22	0.72	0.36 - 1.48		0.81	0.39 - 1.68	
Occupation					0.521			
Student	83	142	1.00	-				
Employed	3	8	1.56	0.40 - 6.04				
Religion					0.862			
Christian	78	135	1.00	-				
Other	8	15	1.08	0.44 - 2.67				
Age at sexual debut					0.041			0.109
Less than 18	55	115	1.00	-		1.00	-	
18 or more	30	34	0.54	0.30 - 0.97		0.61	0.33 - 1.12	
Number of sex partners in the last 6 months					0.055			0.150
0 - 1	79	124	1.00	-		1.00	-	
> 1	7	26	2.37	0.98 - 5.71		1.94	0.79 - 4.80	
Condom use in the last sexual intercourse					1.000			
No	34	60	1.00	-				
Yes	51	90	1.00	0.58 - 1.72				
Had a STI in life					0.665			
No	58	97	1.00	-				
Yes	28	53	1.13	0.65 - 1.98				

HIV infection					0.258
Positive	4	13	1.95	0.61 - 6.17	
Negative	82	137	1.00	-	

† Associated effect of 1 year increase

HPV mono-infection vs multiple-infections

Almost half, 75/168 (44.6%), of HPV infected subjects had a single infection, 48/168 (28.6%) had two concurrent HPV infections and 45/168 (26.8%) had three or more HPV co-infections (Figure 2). HPV-infected male participants had more frequently a single infection (72.2% of the cases) whereas more than 50% of HPV-infected females had two or more HPV co-infections. In the univariate analysis, several factors were shown to be associated with the presence of two or more (multiple) HPV co-infections. Being a woman, younger age (the odds of being infected with HPV reduced by 80% for each year of age), lower educational degree, sexual debut before the age of 18, reported STI in life and the presence of a HIV infection were factors associated with multiple-HPV infections. Nonetheless, in the multivariate analysis only three factors were demonstrated to be significantly associated, namely, being a woman (p=0.001), having started sexual activity before the age of 18 (p=0.008) and the presence of a HIV infection (p=0.003). Condom use in the last sexual intercourse was not significantly associated with protection against HPV infection (p=0.143) (Table 4).

Table 4 – Factors associated with the number (mono or multiple) of HPV infection: unadjusted and adjusted using multinominal logistic regression

	Univariate				p	Multivariate				p
	Mono-infection vs Negative		Multiple-infections vs Negative			Mono-infection vs Negative		Multiple-infections vs Negative		
	OR	CI 95%	OR	CI 95%		OR	CI 95%	OR	CI 95%	
Total Observations	412					406				
Gender										
Male	1.00	-	1.00	-	< 0.001	1.00	-	1.00	-	< 0.001
Female	8.76	4.56 - 16.83	32.33	12.65 - 82.66		7.23	3.64 - 14.33	28.38	10.78 - 74.70	
Age (years)	0.80†	0.69 - 0.94	0.80†	0.69 - 0.92	0.001	0.85†	0.71 - 1.02	0.87†	0.72 - 1.04	0.134
Marital Status										
Single	1.00	-	1.00	-	0.632					
Married/Cohabitating	1.64	0.15 - 18.29	2.66	0.37 - 19.16						
Education										
Secondary or less	1.00	-	1.00	-		1.00	-	1.00	-	
Technical training	0.98	0.54 - 1.79	0.94	0.54 - 1.65	0.040	1.42	0.71 - 2.83	1.77	0.87 - 3.61	0.246
University grade	0.41	0.19 - 0.87	0.47	0.24 - 0.91		0.59	0.26 - 1.33	0.84	0.38 - 1.85	
Occupation										
Student	1.00	-	1.00	-	0.784					
Employed	1.17	0.41 - 3.36	0.74	0.24 - 2.29						
Religion										
Christian	1.00	-	1.00	-	0.764					

Other	1.15	0.47 - 2.84	1.35	0.61 - 3.00						
Age at sexual debut										
Less than 18	1.00	-	1.00	-	0.003	1.00	-	1.00	-	0.008
18 or more	0.82	0.47 - 1.44	0.36	0.20 - 0.67		0.91	0.48 - 1.72	0.33	0.16 - 0.67	
Number of sex partners in life										
0 - 1	1.00	-	1.00	-	0.424					
> 1	0.64	0.29 - 1.43	0.67	0.32 - 1.41						
Number of sex partners in the last 6 months										
0 - 1	1.00	-	1.00	-	0.369					
> 1	0.65	0.34 - 1.23	0.82	0.47 - 1.44						
Condom use in the last sexual intercourse										
No	1.00	-	1.00	-	0.143	1.00	-	1.00	-	0.423
Yes	0.59	0.35 - 1.00	0.80	0.48 - 1.32		0.81	0.45 - 1.48	1.25	0.68 - 2.31	
Had a STI in life										
No	1.00	-	1.00	-	0.003	1.00	-	1.00	-	0.530
Yes	2.33	1.32 - 4.12	2.07	1.21 - 3.55		1.44	0.76 - 2.72	1.18	0.62 - 2.24	
HIV infection										
Positive	0.46	0.06 - 3.79	5.50	2.12 - 14.27	< 0.001	0.44	0.05 - 4.04	6.03	1.73 - 21.02	0.003
Negative	1.00	-	1.00	-		1.00	-	1.00	-	

† Associated effect of 1 year increase

Discussion

This was a cross-sectional study reporting the prevalence of HPV infections and genotype distributions in young adults aged 18–24 in Maputo city, Mozambique. The HPV prevalence in the female population was 63.6% (95CI: 57.1-69.7) and was similar to previous reports of 75.9% in women aged 14–61 years in rural southern Mozambique and other neighbouring countries (11-13, 23). The HPV prevalence in the male population was 10.2% (95CI: 6.1-15.7), which was lower than previous reports for African countries and other regions (2). Although no significant difference was observed in the number of hrHPV infections between women and men, a high susceptibility of women to HPV infections was demonstrated.

We report a lower prevalence of HPV infections in men than in women, which is contrary to what has been shown in neighbouring countries and other regions (24). Studies assessing different male anatomic collection sites show that HPV detection in urethral samples is suboptimal compared to other sampling sites in the male genitalia, but the sensitivity reported for urethral sampling has varied (25, 26). In a study comparing sampling from 7 male genital sites, Giuliano et al reported that detection was highest at the penile shaft (48%), while only 10% of HPV infections were detected when using urethral sampling (26). In contrast, the sensitivity of urethral samples for overall HPV detection was 54.2% in a study that explored expressed prostate secretions as an option for detection of HPV infections in Russian men (27). We found 50/226 (22.1%) urethral samples with undetectable DNA and 158/176 (89.8%) of valid samples tested negative. The first could be related to an inadequate sampling technique and the second to the selected anatomic collection site. Altogether, this could have contributed to the lower male HPV prevalence found in our study.

