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DNA GENOTYPE OF HUMAN PAPILLOMA VIRUS INFECTION AMONG THE ADOLESCENT GIRLS AT KENYATTA NATIONAL HOSPITAL YOUTH CLINIC
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ABSTRACT

***Background*: Adolescents have risky sexual behaviour exposing them to the Human Papilloma Virus (HPV) infection. With clear causal relationship between high risk the HPV (16 and 18) infection and invasive cancer, this study focuses on the distribution of HPV genotypes among the female adolescent and youth at the Kenyatta National Hospital (KNH) youth clinic.**

***Objective*: To determine DNA genotype of HPV infection among adolescent girls at KNH - Youth Clinic and use it as an advocacy tool for the introduction of the HPV vaccine provision in the clinic.**

***Design*: Cross-sectional study.**

***Setting*: Kenyatta National Hospital Youth clinic.**

***Subjects*: Adolescent (and youths) girls aged between 12-24 years.**

***Results*: Two hundred and sixty four participants were recruited into the study for a period of three months. The recruitment was done in the clinic till the required sample size was obtained. The prevalence of cervical HPV infection was 9.8% (95% CI: 6.13 to 13.41). There were multiple serotypes with 27% infected with HPV strain type 18 and 66. Type 16 was 5%, type 18 was 9%, and there were mixed genotype infections in the rest. There were no low risk strains isolated and 18% of the respondents who tested positive for HPV DNA had uncharacterised strains.**

***Conclusion*: The prevalence of HPV among the adolescent girls at KNH youth clinic was 9.8%. Twenty seven percent had co-infection with type 18 and 66.**

INTRODUCTION

Human papilloma virus (HPV) is a common sexually transmitted viral infection. It is estimated that at least 50% of women will be infected with HPV in their lifetime (1). Worldwide it is estimated to affect 291 million women, 105 million being infected with the high risk serotypes 16 and 18 (2). The annual incidence is estimated at 371,000 worldwide (2, 3). The prevalence rates vary from region to region; with estimated rates of 1.4 to 25.6 % in asymptomatic women (4). The World Health Organisation estimates an overall prevalence of 22 % of HPV infection in African countries (5). Rates in Kenyan are unknown although studies among men in high endemic areas have been reported at 50% (6). The distribution of HPV types is also variable. The distribution in invasive lesions in order of reducing prevalence are: 16 ,18 ,33 ,45 ,31 ,58 , 52 , 35 , 59 , 56 , 51 , 39 , 6 , 68 , 73 , 66 , 70. (7, 8). HPV type 16 is consistently the most common serotype in high grade and invasive lesions (8). The prevalence of high risk serotypes in most adolescent populations has been found to be low with rates of 3.4 % or less while low risk serotypes constitute the majority (9,10). Among the low risk serotypes, HPV 6 and 11 are the most common estimated to cause more than 90% of genital warts (4,6,11,) and 25 % of CIN I, whereas high risk types account for the remaining 75% of CIN I. Infection with multiple serotypes is common occurring in 20-30 % of women (12, 13).

Age is an important factor in HPV infection, the highest prevalence and incidence rates being among adolescents' prevalence rates exceeding 30% in most high risk adolescent populations ((3, 14, 15,) there is a linear increase in prevalence by age from 14-25 years with the peak prevalence at 20- 24 years which then declines with advancing age though some societies demonstrate a second peak after the age of sixty years.

In adolescent girls increased incidence is associated with early onset of sexual activity, multiple sexual partners, presence or a history of sexually transmitted infections, anal intercourse, smoking (13,14,16) and less consistently: frequent sexual intercourse , use of combined oral contraceptives, pregnancy and abortions (15 - 19). Having a sexual partner who has multiple partners, (20) homosexuals and non-frequent condom users are also at increased risk (21). Risk factors for infection in men are similar to those in girls (22,23). Lack of circumcision is an additional risk factor both for men and their sexual partners (24, 25). Since the adolescent girls are at a risk of HPV infection, a primary prevention can be achieved by HPV vaccine administration to the prepubertal girls and those who are not yet sexually active. In a study done in 2010, among the parents of the adolescent girls in Nairobi, only 58% accepted vaccine administration to their daughters. This is of concern since vaccination may prevent HPV infection and development of invasive cervical cancer (16).

HPV is a double stranded DNA virus belonging to the papovovirus family, with more than 100 HPV serotypes, 40 of which infect the genital tract of both men and women. The virus infects both keratinised and non-keratinised epithelia such as anogenital tract, oropharynx and lungs, prostate and skin. HPV can broadly be grouped into cutaneous and mucosal types according to their site of infection, and can be further subdivided into low-risk (LR) and high-risk (HR) types depending upon their association with malignancy. High risk HPV types 16 and 18 have been conclusively shown to be associated with invasive cancer of the cervix in 70% of cases (26).

The main route of transmission of high risk (HR) mucosal HPVs is through sexual contact, although the acquisition of the virus cannot be entirely explained by this mode alone. There is evidence of both horizontal and vertical transmission. HR HPVs, particularly HPV-16, have been detected in oral swabs from new-borns, infants and children (14, 28, 29).

