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Detection and typing of Human Papillomavirus in urine from patients attending a sexually transmitted infections clinic in Nairobi County, Kenya

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Abstract
Human papillomavirus (HPV) is a common sexually transmitted infection (STI) that has been etiologically linked to cervical cancer. Different types of samples can be used for cervical screening, including Pap test or biopsy and Liquid Based Cytology, visual inspection using acetic acid or Lugol's iodine, and HPV testing. These methods are invasive. The use of urine as an alternative specimen may be more widely accepted since it is non-invasive and the sample is readily available. The study aimed at detecting and genotyping HPV in urine from patients attending a sexually transmitted infections clinic in Nairobi County. It also aimed at assessing the factors associated with HPV infection. In this cross-sectional study, a structured 'risk factor' questionnaire was administered and HPV from urine specimen was genotyped using the L1 gene. Phylogenetic and molecular evolutionary analyses were conducted. Bivariate analysis and Pearson's chi square (χ^2) tests were used to determine the association between HPV infection and factors associated with HPV. A total of 222 adults (45 males and 177 females) aged 18-49 years were recruited. The prevalence of HPV among males and females was 22.2% (10/45) and 32.8% (58/177) respectively. The prevalence of high-risk types among males and females was 25% (1/4) and 27.5% (11/40) respectively. The high risk HPV genotypes detected among females were: HPV-16 (10%), -66 (7.5%), and -70 (7.5%) while low risk types were HPV 6 (27.5%), followed by -81 (25%), -83 (10%), -11 (7.5%), and -54 (2.5%) respectively. The prevalence of low risk types among males and females was 75% (3/4) and 72.5% (29/40) respectively. The prevalent low-risk HPV type detected in males was HPV type 6 (75%) while HPV-58 (25%) was the only high risk type in males. History of sexually transmitted infections was significantly associated with HPV infection among females (P=0.002). There was also significant association between marital status among males (p=0.046), how often one had used the contraceptives among females (p=0.038) and HPV genotypes at bivariate level. The results indicate high HPV prevalence, high risk and low risk HPVs could be detected in urine from the two populations. Therefore; molecular testing of HPV on urine samples is a method that utilizes a non-invasive technique that may increase screening coverage as it is easy to obtain.
Key words: urine, Human papillomavirus, HPV genotypes, PCR, cervical cancer.

1. Introduction

Human papillomavirus (HPV) is a common STI in the world. Up to 80% of sexually active people will be infected at sometime in their lives with approximately 10-20% developing persistent infection (Einstein *et al.,* 2009). However, majority of genital HPV infections are transient and regress spontaneously within 1-2 years of initial infection (Monsonego *et al.,* 2004). It is estimated by the World Health Organization (WHO) that 530,000 new cases of cervical cancer and 270,000 deaths are reported yearly in the world, with 85% of these deaths occurring in developing countries (Formana *et al.,* 2012). More than 128 genotypes have been characterized based on DNA sequence analysis (Zur Hausen, 2006; de Villiers *et al.,* 2004). Furthermore, HPVs are classified as either cutaneous or mucosal types (International Agency for Research on Cancer, 2011). The cutaneous types are epidermitropic while mucosal types are highly epitheliotropic (Burd, 2003). Mucosal HPV types are further classified into high risk (HR) such as (HPV-16, -18, -31, -33, -34, -35,-39, -45, -51, -52, -56, -58, -59, -66, -68, -69, -70, -73, -59 and -82) and low risk (LR) HPV types (6, 11, 42, 43 and 44) (Trottier & Burchell, 2009; Bouvard *et al.,* 2008). LR HPV types, such as HPV 6 or 11 can cause benign or low-grade abnormalities of the cervix, anogenital warts and recurrent respiratory papillomatosis (RRP) (Munoz *et al.,* 2003). HR-HPV types

such as 16 and 18 are implicated with squamous intraepithelial neoplasias (SILs) of the anogenital area, including cervical, vulvar, vaginal, penile, and anal and oropharyngeal cancers (Parkin &Bray, 2006; Watson *et al.,* 2009; Gillison, 2008). Currently, different types of samples can be used for screening cervical cancer. These may include: conventional cervical cytology (Papanicolaou (Pap smear)) or biopsy and liquid based cytology (LBC), visual inspection using acetic acid or Lugol's iodine (VIA-VILI), and HPV nucleic acid testing (NAT) (Tota *et al.,* 2014). Pap smear screening is the gold standard that is based on detecting abnormal cellular changes of cervical cells or premalignant lesions in asymptomatic women. However, NAT method is more sensitive than conventional cytology as it can be used to detect oncogenic HPV types responsible for lesions with normal cytology or low-grade squamous intraepithelial lesions (LSIL) (Wang *et al.,* 2013). Screening by this method has limitations. It is estimated that only 15-50% of patients with HPV infections are accurately identified by Pap smears (Meisels, 1983; Purola & Savia, 1977). Further, there is poor patient compliance as the efficacy of screening relies on repeated visits and obtaining samples is invasive and requires pelvic examination which is uncomfortable as well as it is time consuming for the health care provider (Reddy & Wasserman, 1997). Moreover, the success of Pap smear is hindered by women's educational levels, misconceptions, prejudices and socio-cultural barriers in low resource settings. Due to these challenges, identification of the disease during late stage, leads to high rates of cervical cancer incidence and mortality (Sankaranarayanan *et al.,* 2001; Cuzick *et al.,* 2008; Tornesello *et al.,* 2014). Perhaps, using alternative screening tools can overcome these challenges and could improve screening coverage among populations from low resource settings. Several studies have showed that HPV DNA could be detected with high sensitivity on urine samples (Brinkman *et al.,* 2002; Melchers *et al.,* 1989). Moreover, HPV DNA testing on urine samples is widely accepted because the sample is easily collected, non-invasive, and readily available especially for mass screening (Manhart *et al.,* 2006). Urine samples have been used to detect HPV in pre-cancer and cancer women (Das *et al.,* 1992). However, for asymptomatic infected men, there is no standard screening method as several sampling sites have been suggested for reliable detection of HPV in men (Melchers *et al.,* 1989). In addition, HPV is readily transmitted from men (reservoir) to women and greatly affects the risk of disease in women (Buckley *et al.,* 1981; Zunzunegui *et al.,* 1986; Agarwal *et al.,* 1993; Bosch *et al.,* 1996; Castellsague *et al.,* 2002). Male sexual behaviour affects rates of HPV infection and disease in female partners; hence an improved understanding of the infection in men is an important component of HPV-related cancer prevention. Thus we aimed at determining the prevalence and genotypes of HPV in urine from patients attending a STI clinic in Nairobi County. Furthermore, this study aimed at assessing the factors associated with HPV infection.