Worldwide, the prevalence of cervical HPV infections in healthy women has been demonstrated to be 11.7%. Nonetheless, in less developed regions, such as East Africa, the prevalence is three times the global figures (33.6%) (28). High-risk HPV16,-18,-52,-31,-58,-

39,-51, and -56 have been found in women with normal cytology. In this study, we demonstrated that hrHPV genotype distribution in Mozambican healthy young women differs from the global figures with HPV52,-35,-16,-53,-58, and -51 being the most prevalent. Catellsagué et al have previously described that hrHPVs 51,-35,-18,-31, -52 were the most commonly found in women aged 14-61 years with normal cytology, in a study conducted in southern Mozambique (13), which is similar to our findings. Furthermore, HPV16 contributed to only 6.8% of female infections in the present study. These findings are concordant with those from Castellsagué et al (13). Globally, HPV16 has been responsible for 22.5% of all infections in women, whereas in sub-Saharan Africa, it seems to have the lowest contribution (13.7%, 11.3%, and 11.1% for Southern, Eastern, and Western Africa, respectively (28). The peak prevalence of HPV infections in women was shown to occur in early ages (before the age of 30 years) (11, 12). Some reports have also described a less pronounced second peak prevalence in older women (around their 50´s). Reasons for the occurrence of the first HPV peak are related to absence of immune responses to HPV and sexual activity in younger women. The second peak is not consistently reported across the globe but it is speculated that hormonal changes my play a role. Although the majority of HPV infections in early ages clear spontaneously (29, 30) some do become chronic infections and may potentially evolve to malignant transformation of the cervix. In the present study we described the epidemiology of HPV infections in both women and men that belong to the "first HPV peak" population.

Our report shows that more than 50% of the study population had two or more concomitant HPV infections with more than 50% of female participants having two or more co-infections compared to only 27.8% of male participants. It has been demonstrated by others that women with multiple HPV infections take longer to clear their infections compared to women with a

single infection (29, 30) thus potentially contributing to the establishment of a persistent infection, a prerequisite for cervical cancer development. Several studies conducted in Mozambique have shown a strong association between HPV16,-33,-35,-45,-18,-31, and -58 with cervical neoplasia (15-18). In another Mozambican study HPV52 was reported to be the second most common hrHPV in women with abnormal cytology and the fourth most common in women with invasive cervical cancer (13). In the present study, we demonstrated a 50% match between the hrHPV types commonly circulating in the female population (HPV35,-16,-52 and -58) and HPVs types commonly present in cervical neoplasia in Maputo, Mozambique.

We confirmed the association between early onset of sexual debut, history of past STI and HIV infection with multiple HPV infections (31, 32). These findings reiterate the need to intensify sexual behaviour education and STI prevention in young populations.

This study shows a high prevalence of HPV infections (66.7%) in the HIV-infected population. A previous report showed a prevalence of HPV infections of 56.6% in HIV-infected African women, which is concordant with our findings (33). Other reports from South Africa have demonstrated an even higher HPV prevalence (75%) in the age group of 17–19 years(34). We demonstrated that HPV infections and multiple HPV infections are more frequent in HIV-infected subjects. Similar descriptions have been previously reported (34, 35). We have shown that HPV35,-52, and -58 were responsible for 60% of all hrHPV infections in this population. McDonald et al. have reported that hrHPV35,-58,-18,-45,-16, and -52 were the most common genotypes in HIV-infected women with normal cytology in neighbouring South Africa (34). Lastly, other reports in Africa have shown that the most frequent genotypes circulating in HIV-infected women were HPV16,-58,-52,-31,-35, and -18

(33). These findings suggest a high homology between the common hrHPV genotypes in Mozambique vs South Africa and other African regions. We have also described a high prevalence of lrHPV infections in the HIV-infected subjects. These findings were also demonstrated elsewhere (36).

Since 2014, Mozambique has been engaged in the HPV vaccine demonstration project which aimed at accessing the country´s preparedness for the introduction of an HPV vaccine in the national vaccination programme. Information regarding HPV types circulating in young populations is crucial for selection of a suitable vaccine. Currently available HPV vaccines, Gardasil®, Cervarix®, and Gardasil®9, can prevent up to seven hrHPV infections. Moderate to high cross-protection against HPV types phylogenetically related to HPV16 and -18 have also been reported with the use of Gardasil® and Cervarix®. Nonetheless, the level of protection has shown to be heterogeneous across the studies and a signal for reduction of vaccine efficacy with extended follow up has also been demonstrated (37). Our study shows that Gardasil®9 has the highest genotype coverage in both Mozambican women and men whereas Gardasil® has the lowest in women and Cervarix® in men. Gardasil®9 can provide protection against HPV52, the most common genotype found in this study. Nonetheless, Gardasil®9 would only cover 37% of the high-risk genotypes found. This has important implications for vaccine-strategic discussions. It should also send signals to vaccine manufacturers concerning future developments of new versions of the HPV vaccines. In the present study we did not assess HPV vaccination status since no HPV vaccine had been introduced or administered within the National Health System.

This study has some limitations. We did not include an older cohort (aged ≥ 25 years) for comparison of circulating HPV genotypes due to funding restrictions. This must be considered in future studies to better describe the epidemiology of HPV infections in

Mozambique. In our study, the questionnaire assessing sexual behavioural captured information about the number of sexual partners categorized into two variables only, either having one or more than one partner. This did not enable us to further analyse the influence of the number of sexual partners on HPV status. The low HPV prevalence in the male population may have been related to the site and technique of sampling and we therefore recommend that additional studies in male populations, in Mozambique, are considered.

Conclusion

This study confirmed the high burden of HPV infections in young women in the Maputo city, Mozambique. The HPV prevalence was demonstrated to be associated with high-risk sexual behaviour, thus confirming the need to intensify sexual education and STI prevention interventions. Additional studies involving other anatomic collection sites in men to confirm the comparatively low prevalence of HPV are needed. For vaccine-strategic discussions, it is important to consider that the current HPV vaccines only cover a proportion of prevailing HPV types in this area.

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Data sharing and Contributorship

Conceived and designed the study: Edna Viegas, Charlotta Nilsson, Kerstin Falk, Nafissa Osman, Ilesh Jani, Sören Andersson

Performed the study and experiments: Edna Viegas, Nália Ismael, Mallin Kaliff, Gabriella Lillsunde-larsson, Nafissa Osman

Analyzed the data: Edna Viegas, Orvalho Augusto

Wrote the paper: Edna Viegas, Charlotta Nilsson, Sören Andersson, Orvalho Augusto, Ilesh Jani, Gabriella Lillsunde-larsson, Kerstin Falk, Torbjörn Ramqvist, Nafissa Osman, Nália Ismael, Mallin Kaliff

Competing Interests

The authors have declared that no competing interests exist.

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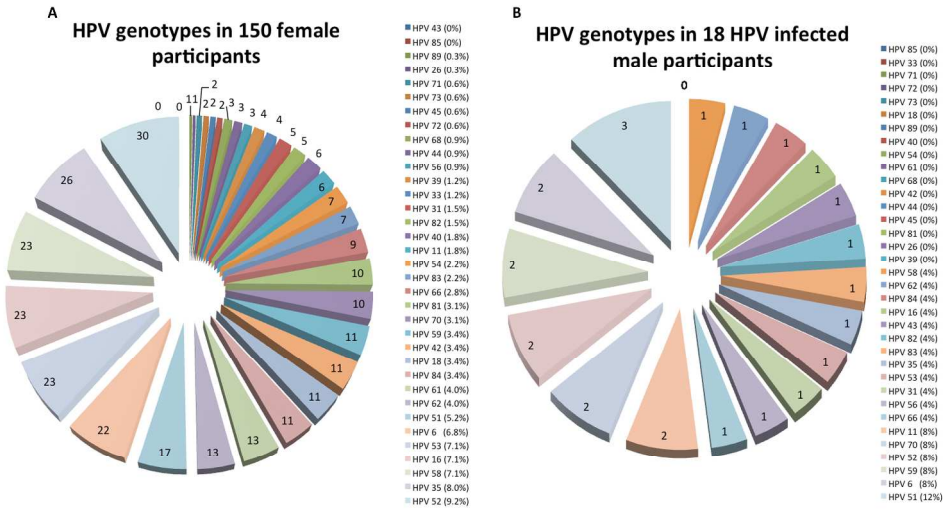
Figure Legend

Figure 1. HPV genotype distribution in female (A) and male (B) participants. For calculation of proportions, the denominator used was the number of HPV infected participants per gender.