2. Methods

The study was a descriptive cross-sectional study that involved the collection of urine from individuals attending the Special Treatment Centre (STC) clinic between August 2013 and March 2014. Prior to the study, approval was granted by KEMRI National Review Board (SSC NO. 2442) and Nairobi County Department of Health Services. The clinic offers a range of STI care and treatment; provides free condoms, Voluntary and Counseling Testing unit (VCT), cervical screening, family planning and other related services. The study population comprised of Commercial Sex Workers (CSWs) and individuals aged between 18 and 49 years presenting with STI related symptoms in Nairobi County who consented to participate. A sample size of 222 was calculated as the representative of the population (both males and females). Patients attending the clinic were conveniently sampled to participate in the study. Potential participants were approached individually in the facility and those willing to participate, were invited for an interview by trained enumerators. Written informed consents were obtained to ensure that they understood everything about the purpose of the study. Data regarding factors associated with HPV infection were collected using a structured questionnaire. After the collection of data, participants were requested to provide approximately 10ml mid-stream self collected urine samples that were collected in 15 ml sterile falcon tubes containing 0.8 ml boric acid. The collected urine samples were then transported to Kenya Medical Research Institute (KEMRI) in a cold chain and stored at -20°C till use.

Each urine sample was initially subjected to centrifugation at 5,000 RPM for 10 minutes and the supernatant was discarded. The cell pellet was re-suspended in 0.5ml Phosphate Buffered Saline (PBS). Nucleic acid (HPV DNA) was extracted from the pellet using the QIAmp DNA Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. Briefly, 200 µL aliquot of urine sample was digested with 20µL of proteinase K, then lysed using 200µL lysis buffer, incubated at 56°C for 15 minutes. DNA was eluted in 50 µL Rnase free water. The extracted DNA was stored at -20 ^{0}C until use. The DNA were amplified in a PCR targeting the L1 gene of HPV using the MY09/11 consensus primers for first round and GP5+/GP6+ modified primers for the second round of amplification (Michelle *et al.,* 2011). For every PCR reaction, a negative control (H_2O) and a positive control (in-house) was used as control The following amplification profile was used for primary PCR: 95^{0}C

initial activation for 30 seconds, 95^0C denaturation for 30 seconds, 48^0C annealing for 30 seconds, and 72^0C extension for 1 min for 40 cycles; followed by a 10 minutes final extension at 72^0C; and a hold step at 4^0C while for secondary PCR: 95^0C initial activation for 30 seconds, 95^0C denaturation for 30 seconds, 43^0C annealing for 30 seconds, and 72^0C extension for 1 min for 40 cycles; followed by a 10 minutes final extension at 72^0C; and a hold step at 4^0C. All amplified products were separated by electrophoresis in a 2% agarose gel stained with ethidium bromide.

All PCR products that had amplified for HPV DNA were purified using QIAquick Purification kit according to the manufacturer's instructions; briefly, 5 volumes of buffer PB was added to 1 volume of PCR reaction and mixed. Followed by the addition of 10 microlitres of 3M Sodium acetate (PH 5.0) and mixed. In addition, washing was done by adding 750 µl buffer PE to the QIAquick column and then centrifuged for 30 seconds. Finally DNA was eluted by adding 50 microlitres of Rnase free water PH (7.0) and the column centrifuged at 13000 RPM for1 minute.

Amplicons were subjected to big dye terminator sequencing. Nucleotide sequences were edited and aligned using BioEdit sequence alignment editor software version 5.0.9. The newly sequenced (query sequences) were aligned along with reference sequences available in the gene bank. This was followed by CLUSTALW multiple sequence alignment. After some manual editing by stripping the ends the phylogenetic and molecular evolutionary analyses were conducted using MEGA6. Maximum parsimony test was used for phylogenetic reconstructions. The phylogenetic tree was constructed using MEGA 6.

Descriptive statistics were used to give proportions and frequencies. Bivariate analysis, Pearson's chi square (χ^2) test was used to determine the association between HPV infection and factors associated with HPV. $P \leq 0.05$ was considered statistically significant.