Figure 2. Distribution of HPV infections in female and male participants. The denominator is the total number of infected women and men, respectively.



HPV genotype distribution in female (A) and male (B) participants. For calculation of proportions, the denominator used was the number of HPV infected participants per gender.

273x159mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Indicated in the abstract page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2, 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	8, 20-24
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11 Table 1
		(b) Indicate number of participants with missing data for each variable of interest	10 Table 1
		(c) Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures over time	Not applicable
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear	11-20 Table 3 and 4

		which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	10, 15-16, 19-20 Tables 1, 3 and 4
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	21-24
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24-25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	25
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	26

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Human papillomavirus prevalence and genotype distribution among young women and men in Maputo city, Mozambique

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Human papillomavirus prevalence and genotype distribution among young women and men in Maputo city, Mozambique

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Abstract

Objectives

Human papillomavirus (HPV) is a well-known cause of cervical cancer, the second most frequent cancer in female African populations. This study aimed at determining the prevalence of HPV infection and the genotype distribution in young adults aged 18-24, in Maputo city, Mozambique, and to assess the suitability of commercially available HPV vaccines.

Methods

This cross-sectional study was conducted between 2009 and 2011 at a youth clinic in Maputo Central Hospital. Cervical and urethral samples were obtained from 236 women and 176 men, respectively. Demographic and behavioural data were collected using structured questionnaires. HPV genotyping was performed for 35 different high, probably or possibly high and low-risk HPV types using the Clart® Human Papillomavirus 2.

Results

HPV prevalence was 168/412 (40.8%; 95%CI: 36.0-45.5) and was significantly higher in women than in men (63.6% vs 10.2%). HPV52 was the most frequent type found in women, followed by HPV35, -16, -53, -58, -6, and -51. In men, HPV51 ranked the highest, followed by HPV6, -11, -52, -59, and -70. HIV infection and sexual debut before 18 years of age were associated with multiple HPV infections (OR 3.03; 95%CI: 1.49-6.25 and OR 6.03; 95%CI: 1.73-21.02, respectively). Women had a significantly higher HPV infection prevalence than men ($p<0.001$). The 9-valent HPV vaccine would cover 36.8% of the high-risk genotypes circulating in women in this study, compared to 26.3% and 15.8% coverage by the bivalent and quadrivalent vaccines, respectively.

Conclusion

This study confirmed the high burden of HPV infections in young women, in Maputo city, Mozambique. The HPV prevalence was associated with high-risk sexual behaviour. Sexual education and STI prevention interventions should be intensified in Mozambique. Only a proportion of the high-risk HPV genotypes (37%) were covered by currently available vaccines.

Strengths and limitations

- This is the first study to describe the Human Papillomavirus (HPV) prevalence and genotype distribution in young adults (aged 18-24) in Mozambique.
- This study provides insights on the HPV types circulating in young populations, in Maputo city, which is an important public health information particularly for discussions on HPV vaccine introduction in the country.
- The absence of an older cohort (aged ≥ 25 years) for comparison of circulating HPV genotypes was a limitation of this study.
- The lower HPV prevalence in the male population may have been related to the site of sample collection, therefore additional studies in male population, in Mozambique, using different anatomic collection sites should be considered.

Key messages

- There is a high burden of HPV infection in young women, in Maputo city, Mozambique.
- High-risk sexual behavior was demonstrated to be associated with high prevalence of HPV infection.
- There is a need to intensify sexual education and STI prevention for young adults.

- Currently available HPV vaccines would only cover a proportion (37%) of the prevalent high-risk genotypes.

Introduction

Globally, cervical cancer (CC) is the fourth most common cancer in women and the second most common cancer in female African populations (1). More than 75% of cases occur in less developed areas. East Africa is the most affected region with an age-standardized incidence rate of 42.7 per 100.000 women. Mozambique has the second highest incidence of CC after Malawi (2).

Human papilloma virus (HPV) infection has been implicated in virtually all CC cases. To date, 201 different HPV types have been identified (3). More than 40 of these types can infect the anogenital tract and have been classified as low-risk (lrHPV6,-11,-40,-42,-43,-44,-54,-61,-62,-71,-72,-81,-83,-84, and -89), probably or possibly high-risk (pHR-HPV26,-30,-34,-53,-66,-67,-68,-69,-70,-73,-82,-85, and -97) and high-risk HPV types (hr-HPV16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58, and -59) depending on their ability to lead to malignant progression (4-6).

Worldwide, HPV16 has been responsible for more than half (54.4%) of the CC cases followed by HPV18 (16.5%), HPV58 (5.1%), HPV33 (4.7%), HPV45 (4.4%), HPV31 (3.6%), HVP52 (3.4%) and HPV35 (1.9%) (7). However, only a small proportion of infected women develop the disease. Persistent infection with hr-HPV types and integration of HPV-DNA in the host cells are usually required for malignant transformation of the cervix. The time between infection and initial pre-cancer lesions is around seven to ten years. Cervical cancer is more frequently diagnosed in middle-age women (40-64 years) thus suggesting that infections are taking place early in life. Globally, around 20% of all cervical cancers are

being diagnosed between the ages of 15-39 years (8). This suggests that a proportion of infections may be occurring even earlier.

Previous reports have shown a high prevalence of HPV infections in younger populations (9, 10) and reports in southern Africa demonstrated a high prevalence of HPV infections among women of 30 years or younger (11, 12). Limited information is available about the HPV epidemiology in Mozambique particularly in men and young populations in urban settings. Castellsagué et al have shown that the HPV prevalence is high (75%) in women aged 14-61 years in rural southern Mozambique (13). Cervical cancer is the most frequent cancer in Mozambican women (14). Others have described the contribution of HPV infection in the development of cervical cancers, in Mozambique (15-18).

By the end of 2016 HPV vaccination had only been introduced in 65 countries worldwide, seven being in the African continent. In Mozambique, HPV vaccine has yet not been introduced in the national expanded program on immunization. Currently 23 African countries, including Mozambique, are conducting or have recently finalized their HPV vaccine pilot projects (1).

Currently, the following three HPV vaccines are available: Gardasil® (which confers protection against HPV6,-11,-16, and -18 and cross-protection against HPV31); Cervarix® (which prevents against HPV16 and -18 and provides high and moderate cross-protection against HPV31,-45 and -33, respectively); and Gardasil®9 (which protects against HPV6,-11,-16,-18,-31,-33,-45,-52, and -58). Gardasil®9 is a second generation 9-valent vaccine that can theoretically prevent 87% of all hrHPV infections in Africa (19). Nonetheless, the HPV distribution varies between African regions, and the identification of country-specific profiles and their adequacy to the currently available vaccines remains of great importance when deciding on vaccine introduction.

In the present study, we aimed to (1) determine the prevalence and distribution of HPV infections in sexually active young adults; and (2) determine the suitability of the current HPV vaccines in the context of the Mozambican epidemic.

Subjects and Methods

This report adheres to the STROBE guidelines for the reporting of observational studies.