3. Results

Demographic and behavioural characteristics of patients attending STC

The demographic characteristics of patients are presented in (Table 1). A total of 222 adults aged 18-49 years with a mean age±SD of 32±SD=1.2 years were recruited. Forty five (20.3%) were males (mean age 34±SD=2.73 years) and 177 (79.7%) were females (mean age 31±SD=1.33) (Table 1). The overall prevalence of HPV was 22.2% and 32.8% among males and females respectively (Figure 1). Slightly less than half of males, 33.3% (15/45) were aged 40-49 years while 33.3% (59/177) females were aged 30-39 years. More than half of both males 66.7% (30/45) and females 54.2% (96/177) were married. Majority of males 48.9% (n=22) and females 55.4% (n=98) had no children. Most males 95.6% (43/45) and females 98.3% (174/177) were Christians. Majority of females 33.6% (56/177) had tertiary education. Less than half 33.3% (15/45) of males were formal employees while most females were self employed, 40.7% (72/177). Slightly half 82.2% (37/45) of males lived in permanent houses whereas more than half of females 71.2% (126/177) also lived in permanent houses. Most males 100% (n=45) and females 98.3% (n=174) were sexually active. Slightly half of males, 48.9% (n=22) had sex before18 years while majority of females 56.5% (n=100) were aged 19-24 years. Eighty six point seven percent (n=39) and 73.4% (n=130) males and females had two or more sex partners respectively. Most males 35.6% (n=16) sometimes used condom during sex while most females 44.1% (n=78) never used condom during coitus. More than half males and females reported to have not used contraceptives at 95.6% (n=43) and 58.2% (n=103) respectively. Most males 82.2% (n=37) and females 98.3% (n=174) were not smoking. However, a large proportion 55.6% (n=25) of males were drinking alcohol while most females, 83.6% (n=148) were not taking alcohol.

Distribution of HPV genotypes among patients attending STC in Nairobi

The most frequently detected genotype was HPV-6 (75%), which is classified as low risk type while HPV-58 (25%) was the only high-risk type detected among males. Among females, HPV types 16 (10%), 66 (7.5%), 70 (7.5%), and 85 (2.5%) were the high-risk types that were detected. Among the Low risk types, HPV-6 (27.5%) was the most prevalent type detected followed by HPV-81 (25%), HPV-83 (10%), HPV-11 (7.5%) and 54 (2.5%) respectively. The summary of both high and low risk HPV types are shown in (Table 2).

Factors associated with HPV infection among patients attending STC in Nairobi

Factors associated with HPV infection is presented in (Table 3). Age as a factor for HPV DNA positivity was not significantly associated with HPV infection. However, history of STIs and diseases was significantly associated with HPV infection among females only ($\chi2=9.894$; $p=0.002$) at bivariate level. More than half 63.8% (37/58) of females reported to have had history of STIs whereas most males 90% (9/10) had history of STIs before. Cervical screening and HPV vaccination was also not significantly associated with HPV positivity among females. However, no risk factor was found to contribute independently to HPV infection on multivariate analyses.

HPV genotypes associated with factors for HPV infection among patients attending STC

The prevalence of high-risk types among males and females was found to be 25% (1/4) and 27.5% (11/40) respectively. Further, the prevalence of low risk types among males and females was 75% (3/4) and 72.5% (29/40) respectively. How often one had used the contraceptives was significantly associated with HPV types among females (p=0.038). HPV genotypes in relation to factors for HPV DNA positivity are shown in (Table 4).

HPV nucleotide accession numbers

The nucleotide sequences that were determined in the present study were deposited in the Gene Bank and these are their accession numbers: [GenBank: KR674038-KR674081]. The accession numbers for the reference HPV nucleotide sequences are as follows: HPV-85 [GenBank:AF131950.1]; HPV-54 [GenBank:AF436129.1]; HPV-16 [GenBank:AF548834.1]; HPV-83 [GenBank:AJ617544.1]; HPV-11 [GenBank:EF626589.1]; HPV-58 [GenBank:JN383597.1]; HPV-70 [GenBank:JN617895.1]; HPV-6 [GenBank:JN617897.1]; HPV-66 [GenBank:JN661519.1] and HPV-81 [GenBank:KM501530.1]. In figure 2, the phylogenetic tree is shown.

4. Discussion

Few studies have been conducted on the prevalence and genotyping of HPV in Sub-Saharan Africa, including Kenya. Most of the data that are available are from HPV studies in women as data on infections in men are very limited. Most research has focused on HPV infection in women because of the association of HPV infection and cervical cancer using cervical smears but in men HPV is mostly asymptomatic. Therefore, to the best of our knowledge this is the first study in Kenya to report HPV infection and genotypes in urine.

Among the 222 patients aged between 18-49 years who were enrolled, the crude prevalence was 22.2% (10/45) and 32.8% (58/177) in males and females respectively (OR at 95% CI; (0.272-1.266) $\chi 2=1.878$; p=0.171). The prevalence was high as well as low as compared to previous studies. There was an agreement in a study that was conducted among Japanese men who attended an STD clinic and the present study since they found an HPV prevalence rate of 22.1% in urine samples (Nakashima *et al.,* 2014). While the present study found 22.2% HPV prevalence among the same population though the study populations and geographic locations was different.

Smits *et al.,* 2005 reported HPV prevalence in urine samples from 104 HIV infected and 115 uninfected men. The observed prevalence rates of HPV infection among HIV positive men was 39.4%. This was high as compared to the present study that recorded 22.2% prevalence among men. In the HIV positive men HPV 52 was the most predominant genotype observed in 13 men (12.5%), followed by HPV types 18 (6.7%), 35 (5.8%), and 70 (4.8%). HPV 6 and 11 were only observed in two HIV-positive men and were absent in the HIV-negative group. In the present study, LR HPV types were found slightly more often than HR types in males. Among the LR types, HPV 6 (75%) was the most frequently detected among males while HPV 58 (25%) was the only HR type detected. In females, high risk types were the most frequently detected as compared to low risk types. The most prevalent HR HPV types detected among females were: HPV-16 (10%), 66 (7.5%), and 70 (7.5%) while HPV-85 (2.5%) was the least prevalent type detected. The most predominant LR HPV type detected in females was HPV type 6 (27.5%), followed by 81 (25%), 83 (10%), 11 (7.5%), and 54 (2.5%) respectively.

The present study also found a high prevalence of HPV infection at 22.2% and 32.8% among males and females attending a STI clinic respectively as compared to a study by Bianchi *et al.,* 2013 that reported a very low prevalence rate of 1.5% (13/870) of HPV infection among Italian adolescents, and found a significantly lower prevalence in boys 2/501 (0.4%) than in girls 11/369 (3.0%). In the two HPV DNA positive boys the infection was attributed to HPV-70 while in girls 63.6% (95%CI 33.6-87.2) of the 11 HPV-positive girls had a single infection and 4 (36.4%; 95% CI 12.8-66.4) were infected with two genotypes. A total of 8 different genotypes were detected among 15 infections identified in females: HPV-16 (4/15, 26.7%), HPV-70 (3/15, 20.0%), HPV-6 and HPV-11 (2/15 each, 13.3%) and HPV-52, HPV-54, HPV-66 and HPV-87 (1/15 each, 6.7%). The only HPV positive girl among the 11-14-year age-group was infected with HPV-70.

Paired 50 urine and 50 cervical samples from women patients study was compared to the present study. Thirty four percent (17/50) cases studied were positive for HPV DNA in the cervical samples, and 22% (11/50) cases were positive for HPV DNA in urine samples. This positivity is low as compared to the present study that determined 32.8% prevalence among females. This may be attributed to small sample size, different populations studied and different geographic distribution. When considering the viral type there was concordance in 7 of 9 cases. HPV type 16 was detected in both cervical and urine samples. In 1 case, type 54 was detected in the cervical sample and type 77 in the urine sample; and in the remaining case, type 66 was detected in the cervical sample and type 68 in the urine sample (Alameda *et al.,* 2007).

In another study that consisted of 101 HIV-positive women recruited from the HIV Outpatient Clinic in New Orleans, La., who provided their urine samples and matching cervical swab samples. The overall prevalence of detection of any HPV DNA was 48% for the urine specimens and 58% for the cervical swab specimens. These

rates are higher as compared to the present study especially urine sampling that determined HPV prevalence rate at 32.8% among females. The differences in prevalence between these studies and the present study may be due to the different study populations that were evaluated. The prevalence rates of HPV positivity (32.8%) found in the present study also differ from those found in a study conducted by Sellors *et al.,* 2000 with a population of women who were attending a colposcopy clinic. The overall prevalence of HPV of any type in the study was 35% in urine specimens and 63% in cervical swab specimens respectively. The difference between the two studies can again be explained in part by the different populations examined (Brinkman *et al.,* 2002).

5. Conclusions

High risk and low risk HPV can be detected in urine of sexually active individuals as it can be transported by urine, probably in the exfoliated infected HPV cells. The prevalence of HPV was also found to be high in both males and females attending STC despite small sample size of men who participated. Nucleic acid testing (NAT) using urine is a non-invasive and easier method to determine HPV infections among populations with poor gynaecologic attention. This is important for monitoring population prevalence and strategy for HPV vaccination especially among men and women.

Future studies should focus on paired urine and cervical smears/endocervical swabs for women and for men penile/urethral swabs for comparison of HPV genotypes from different anatomical sites.

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Table 1: Demographic and behavioural characteristics of patients attending STC