Study design and population

This was a cross-sectional ancillary study of a human immunodeficiency virus (HIV) incidence study conducted at a youth clinic in Maputo city, Mozambique, from August 2009 to October 2011 (20). In the parent study, briefly, 1,380 males and females aged 18–24 years were screened for HIV, syphilis and hepatitis B virus. HIV-seronegative subjects were enrolled and followed for one year with quarterly visits to the clinic for assessment of HIV serostatus. Baseline demographic and behavioural data were collected using structured questionnaires. In the present study, samples from male and female subjects were collected at one time point, at screening or at one of the follow-up visits. We aimed at sampling 500 individuals with equal gender distribution (250 females and 250 males) based on the possibility of recruiting male subjects at the youth clinic, which mainly caters to women. Cervical samples were collected in female participants via speculoscopy, using a Rovers® Viba-Brush (Rovers Medical Devices B.V., Oss., The Netherlands). Urethral samples were collected from male participants by gently inserting a cotton swab approximately 2–4 cm in the urethral meatus and rotating it in one direction. Both the brushes and swabs were immerged in 5 mL of SurePath cell-preservation solution (TriPath Imaging, Burlington, NC,

USA), transported to the study laboratory within the same day of collection and stored at +4 ºC to +8ºC for three months and then at -80ºC.

Laboratory testing

DNA was extracted from an initial sample volume of 1.5 mL using a QIAamp DNA Mini Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions and eluted to a final volume of 200 µl.

HPV genotyping was performed using the Clart® Human Papillomavirus 2 (Genomica, Madrid, Spain), a low-density microarray platform based on PCR amplification of a 450 bp fragment within an HPVL1 highly conserved region from 35 different HPV types (HPV6,-11,-16,-18,-26,-31,-33,-35,-39,-40,-42,-43,-44,-45,-51,-52,-53,-54,-56,-58,-59,-61,-62,-66,-68,-70,-71,-72,-73,-81,-82,-83,-84,-85, and -89) and a human gene control (CTFR). Individual genotyping results were analysed on the Clinical Array Reader (Genomica, Madrid, Spain). Adequacy of samples was assessed by amplification of the CTFR. Samples with undetectable DNA were rerun and the second result was considered final. The Clart® Human Papillomavirus 2 assay complies with the European Union (EU) safety, health or environmental requirements, with the EU legislation and with the European In-Vitro Diagnostic Devices Directive. This assay has been shown to have a similar performance as that of other well-established HPV screening assays, such as the FDA-approved Hybrid Capture 2 test (21, 22).

DNA extraction was performed at the Instituto Nacional de Saúde laboratories in Maputo, Mozambique. DNA Samples were then shipped to the Molecular Biology laboratories at the Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University Hospital, Örebro, Sweden, for HPV genotyping.

HIV testing was performed as previously described on site in Maputo (20).

Statistical analysis

Demographic and risk behavioural data were entered into a MySQL database version 5.1 (MySQL AB, 2008) and HPV laboratory results in a Microsoft Office Excel 2010 spreadsheet (Microsoft, Redmond, WA). For processing and analysis, data were imported into Stata version 14 (StataCorp. 2015. Stata: Release 14. Statistical Software. College Station, TX: StataCorp LP).

All analyses were stratified by gender. Descriptive statistics were employed as follows: frequencies for categorical variables, and means and standard deviations (SD) for quantitative variables. We used list wise deletion as the missing values were negligible. Here, we report the prevalence of HPV infections and HPV genotypes per gender and HIV status. Bivariate logistic analysis between sociodemographic and sexual behavioural characteristics and presence of any HPV was conducted. Age of individuals and age of sexual debut and characteristics whose p-value on bivariate logistic regression analysis was below 0.25 were included in the multivariable logistic regression. Additionally, to identify characteristics that would contribute to multiple HPV genotype infections a multinomial logistic regression (MNLR) was used. For this analysis, males and females are not stratified given the sample constraints. We first grouped the participants as follows: a) subjects without an HPV infection; b) subjects with one HPV infection (mono-infection); and c) subjects with at least two HPV infections (multiple infections). The MNLR per level of the factor under consideration provided two odds-ratios (one for mono-infected versus not-infected subjects and another for the subjects with multiple-infections versus not-infected subjects). We report both the unadjusted and adjusted odds-ratios and their respective 95% CI. For adjustment, we included factors whose p-values through the global likelihood ratio test were below 0.25 in

the MNLR bivariate analysis; gender and age were forced factors to include in the model. The significance level was set at 5%.

Ethics statement

Ethical approval was granted by the National Health Bioethics Committee of Mozambique (Ref. 148/CNBS of May 8, 2009 and Ref. 18/CNBS/11). Testing performed in Sweden was approved by the Regional Ethical Review Board Uppsala. Study investigators followed the GCP-ICH guidelines. Subjects signed an informed consent form prior to any study activities.

Results

In total, 489 participants (263 females and 226 males) were enrolled, but samples from 77 (15.7%) subjects had undetectable DNA levels and were excluded from the data analysis, being 27/263 (10.3%) from females and 50/226 (22.1%) from males.

Demographic and behavioural characteristics

Table 1 shows the socio demographic and sexual behavioural characteristics of study participants by sex. Of the 412 subjects with valid HPV results, 236 (57.3%) were females, and 176 (42.7%) were males. The mean age of study participants was 21.1 years (SD: 1.71) with males being slightly older than females (21.5 vs 20.8). Almost all participants were students (94.2%) and all had some formal education. Male subjects were more educated than females with more than half having secondary or higher educational degrees (56.8% vs 41.9%). Nearly all participants were single (98.8%). The median age at sexual debut was 17 years (interquartile range: 15–18) being 16 years (interquartile range: 14–18) for males and 17 years (interquartile range: 16–18) for females. The majority of subjects (89.3%) had more than one sexual partner in life (97.2% of males and 83.5% of females) and 24% reported two

or more sexual partners in the last 6 months (37.5% of males and 14% of females). Around one-fourth of the study population reported having at least one episode of sexually transmitted infection (STI) in life and this was seen more frequently in women than in men (34.3% vs 13.6%). Only 66.0% of subjects reported having used a condom at the time of their last sexual intercourse with females reporting a lower frequency compared to males (59.7% vs 74.4%). HIV infection was diagnosed in 21 (5.1%) participants, of which 17 (81.0%) were females.

Table 1 – Sociodemographic and sexual behavioural characteristics of study participants by sex.