	Gender				
	Male (n=45)		Female (n=177)		
Variable	**Frequency**	**Percent**	**Frequency**	**Percent**	**Total**
Age(years)					
18-24	7	15.6	44	24.9	51
25-29	10	22.2	45	25.4	55
30-39	13	28.9	59	33.3	72
40-49	15	33.3	29	16.4	44
Marital status					
Married	30	66.7	96	54.2	126
Single	11	24.4	69	39	80
Separated	4	8.9	11	6.2	15
Widowed	-	-	1	0.6	1
Religion					
Christians	43	95.6	174	98.3	217
Muslims	2	4.4	3	1.7	5
Education level					
No education	-	-	3	1.7	3
Primary	15	33.3	60	33.9	75
Secondary	15	33.3	58	32.8	73
Tertiary	15	33.3	56	31.6	71
Occupation					
Agricultural labourer	2	4.4	3	1.7	5
Formal employee	15	33.3	42	23.7	57
Students	2	4.4	36	20.3	38
Unemployed	4	8.9	14	7.9	18
Self employed	15	33.3	72	40.7	87
Other cadres	7	15.6	10	5.6	17
Housing					
Temporary	3	6.7	32	18.1	35
Semi-permanent	5	11.1	19	10.7	24
Permanent	37	82.2	126	71.2	163
Sexually active					
Yes	45	100	174	98.3	219
No	-	-	3	1.7	3
Age at 1st sex					
Below 18 years	22	48.9	67	37.9	89
19-24 years	20	44.4	100	56.5	120
Above 25 years	3	6.7	10	5.6	13
No. of sex partners					
None	-	-	1	0.6	1
One	6	13.3	46	26	52
Two or more	39	86.7	130	73.4	169
Condom use					
Never	14	31.1	78	44.1	92
Rarely	8	17.8	17	9.6	25
Sometimes	16	35.6	55	31.1	71
Often	7	15.6	26	14.7	33
Missing	-	-	1	0.6	1
Sex frequency/day					
None	4	8.9	25	14.1	29
Once	23	51.1	106	59.9	129
Twice	10	22.2	28	15.8	38
More than twice	8	17.8	17	9.6	25
Missing			1	0.6	1
Genital hygiene					
Yes	38	84.4	150	84.7	188

No	7	15.6	26	14.7	33
Missing	-	-	1	0.6	1
Parity					
None	22	48.9	98	55.4	120
One	10	22.2	42	23.7	52
Two	8	17.8	20	11.3	28
Three	4	8.9	9	5.1	13
>3	1	2.2	7	4	8
Missing	-	-	1	0.6	1
Contraceptives use					
Yes	2	4.4	74	41.8	76
No	43	95.6	103	58.2	146
History of STIs					
Yes	29	64.4	83	46.9	112
No	16	35.6	94	53.1	110
Cervical screening					
Yes	-	-	45	25.4	45
No	-	-	132	74.6	132
HPV vaccination					
Yes	-	-	2	1.1	2
No	-	-	175	98.9	175
Smoking habits					
Yes	8	17.8	3	1.7	11
No	37	82.2	174	98.3	211
Alcohol uptake					
Yes	25	55.6	29	16.4	54
No	20	44.4	148	83.6	168

Table 2: Overall distributions of HPV types in males and females participants

	Gender			
	Male		**Female**	
HPV types	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**
6 (LR)	3	75	11	27.5
11(LR)			3	7.5
16(HR)			4	10
54(LR)			1	2.5
58(HR)	1	25	-	-
66(HR)			3	7.5
70(HR)			3	7.5
81(LR)			10	25
83(LR)			4	10
85(HR)			1	2.5
Total	4	100	40	100

Table 3: Factors associated with HPV infection among patients attending STC

	HPV DNA status by gender					
	Male (n=45)			**Female (n=177)**		
Variable	**positive**	**Negative**	**p-value**	**Positive**	**Negative**	**p-value**
Age in yrs			0.927			0.807
18-24	1(10%)	6(17.1%)		14(24.1%)	30(25.2%)	
25-29	2(20%)	8(22.9%)		14(24.1%)	31(26.1%)	
30-39	3(30%)	10(28.6%)		22(37.9%)	37(31.1%)	
40-49	4(40%)	11(31.4%)		8(13.8%)	21(17.6%)	
Marital status			0.515			0.22
Married	7(70%)	23(65.7%)		27(46.6%)	69(58%)	
Single	3(30%)	8(22.9%)		27(46.6%)	42(35.3%)	
Separated	0(0%)	4(11.4%)		3(5.2%)	8(6.7%)	
Widowed	0(0%)	0(0%)		1(0.6%)	0(0%)	
Religion			0.334			0.983
Christians	9(90%)	34(97.1%)		57(98.3%)	117(98.3%)	
Muslims	1(10%)	1(2.9%)		1(1.7%)	2(1.7%)	
Education level			0.598			0.127
None	0(0%)	0(0%)		0(0%)	3(2.5%)	
Primary	2(20%)	13(37.1%)		14(24.1%)	46(38.7%)	
Secondary	4(40%)	11(31.4%)		23(39.7%)	35(29.4%)	
Tertiary	4(40%)	11(31.4%)		21(36.2%)	35(29.4%)	
Occupation			0.439			0.463
Agric. Labourer	1(10%)	1(2.9%)		0(0%)	3(2.5%)	
Formal employee	1(10%)	14(40%)		11(19%)	31(26.1%)	
Students	1(10%)	3(8.6%)		6(10.3%)	8(6.7%)	
Unemployed	0(0%)	2(5.7%)		15(25.9%)	21(17.6%)	
Self employed	5(50%)	10(28.6%)		22(37.9%)	50(42%)	
Others	2(20%)	5(14.3%)		4(6.9%)	6(5%)	
Housing			0.411			0.720
Temporary	0(0%)	3(8.6%)		12(20.7%)	20(16.8%)	
Semi-permanent	2(20%)	3(8.6%)		7(12.1%)	12(10.1%)	
Permanent	8(80%)	29(82.9%)		39(67.2%)	87(73.1%)	
Sexually active?			-			0.223
Yes	10(100%)	35(100%)		58(100%)	116(97.5%)	
No	0(0%)	0(0%)		0(0%)	3(2.5%)	
Age at first sex			0.065			0.130
<18 years	6(60%)	16(45.7%)		23(39.7%)	44(37%)	
19-24 years	2(20%)	18(51.4%)		29(29%)	71(59.7%)	
>25 years	2(20%)	1(2.9%)		6(60%)	4(3.4%)	
No. of sexual partners			0.725			0.573
None	0(0%)	0(0%)		0(0%)	1(0.8%)	
One	1(10%)	5(14.3%)		13(28.3%)	33(27.7%)	
2 or more	9(90%)	30(85.7%)		45(34.6%)	85(71.4%)	
Condom use			0.866			0.099
Never	4(40%)	10(28.6%)		18(31%)	60(50.8%)	
Rarely	2(20%)	6(17.1%)		7(12.1%)	10(8.5%)	
Sometimes	3(30%)	13(37.1%)		23(39.7%)	32(27.1%)	
Often	1(10%)	6(17.1%)		10(17.2%)	16(13.6%)	
Sex frequency in a day			0.58			0.492
None	1(10%)	3(8.6%)		7(12.1%)	18(15.3%)	
Once	5(50%)	18(51.4%)		32(55.2%)	74(62.7%)	
Twice	1(10%)	9(25.7%)		12(20.7%)	16(13.6%)	
>twice	3(30%)	5(14.3%)		7(12.1%)	10(8.5%)	
Genital hygiene			0.660			0.246
Yes	8(80%)	30(85.7%)		52(89.7%)	98(83.1%)	
No	2(20%)	5(14.3%)		6(10.3%)	20(16.9%)	