	Total		Male		Female	
	N	%	N	%	N	%
Total	412	100.0	176	100.0	236	100.0
Age (years)						
Mean (SD)	21.1 (1.71)		21.5 (1.67)		20.8 (1.68)	
Marital Status						
Single	407	98.8	175	99.4	232	98.3
Married/Cohabitating	5	1.2	1	0.6	4	1.7
Education						
Secondary or less	213	51.7	76	43.2	137	58.1
Technical training	109	26.5	50	28.4	59	25.0
University grade	90	21.8	50	28.4	40	16.9
Occupation						
Student	388	94.2	163	92.6	225	95.3
Employed	23	5.6	12	6.8	11	4.7
Missing	1	0.2	1	0.6	0	0.0
Religion						
Christian	375	91.0	162	92.0	213	90.3
Other	37	9.0	14	8.0	23	9.7
Age at sexual debut						
Less than 18	285	69.2	115	65.3	170	72.0
18 or more	122	29.6	58	33.0	64	27.1
Missing	5	1.2	3	1.7	2	0.8
Number of sex partners in life						
0 - 1	44	10.7	5	2.8	39	16.5
> 1	368	89.3	171	97.2	197	83.5
Number of sex partners in the last 6 months						
0 - 1	313	76.0	110	62.5	203	86.0

> 1	99	24.0	66	37.5	33	14.0
Condom use in the last sexual intercourse						
No	139	33.7	45	25.6	94	39.8
Yes	272	66.0	131	74.4	141	59.7
Missing	1	0.2	0	0.0	1	0.4
Had a STI in life						
No	305	74.0	150	85.2	155	65.7
Yes	105	25.5	24	13.6	81	34.3
Missing	2	0.5	2	1.1	0	0.0
HIV infection						
Positive	21	5.1	4	2.3	17	7.2
Negative	391	94.9	172	97.7	219	92.8

HPV prevalence and genotype distributions

The overall HPV prevalence was 40.8% (168 infected subjects; 95% CI: 36.0–45.5) and was significantly higher in women than in men [150/236 (63.6%, 95% CI: 57.1-69.7) vs 18/176 (10.2%, 95% CI: 6.2-15.7), p<0.001]. The HPV prevalence among HIV infected subjects (14/21, 66.7%) was 69% higher than in the HIV negative subjects (154/391, 39.4%; p=0.013). Figure 1 and Table 2 show the distribution of HPV genotypes in male and female participants. Of the 412 enrolled participants, 115 (27.9%), 54 (13.1%) and 89 (21.6%) were infected with one or more high-risk, probably high-risk and low-risk HPV types, respectively. High-risk and pHR-HPV types were present in 124/150 (82.7%, 95% CI: 75.6-88.4) and 14/18 (77.8%, 95% CI: 52.4-93.6) of HPV-infected women and men, respectively (p=0.533). Overall, HPV52 was the most frequent type found (9.1%), followed by HPV35,-6,-16,-53,-58, and -51. Altogether, these HPV types accounted for 49.9% of the infections in women and men. In female participants, the most common genotypes were similar to the overall distribution (HPV52,-35,-16,-53,-58,-6, and -51) and accounted for half (50.3%) of the infections in women (Figure 1A). In contrast, male participants´ HPV infections were less diverse with HPV51 being the most frequent type found, followed by HPV6,-11,-52,-59, and -70 in equal proportions. These six types were responsible for 52% of the infections in men

(Figure 1B). High-risk HPV genotypes were identified in 11/21 (52.4%) of HIV-infected subjects. Three hrHPVs (HPV35, -52, and -58) accounted for 60% of all hrHPV infections in this population. There were no differences in the number of subjects infected with hrHPVs and pHR-HPVs in the HIV infected (p=0.552) and uninfected populations (p=1.000). In contrast, lrHPVs were more frequently observed in the HIV-infected subjects (p=0.022).

Table 2 – HPV prevalence and HPV genotype distribution by sex

	Total	Gender		HIV Serostatus		HPV genotype coverage		
		Male	Female	Negative	Positive	Gardasil®	Cervarix®	Gardasil®9
Total Subjects, N	412	176	236	391	21			
Number of HPV infections, N	351	25	326	305	46			
Subjects with at least 1 HPV infection[a], N (%)	168 (40.8)	18 (10.2)	150 (63.6)	154 (39.4)	14 (66.7)			
Subjects with High-Risk HPV infections[b], N (%)	115 (27.9)	10 (5.7)	105 (44.5)	104 (26.6)	11 (52.4)			
16	24 (5.8)	1 (0.6)	23 (9.7)	22 (5.6)	2 (9.5)	++++	++++	++++
18	11 (2.7)	0 (0.0)	11 (4.7)	10 (2.6)	1 (4.8)	++++	++++	++++
31	6 (1.5)	1 (0.6)	5 (2.1)	5 (1.3)	1 (4.8)	++++[ρ]	++++[§]	++++
33	4 (1.0)	0 (0.0)	4 (1.7)	2 (0.5)	2 (9.5)	++++[ρ]	++++[ρ]	++++
35	27 (6.6)	1 (0.6)	26 (11.0)	22 (5.6)	5 (23.8)			
39	4 (1.0)	0 (0.0)	4 (1.7)	4 (1.0)	0 (0.0)			
45	2 (0.5)	0 (0.0)	2 (0.8)	1 (0.3)	1 (4.8)		++++[§]	++++
51	20 (4.9)	3 (1.7)	17 (7.2)	19 (4.9)	1 (4.8)			
52	32 (7.8)	2 (1.1)	30 (12.7)	28 (7.2)	4 (19.0)			++++
56	4 (1.0)	1 (0.6)	3 (1.3)	4 (1.0)	0 (0.0)			
58	24 (5.8)	1 (0.6)	23 (9.7)	21 (5.4)	3 (14.3)			++++
59	13 (3.2)	2 (1.1)	11 (4.7)	13 (3.3)	0 (0.0)			
Total number of infections[c]	171 (48.7)	12 (48.0)	159 (48.8)	151 (49.5)	20 (43.5)			
Subjects with Probably or Possibly High-Risk HPV infections, N (%)	54 (13.1)	5 (2.8)	49 (20.8)	50 (12.8)	4 (19.0)			
26	1 (0.2)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)			
53	24 (5.8)	1 (0.6)	23 (9.7)	22 (5.6)	2 (9.5)			

66	10 (2.4)	1 (0.6)	9 (3.8)	9 (2.3)	1 (4.8)		
68	3 (0.7)	0 (0.0)	3 (1.3)	3 (0.8)	0 (0.0)		
70	12 (2.9)	2 (1.1)	10 (4.2)	11 (2.8)	1 (4.8)		
73	2 (0.5)	0 (0.0)	2 (0.8)	2 (0.5)	0 (0.0)		
82	6 (1.5)	1 (0.6)	5 (2.1)	6 (1.5)	0 (0.0)		
85	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Total number of infections[c]	58 (16.5)	5 (20.0)	53 (16.3)	54 (17.7)	4 (8.7)		
Subjects with Low-Risk HPV infections, N (%)	89 (21.6)	7 (4.0)	82 (34.7)	79 (20.2)	12 (57.1)		
6	24 (5.8)	2 (1.1)	22 (9.3)	21 (5.4)	3 (14.3)	++++	++++
11	8 (1.9)	2 (1.1)	6 (2.5)	6 (1.5)	2 (9.5)	++++	++++
40	6 (1.5)	0 (0.0)	6 (2.5)	5 (1.3)	1 (4.8)		
42	11 (2.7)	0 (0.0)	11 (4.7)	10 (2.6)	1 (4.8)		
43	1 (0.2)	1 (0.6)	0 (0.0)	1 (0.3)	0 (0.0)		
44	3 (0.7)	0 (0.0)	3 (1.3)	3 (0.8)	0 (0.0)		
54	7 (1.7)	0 (0.0)	7 (3.0)	4 (1.0)	3 (14.3)		
61	13 (3.2)	0 (0.0)	13 (5.5)	11 (2.8)	2 (9.5)		
62	14 (3.4)	1 (0.6)	13 (5.5)	11 (2.8)	3 (14.3)		
71	2 (0.5)	0 (0.0)	2 (0.8)	2 (0.5)	0 (0.0)		
72	2 (0.5)	0 (0.0)	2 (0.8)	1 (0.3)	1 (4.8)		
81	10 (2.4)	0 (0.0)	10 (4.2)	7 (1.8)	3 (14.3)		
83	8 (1.9)	1 (0.6)	7 (3.0)	7 (1.8)	1 (4.8)		
84	12 (2.9)	1 (0.6)	11 (4.7)	10 (2.6)	2 (9.5)		
89	1 (0.2)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)		
Total number of infections[c]	122 (34.8)	8 (32.0)	114 (35.0)	100 (32.8)	22 (47.8)		