Parity			0.098			0.634
None	4(40%)	18(51.4%)		29(50%)	69(58%)	
One	4(40%)	6(17.1%)		18(31%)	24(20.2%)	
Two	0(0%)	8(22.9%)		7(12.1%)	13(10.9%)	
Three	1(10%)	3(8.6%)		2(3.4%)	7(5.9%)	
>three	1(10%)	0(0%)		2(3.4%)	5(4.2%)	
Contraceptive use			0.439			0.807
Yes	0(0%)	2(5.7%)		25(43.1%)	49(41.2%)	
No	10(100%)	33(94.3%)		33(56.9%)	70(58.8%)	
History of STIs			0.056			0.002*
Yes	9(90%)	20(57.1%)		37(63.8%)	46(38.7%)	
No	1(10%)	15(42.9%)		21(36.2%)	73(61.3%)	
Cervical screening			-			0.231
Yes	0(0%)	0(0%)		18(31%)	27(22.7%)	
No	0(0%)	0(0%)		40(69%)	92(77.3%)	
HPV vaccination						0.602
Yes	0(0%)	0(0%)		1(1.7%)	1(0.8%)	
No	10(100%)	35(100%)		57(98.3%)	118(99.2%)	
Smoking habits			0.835			0.983
Yes	2(20%)	6(17.1%)		1(1.7%)	2(1.7%)	
No	8(80%)	29(82.9%)		57(98.3%)	117(98.3%)	
Alcohol uptake			0.297			0.130
Yes	7(70%)	18(51.4%)		13(22.4%)	16(13.4%)	
No	3(30%)	17(48.6%)		45(77.6%)	103(86.5%)	

** Significant p<0.05*

Table 4: HPV types associated with factors for HPV DNA positivity among patients attending STC

	HPV genotypes by gender					
	Male			**Female**		
Variable	**High-risk**	**Low-risk**	**p-value**	**High-risk**	**Low-risk**	**p-value**
Age in yrs			0.135			0.327
18-24	0(0%)	0(0%)		2(18.2%)	6(20.7%)	
25-29	1(100%)	0(0%)		5(45.5%)	6(20.7%)	
30-39	0(0%)	1(33.3%)		4(36.4%)	13(44.8%)	
40-49	0(0%)	2(66.7%)		0(0%)	4(13.8%)	
Marital status			**0.046***			0.796
Married	0(0%)	3(100%)		6(54.5%)	14(48.3%)	
Single	1(100%)	0(0%)		5(45.5%)	14(48.3%)	
Separated	0(0%)	0(0%)		0(0%)	1(3.4%)	
Widowed	0(0%)	0(0%)				
Religion			-			-
Christians	1(100%)	3(100%)		11(100%)	29(100%)	
Muslims	0(0%)	0(0%)		0(0%)	0(0%)	
Education level			0.513			0.778
None	0(0%)	0(0%)		0(0%)	0(0%)	
Primary	0(0%)	1(33.3%)		3(27.3%)	5(17.2%)	
Secondary	1(100%)	1(33.3%)		4(36.4%)	12(41.4%)	
Tertiary	0(0%)	1(33.3%)		4(36.4%)	12(41.4%)	
Occupation			0.505			0.609
Agric. Labourer	0(0%)	0(0%)		0(0%)	0(0%)	
Formal employee	0(0%)	0(0%)		1(9.1%)	8(27.6%)	
Student	0(0%)	0(0%)		1(9.1%)	5(17.2%)	
Unemployed	0(0%)	0(0%)		3(27.3%)	6(20.7%)	
Self employed	1(100%)	2(66.7%)		5(45.5%)	9(31%)	
Other cadres	0(0%)	1(33.3%)		1(9.1%)	1(3.4%)	
Housing			0.505			0.973
Temporary	0(0%)	0(0%)		2(18.2%)	6(20.7%)	

Semi-permanent	0(0%)	1(33.3%)		1(9.1%)	3(10.3%)	
Permanent	1(100%)	2(66.7%)		8(72.7%)	20(69%)	
Sexually active?			-			-
Yes	1(100%)	3(100%)		11(100%)	29(100%)	
No	0(0%)	0(0%)		0(0%)	0(0%)	
Age at first sex			0.505			0.910
<18 years	1(100%)	2(66.7%)		5(45.5%)	11(37.9%)	
19-24 years	0(0%)	0(0%)		5(45.5%)	15(51.7%)	
>25 years	0(0%)	1(33.3%)		1(9.1%)	3(10.3%)	
No. of sexual partners			-			0.687
None	0(0%)	0(0%)		0(0%)	0(0%)	
One	0(0%)	0(0%)		2(18.2%)	7(24.1%)	
2 or more	1(100%)	3(100%)		9(81.8%)	22(75.9%)	
Condom use			0.248			0.205
Never	0(0%)	2(66.7%)		1(9.1%)	10(34.5%)	
Rarely	0(0%)	0(0%)		0(0%)	3(10.3%)	
Sometimes	1(100%)	1(33.3%)		7(63.6%)	11(37.9%)	
Often	0(0%)	0(0%)		3(27.3%)	5(17.2%)	
Sex frequency in a day			0.505			0.092
None	0(0%)	0(0%)		1(9.1%)	2(6.9%)	
Once	1(100%)	2(66.7%)		6(54.5%)	16(55.2%)	
Twice	0(0%)	1(33.3%)		1(9.1%)	10(34.5%)	
>twice	0(0%)	0(0%)		3(27.3%)	1(3.4%)	
Genital hygiene			0.248			0.114
Yes	1(100%)	1(33.3%)		9(81.8%)	28(96.6%)	
No	0(0%)	2(66.7%)		2(18.2%)	1(3.4%)	
Parity			0.135			0.933
None	1(100%)	0(0%)		6(54.5%)	14(48.3%)	
One	0(0%)	2(66.7%)		4(36.4%)	10(34.5%)	
Two	0(0%)	0(0%)		1(9.1%)	3(10.3%)	
Three	0(0%)	0(0%)		0(0%)	1(3.4%)	
>3	0(0%)	1(33.3%)		0(0%)	1(3.4%)	
Contraceptives use			-			0.629
Yes	0(0%)	0(0%)		4(36.4%)	13(44.8%)	
No	1(100%)	3(100%)		7(63.6%)	16(55.2%)	
If yes, how often						**0.038***
Regular	-	-		2(66.7%)	12(100%)	
Sometimes	-	-		1(33.3%)	0(0%)	
History of STIs			-			0.911
Yes	1(100%)	3(100%)		7(63.6%)	19(65.5%)	
No	0(0%)	0(0%)		4(36.4%)	10(34.5%)	
Cervical screening			-			0.687
Yes	-	-		2(18.2%)	7(24.1%)	
No	-	-		9(81.8%)	22(75.9%)	
HPV vaccination			-			0.100
Yes	0(0%)	0(0%)		1(9.1%)	0(0%)	
No	1(100%)	3(100%)		10(90.9%)	29(100%)	
Smoking habits			0.505			-
Yes	0(0%)	1(33.3%)		0(0%)	0(0%)	
No	1(100%)	2(66.7%)		10(100%)	29(100%)	
Alcohol uptake			0.505			0.687
Yes	1(100%)	2(66.7%)		2(18.2%)	7(24.1%)	
No	0(0%)	1(33.7%)		9(81.8%)	22(75.9%)	