++++ Genotype covered by the vaccine
++++§ Genotype highly covered through vaccine cross-protection
++++p Genotype moderately covered through vaccine cross-protection
[a]The denominator is total subjects
[b]The denominator is total subjects
[c]The denominator is number of HPV infections

Vaccine-matched HPV genotypes

Table 2 shows the distribution of HPV genotypes in women and men, and their correspondence to the genotypes present in the current HPV vaccines. Genotypes associated with vaccine cross-protection were included in the analysis and considered as covered by the vaccine. The total number of circulating HPV genotypes matching the vaccine genotypes were 5/33 (15.2%; Gardasil®: HPV6,-11,-16,-18, and -31), 5/33 (15.2%; Cervarix®: HPV16,-18,-31,-33, and -45) and 9/33 (27.3%; Gardasil®9: HPV6,-11,-16,-18,-31,-33,-45,-52, and -58), for the female population, and 4/18 (22.2%; Gardasil®: HPV6,-11,-16, and -31), 2/18 (11.1%; Cervarix®: HPV6 and -31) and 6/18 (33.3%; Gardasil®9: HPV6,-11,-16,-31,-52, and -58) for the male population. The three vaccines can cover 3/19 (15.8%; Gardasil®: HPV16,-18, and -31), 5/19 (26.3%; Cervarix®: HPV16,-18,-31,-33, and -45) and 7/19 (36.8%; Gardasil®9: HPV16,-18,-31,-33,-45,-52, and -58) hrHPVs circulating in women and 2/12 (16.7%; Gardasil®: HPV16 and -31), 2/12 (16.7%; Cervarix®: HPV16 and -31) and 4/12 (33.3%; Gardasil®9: HPV16,-31,-52, and -58) in men.

Factors associated with HPV infection

Overall, women had a significantly higher HPV infection prevalence than men ($p<0.001$). Although the odds of being infected with HPV reduced by 80% for each year of age, in the multivariate analysis this was not significant. Sexual debut before the age of 18 years, history of STI and infection with HIV were significantly associated with the presence of HPV infection ($p=0.008$; $p<0.001$; $p=0.013$, respectively) in the univariate analysis. When stratifying by gender (Tables 3A and 3B), the univariate analysis show that women who initiated sexual activity before the age of 18 were significantly more at risk of having a HPV infection ($p=0.041$) and a marginally significance association was seen in women who reported to have had more than 2 sexual partners in the last 6 months ($p=0.055$) but this was

not seen in the multivariate analysis. No significant associations were seen for male participants both in the univariate and multivariate analysis, but a marginally significant association was seen for a reported history of STI and HPV infection.

Table 3A – Factors associated with any HPV infection among male participants: unadjusted and adjusted

	Any HPV infection		Univariate			Multivariate		
	Negative (N)	Positive (N)	OR	CI 95%	p	OR	CI 95%	p
Total Observations	158	18				171		
Age (years)			0.90†	0.67 - 1.20	0.465	0.92†	0.66 - 1.27	0.605
Education					0.173			0.239
Primary and Secondary	66	10	1.00	-		1.00	-	
Technical training	44	6	0.90	0.31 - 2.65		1.02	0.31 - 3.34	
University grade	48	2	0.28	0.06 - 1.31		0.27	0.05 - 1.32	
Occupation					0.813			
Student	146	17	1.00	-				
Employed	11	1	0.78	0.09 - 6.43				
Religion					0.618			
Christian	146	16	1.00	-				
Other	12	2	1.52	0.31 - 7.41				
Age at sexual debut					0.268			0.191
Less than 18	101	14	1.00	-		1.00	-	
18 or more	54	4	0.53	0.17 - 1.70		0.43	0.12 - 1.52	
Number of sex partners in the last 6 months					0.254			
0 - 1	101	9	1.00	-				
> 1	57	9	1.77	0.67 - 4.72				
Condom use in the last sexual intercourse					0.822			
No	40	5	1.00	-				
Yes	118	13	0.88	0.30 - 2.63				
Had a STI in life					0.097			0.057
No	137	13	1.00	-		1.00	-	
Yes	19	5	2.77	0.89 - 8.65		3.21	0.97 - 10.64	

HIV infection					0.347
Positive	3	1	3.04	0.30 - 30.86	
Negative	155	17	1.00	-	

† Associated effect of 1 year increase

Table 3B – Factors associated with any HPV infection among female participants: unadjusted and adjusted

	Any HPV infection		Univariate			Multivariate		
	Negative (N)	Positive (N)	OR	CI 95%	p	OR	CI 95%	p
Total Observations	86	150				234		
Age (years)			0.87†	0.74 - 1.02	0.091	0.88†	0.75 - 1.04	0.139
Education					0.246			0.267
Primary and Secondary	51	86	1.00	-		1.00	-	
Technical training	17	42	1.47	0.76 - 2.84		1.57	0.80 - 3.11	
University grade	18	22	0.72	0.36 - 1.48		0.81	0.39 - 1.68	
Occupation					0.521			
Student	83	142	1.00	-				
Employed	3	8	1.56	0.40 - 6.04				
Religion					0.862			
Christian	78	135	1.00	-				
Other	8	15	1.08	0.44 - 2.67				
Age at sexual debut					0.041			0.109
Less than 18	55	115	1.00	-		1.00	-	
18 or more	30	34	0.54	0.30 - 0.97		0.61	0.33 - 1.12	
Number of sex partners in the last 6 months					0.055			0.150
0 - 1	79	124	1.00	-		1.00	-	
> 1	7	26	2.37	0.98 - 5.71		1.94	0.79 - 4.80	
Condom use in the last sexual intercourse					1.000			
No	34	60	1.00	-				
Yes	51	90	1.00	0.58 - 1.72				
Had a STI in life					0.665			
No	58	97	1.00	-				

Yes	28	53	1.13	0.65 - 1.98	
HIV infection					0.258
Positive	4	13	1.95	0.61 - 6.17	
Negative	82	137	1.00	-	

† Associated effect of 1 year increase

HPV mono-infection vs multiple-infections

Almost half, 75/168 (44.6%), of HPV infected subjects had a single infection, 48/168 (28.6%) had two concurrent HPV infections and 45/168 (26.8%) had three or more HPV co-infections (Figure 2). HPV-infected male participants had more frequently a single infection (72.2% of the cases) whereas more than 50% of HPV-infected females had two or more HPV co-infections. In the univariate analysis, several factors were shown to be associated with the presence of two or more (multiple) HPV co-infections. Being a woman, younger age (the odds of being infected with HPV reduced by 80% for each year of age), lower educational degree, sexual debut before the age of 18, reported STI in life and the presence of a HIV infection were factors associated with multiple-HPV infections. Nonetheless, in the multivariate analysis only three factors were demonstrated to be significantly associated, namely, being a woman (p=0.001), having started sexual activity before the age of 18 (p=0.008) and the presence of a HIV infection (p=0.003). Condom use in the last sexual intercourse was not significantly associated with protection against HPV infection (p=0.143) (Table 4).

Table 4 – Factors associated with the number (mono or multiple) of HPV infection: unadjusted and adjusted using multinominal logistic regression

	Univariate				p	Multivariate				p
	Mono-infection vs Negative		Multiple-infections vs Negative			Mono-infection vs Negative		Multiple-infections vs Negative		
	OR	CI 95%	OR	CI 95%		OR	CI 95%	OR	CI 95%	
Total Observations	412					406				
Gender										
Male	1.00	-	1.00	-	< 0.001	1.00	-	1.00	-	< 0.001
Female	8.76	4.56 - 16.83	32.33	12.65 - 82.66		7.23	3.64 - 14.33	28.38	10.78 - 74.70	
Age (years)	0.80†	0.69 - 0.94	0.80†	0.69 - 0.92	0.001	0.85†	0.71 - 1.02	0.87†	0.72 - 1.04	0.134
Marital Status										
Single	1.00	-	1.00	-	0.632					
Married/Cohabitating	1.64	0.15 - 18.29	2.66	0.37 - 19.16						
Education										
Secondary or less	1.00	-	1.00	-		1.00	-	1.00	-	
Technical training	0.98	0.54 - 1.79	0.94	0.54 - 1.65	0.040	1.42	0.71 - 2.83	1.77	0.87 - 3.61	0.246
University grade	0.41	0.19 - 0.87	0.47	0.24 - 0.91		0.59	0.26 - 1.33	0.84	0.38 - 1.85	
Occupation										
Student	1.00	-	1.00	-	0.784					
Employed	1.17	0.41 - 3.36	0.74	0.24 - 2.29						
Religion										
Christian	1.00	-	1.00	-	0.764					

Other	1.15	0.47 - 2.84	1.35	0.61 - 3.00						
Age at sexual debut										
Less than 18	1.00	-	1.00	-	0.003	1.00	-	1.00	-	0.008
18 or more	0.82	0.47 - 1.44	0.36	0.20 - 0.67		0.91	0.48 - 1.72	0.33	0.16 - 0.67	
Number of sex partners in life										
0 - 1	1.00	-	1.00	-	0.424					
> 1	0.64	0.29 - 1.43	0.67	0.32 - 1.41						
Number of sex partners in the last 6 months										
0 - 1	1.00	-	1.00	-	0.369					
> 1	0.65	0.34 - 1.23	0.82	0.47 - 1.44						
Condom use in the last sexual intercourse										
No	1.00	-	1.00	-	0.143	1.00	-	1.00	-	0.423
Yes	0.59	0.35 - 1.00	0.80	0.48 - 1.32		0.81	0.45 - 1.48	1.25	0.68 - 2.31	
Had a STI in life										
No	1.00	-	1.00	-	0.003	1.00	-	1.00	-	0.530
Yes	2.33	1.32 - 4.12	2.07	1.21 - 3.55		1.44	0.76 - 2.72	1.18	0.62 - 2.24	
HIV infection										
Positive	0.46	0.06 - 3.79	5.50	2.12 - 14.27	< 0.001	0.44	0.05 - 4.04	6.03	1.73 - 21.02	0.003
Negative	1.00	-	1.00	-		1.00	-	1.00	-	

† Associated effect of 1 year increase

Discussion

This was a cross-sectional study reporting the prevalence of HPV infections and genotype distributions in young adults aged 18–24 in Maputo city, Mozambique. The HPV prevalence in the female population was 63.6% (95CI: 57.1-69.7) and was similar to previous reports of 75.9% in women aged 14–61 years in rural southern Mozambique and other neighbouring countries (11-13, 23). The HPV prevalence in the male population was 10.2% (95CI: 6.1-15.7), which was lower than previous reports for African countries and other regions (2). Although no significant difference was observed in the number of hrHPV infections between women and men, a high susceptibility of women to HPV infections was demonstrated.

We report a lower prevalence of HPV infections in men than in women, which is contrary to what has been shown in neighbouring countries and other regions (24). Studies assessing different male anatomic collection sites show that HPV detection in urethral samples is suboptimal compared to other sampling sites in the male genitalia, but the sensitivity reported for urethral sampling has varied (25, 26). In a study comparing sampling from 7 male genital sites, Giuliano et al reported that detection was highest at the penile shaft (48%), while only 10% of HPV infections were detected when using urethral sampling (26). In contrast, the sensitivity of urethral samples for overall HPV detection was 54.2% in a study that explored expressed prostate secretions as an option for detection of HPV infections in Russian men (27). We found 50/226 (22.1%) urethral samples with undetectable DNA and 158/176 (89.8%) of valid samples tested negative. The first could be related to an inadequate sampling technique and the second to the selected anatomic collection site. Altogether, this could have contributed to the lower male HPV prevalence found in our study.

Worldwide, the prevalence of cervical HPV infections in healthy women has been demonstrated to be 11.7%. Nonetheless, in less developed regions, such as East Africa, the prevalence is three times the global figures (33.6%) (28). High-risk HPV16,-18,-52,-31,-58,-

39,-51, and -56 have been found in women with normal cytology. In this study, we demonstrated that hrHPV genotype distribution in Mozambican healthy young women differs from the global figures with HPV52,-35,-16,-53,-58, and -51 being the most prevalent. Catellsagué et al have previously described that hrHPVs 51,-35,-18,-31, -52 were the most commonly found in women aged 14-61 years with normal cytology, in a study conducted in southern Mozambique (13), which is similar to our findings. Furthermore, HPV16 contributed to only 6.8% of female infections in the present study. These findings are concordant with those from Castellsagué et al (13). Globally, HPV16 has been responsible for 22.5% of all infections in women, whereas in sub-Saharan Africa, it seems to have the lowest contribution (13.7%, 11.3%, and 11.1% for Southern, Eastern, and Western Africa, respectively (28). The peak prevalence of HPV infections in women was shown to occur in early ages (before the age of 30 years) (11, 12). Some reports have also described a less pronounced second peak prevalence in older women (around their 50´s). Reasons for the occurrence of the first HPV peak are related to absence of immune responses to HPV and sexual activity in younger women. The second peak is not consistently reported across the globe but it is speculated that hormonal changes my play a role. Although the majority of HPV infections in early ages clear spontaneously (29, 30) some do become chronic infections and may potentially evolve to malignant transformation of the cervix. In the present study we described the epidemiology of HPV infections in both women and men that belong to the "first HPV peak" population.

Our report shows that more than 50% of the study population had two or more concomitant HPV infections with more than 50% of female participants having two or more co-infections compared to only 27.8% of male participants. It has been demonstrated by others that women with multiple HPV infections take longer to clear their infections compared to women with a

single infection (29, 30) thus potentially contributing to the establishment of a persistent infection, a prerequisite for cervical cancer development. Several studies conducted in Mozambique have shown a strong association between HPV16,-33,-35,-45,-18,-31, and -58 with cervical neoplasia (15-18). In another Mozambican study HPV52 was reported to be the second most common hrHPV in women with abnormal cytology and the fourth most common in women with invasive cervical cancer (13). In the present study, we demonstrated a 50% match between the hrHPV types commonly circulating in the female population (HPV35,-16,-52 and -58) and HPVs types commonly present in cervical neoplasia in Maputo, Mozambique.

We confirmed the association between early onset of sexual debut, history of past STI and HIV infection with multiple HPV infections (31, 32). These findings reiterate the need to intensify sexual behaviour education and STI prevention in young populations.