** Significant p<0.05*

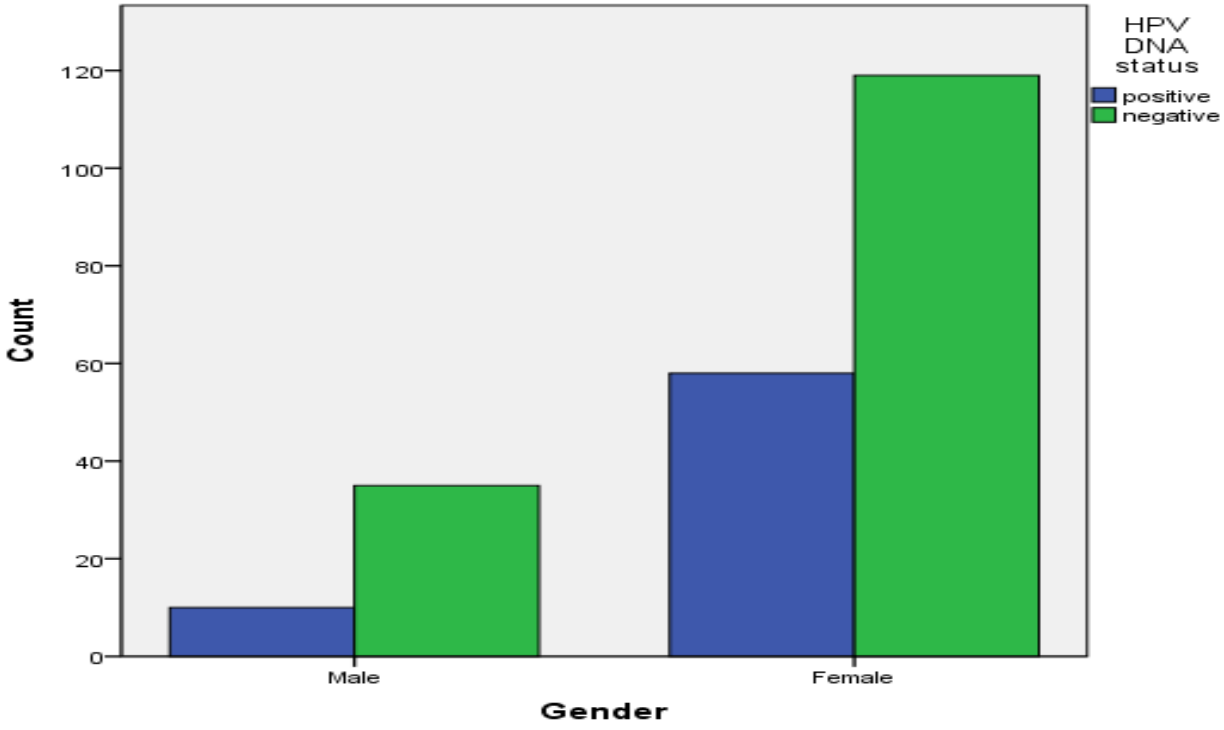

Figure 1: Prevalence of HPV according to gender 22.2% (10/45) among men and 32.8% (58/177) among women.

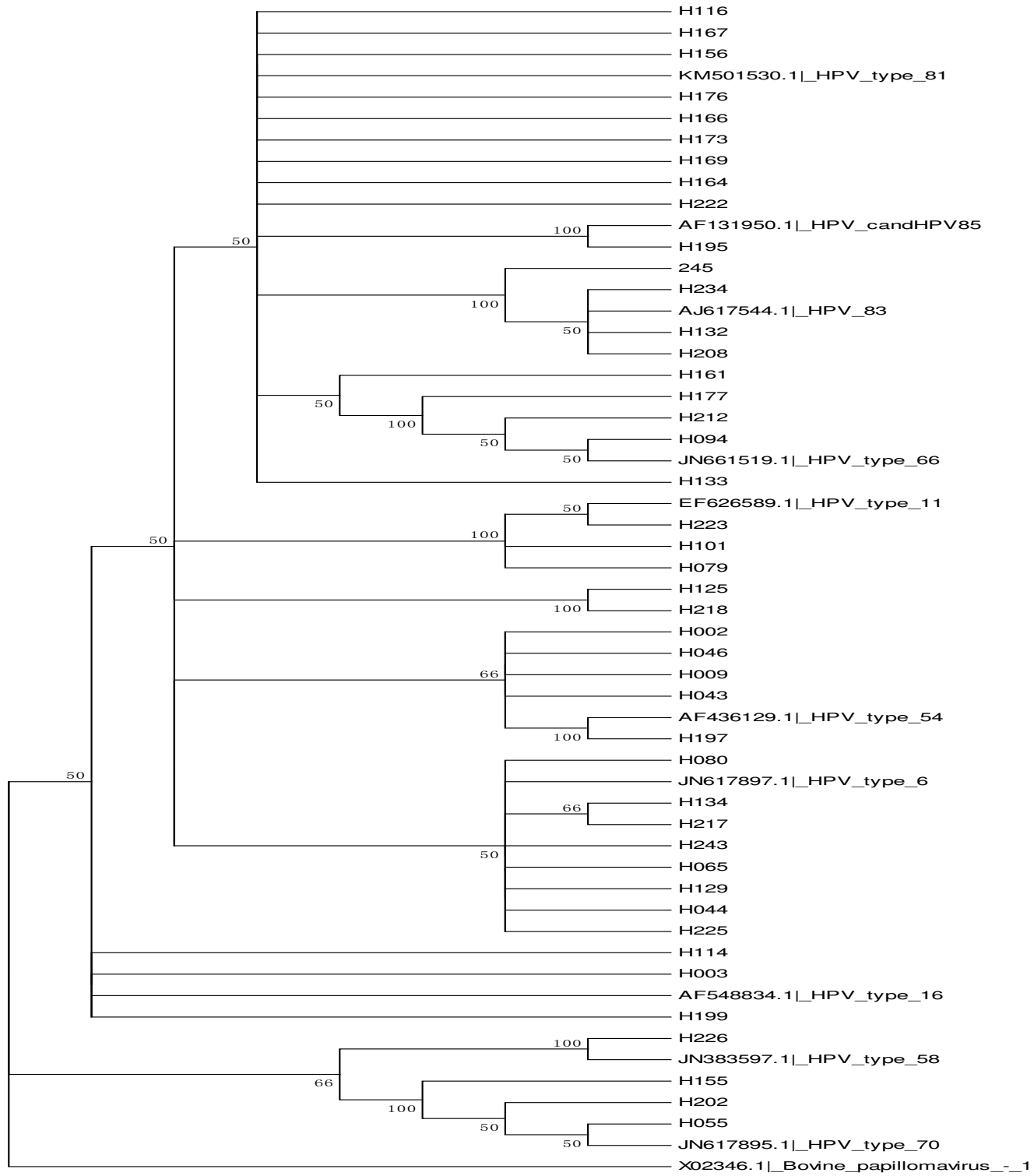

Figure 2: Phylogenetic analysis of L1 nucleotide sequences amplified by GP5+/GP6+ primers in HPV isolates from patients attending the STC clinic in Nairobi County.

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