This study shows a high prevalence of HPV infections (66.7%) in the HIV-infected population. A previous report showed a prevalence of HPV infections of 56.6% in HIV-infected African women, which is concordant with our findings (33). Other reports from South Africa have demonstrated an even higher HPV prevalence (75%) in the age group of 17–19 years(34). We demonstrated that HPV infections and multiple HPV infections are more frequent in HIV-infected subjects. Similar descriptions have been previously reported (34, 35). We have shown that HPV35,-52, and -58 were responsible for 60% of all hrHPV infections in this population. McDonald et al. have reported that hrHPV35,-58,-18,-45,-16, and -52 were the most common genotypes in HIV-infected women with normal cytology in neighbouring South Africa (34). Lastly, other reports in Africa have shown that the most frequent genotypes circulating in HIV-infected women were HPV16,-58,-52,-31,-35, and -18

(33). These findings suggest a high homology between the common hrHPV genotypes in Mozambique vs South Africa and other African regions. We have also described a high prevalence of lrHPV infections in the HIV-infected subjects. These findings were also demonstrated elsewhere (36).

Since 2014, Mozambique has been engaged in the HPV vaccine demonstration project which aimed at accessing the country´s preparedness for the introduction of an HPV vaccine in the national vaccination programme. Information regarding HPV types circulating in young populations is crucial for selection of a suitable vaccine. Currently available HPV vaccines, Gardasil®, Cervarix®, and Gardasil®9, can prevent up to seven hrHPV infections. Moderate to high cross-protection against HPV types phylogenetically related to HPV16 and -18 have also been reported with the use of Gardasil® and Cervarix®. Nonetheless, the level of protection has shown to be heterogeneous across the studies and a signal for reduction of vaccine efficacy with extended follow up has also been demonstrated (37). Our study shows that Gardasil®9 has the highest genotype coverage in both Mozambican women and men whereas Gardasil® has the lowest in women and Cervarix® in men. Gardasil®9 can provide protection against HPV52, the most common genotype found in this study. Nonetheless, Gardasil®9 would only cover 37% of the high-risk genotypes found. This has important implications for vaccine-strategic discussions. It should also send signals to vaccine manufacturers concerning future developments of new versions of the HPV vaccines. In the present study we did not assess HPV vaccination status since no HPV vaccine had been introduced or administered within the National Health System.

This study has some limitations. Power calculation was applied for the HIV incidence in the parent study but not for the HPV ancillary study. We did not include an older cohort (aged ≥ 25 years) for comparison of circulating HPV genotypes due to funding restrictions. This must

be considered in future studies to better describe the epidemiology of HPV infections in Mozambique. In our study, the questionnaire assessing sexual behavioural captured information about the number of sexual partners categorized into two variables only, either having one or more than one partner. This did not enable us to further analyse the influence of the number of sexual partners on HPV status. The low HPV prevalence in the male population may have been related to the site and technique of sampling and we therefore recommend that additional studies in male populations, in Mozambique, are considered.

Conclusion

This study confirmed the high burden of HPV infections in young women in the Maputo city, Mozambique. The HPV prevalence was demonstrated to be associated with high-risk sexual behaviour, thus confirming the need to intensify sexual education and STI prevention interventions. Additional studies involving other anatomic collection sites in men to confirm the comparatively low prevalence of HPV are needed. For vaccine-strategic discussions, it is important to consider that the current HPV vaccines only cover a proportion of prevailing HPV types in this area.

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Data sharing and Contributorship

Conceived and designed the study: Edna Viegas, Charlotta Nilsson, Kerstin Falk, Nafissa Osman, Ilesh Jani, Sören Andersson

Performed the study and experiments: Edna Viegas, Nália Ismael, Mallin Kaliff, Gabriella Lillsunde-larsson, Nafissa Osman

Analyzed the data: Edna Viegas, Orvalho Augusto

Wrote the paper: Edna Viegas, Charlotta Nilsson, Sören Andersson, Orvalho Augusto, Ilesh Jani, Gabriella Lillsunde-larsson, Kerstin Falk, Torbjörn Ramqvist, Nafissa Osman, Nália Ismael, Mallin Kaliff

Competing Interests

The authors have declared that no competing interests exist.

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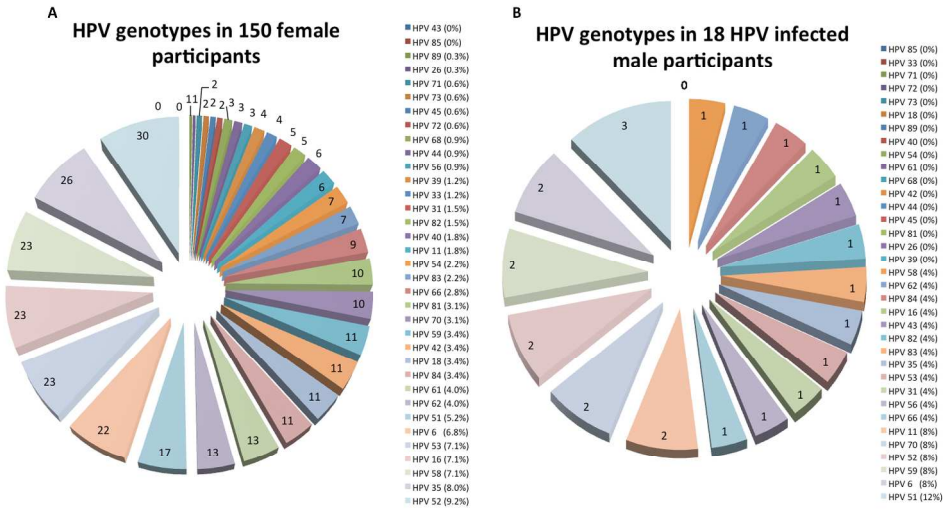
Figure Legend

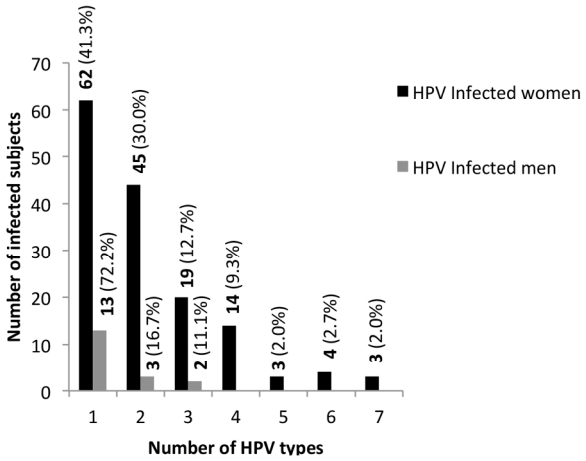
Figure 1. HPV genotype distribution in female (A) and male (B) participants. For calculation of proportions, the denominator used was the number of HPV infected participants per gender.

Figure 2. Distribution of HPV infections in female and male participants. The denominator is the total number of infected women and men, respectively.



HPV genotype distribution in female (A) and male (B) participants. For calculation of proportions, the denominator used was the number of HPV infected participants per gender.

273x159mm (300 x 300 DPI)



Distribution of HPV infections in female and male participants. The denominator is the total number of infected women and men, respectively.

179x113mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Indicated in the abstract page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2, 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	8, 20-24
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11 Table 1
		(b) Indicate number of participants with missing data for each variable of interest	10 Table 1
		(c) Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures over time	Not applicable
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear	11-20 Table 3 and 4

		which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	10, 15-16, 19-20 Tables 1, 3 and 4
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	21-24
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24-25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	25
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	26

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.