HIV infection does play an important role in the progression of HPV infection to invasive cancer (26). There is, therefore, a need to determine HIV serostatus in all people with HPV infection (30,31). The adolescents are a vulnerable group for HPV and HIV infection, to their behavioural attitudes and peer pressure. It is, therefore, important to determine the prevalence of HR HPV infection in this age group. This will help in strengthening the need for advocacy for introduction of HPV vaccine use from this age group. Most HPV infections are sub-clinical and transient; about 90 % will resolve within nine months but may last up to two years (3, 14, 32, and 33) due to development of cell mediated immunity. After infection HPV remains in a quiescent state that lasts two to twelve month then is either cleared or persists below sensitivity level (latent phase). About 10-20 % of infections may persist and develop into genital warts or CIN 1 within three to six months. Most of this regress but some may progress to pre-cancer lesions (CIN 2, 3) in four to five years (33, 34). Some high grade lesions may arise directly from initial HPV infection (10). Progression to invasive cancer takes one to fifteen years; the development of invasive cancer is age related with peaks at 50 years, CIN I at 28 years and CIN 2, 3 at 42 years (15, 34). The trends seen in our region show that development of invasive cancers occurs at an earlier age.

MATERIAL AND METHOD

Study population: These were adolescent / young girls aged 12-26 years who attended the Youth clinic within the study period. The clinic attends to an average of 600 adolescent and youths per month,(60% are females), who present for various reasons including seeking treatment for sexually transmitted infections, drug abuse, teenage pregnancies and consultation for menstrual abnormalities among others. All adolescents / young females who were eligible and gave an informed consent or those whose parents or guardians were available to give the written consent were recruited into the study. Each client was individually counselled and informed consent was obtained. Simple random sampling was used, every third female client reporting to the Youth centre and met the inclusion criteria and consented was recruited into the study.

A questionnaire was administered in the clinic in a private room before she was attended to by the clinic doctor and the following information was obtained; Respondent's characteristics- age, marital status, level of education, gynaecological history, sexual history and social history, knowledge on HPV transmission. Consequences of HPV infection and prevention. The specimen collection procedure was explained and the clients were directed to a private room for specimen collection. The client was put in lithotomy position, sterile procedures were observed, speculum was introduced into the vagina and a cervical swab was taken by using a cervical brush to determine the presence and genotype of HPV infection. The swabs were placed in a labeled glass tube containing a preservative and stored in a cold box. The cold box containing specimens was transported to KEMRI HPV Laboratory for DNA analysis on the same day of collection within 15 hours. Laboratory forms accompanying participant's specimen were given unique number for each of the respondents. This unique number was also labeled clearly in the respondent questionnaire.

Processing of the cervical swab for HPV

DNA Extraction: The extraction of the DNA was carried out using the AL Buffer as a lytic buffer to the

cervical cell previously obtained from the respondent. The proteinase K was introduced into the sample for the purpose of destruction of the RNA found in the cell contents to its respective bases. Introduction of absolute ethanol brought about precipitation of all the proteins in the mixture that was then followed by centrifugation of the same so as to sediment the proteins. The supernatant which contains the DNA templates and the suspended bases of the destroyed RNA were then subjected to the spin column onto which the DNA absorbed as the rest of the stuff is washed down the column into the collecting tube. After washing the absorbed DNA twice with AW1 and AW2 respectively, the DNA was then eluded into a 1.5ml eppendoff tube using the AE buffer or distilled water. HPV amplification was then done using a thermo cycler that uses the pre-programmed thermocyclic cycles ranging in temperatures from 95^0C, 50^0C, 72^0C, and 4^0C respectively till the DNA templates of interests' desired detectable range was achieved.

Quality Assurance: The laboratory number of the specimen was counter checked to ensure correspondence. The standardised protocol from QIAGEN that is internationally recognised was used for processing the samples for DNA extraction, amplification, electrophoresis and detection of HPV. Genotyping of various types of HPV was done by using the standardised protocol for hybridisation.

Data Management: Data entry and cleaning was done, each entry had the unique study number so as to protect the privacy of the study participants. The information was stored and analysed using Statistical Package for Social Scientists version 17 (SPSS, Chicago). The data collection questionnaires were filed and stored in a safe cabinet where verification of results can be done whenever necessary.

Ethical considerations: Approval from Kenyatta National Hospital and University of Nairobi Ethics and Research committee was granted for the study.

RESULTS

A total of 264 female participants were recruited into the study. Seven did not have HPV results. The mean age was 22.6 years (SD=23 years) (Table 1).

Table 1
Demographic characteristics of the adolescents that participated in the study

Patient characteristic	HPV negative	HPV positive
Median age (Min, Max) n=256	22.7 (16,25)	22.0 (17,25)
Marital status	n(%)	n(%)
Single cohabiting	79(34)	10(40)
Single not cohabiting	119(52)	13(52)
Married	28(12)	0
Divorced	3(1)	1(4)
Widowed	2(1)	1(4)
Education level		
Primary	19(8.3)	1(4)
Secondary	19(8.3)	2(8)
College	185(81.1)	21(84)
University	5(2.2)	1(4)
Spouse's Education level		
None	2	0
Primary	6	0
Secondary	12	0
Tertiary	48	8
University	3	0

Most of the study participants had college education. Those who were single and yet cohabiting were 34.8%. The majority of the respondents (86.3%) were single.

Out of the 256 participants who were tested, 25 (9.77%) had positive HPV DNA results. The prevalence of HPV infection was found to have been 9.77% (95% CI: 6.13 to 13.41).this has been illustrated in (Fig 1).

Figure 1
Prevalence of HPV infection among adolescents at KNH (n=256)

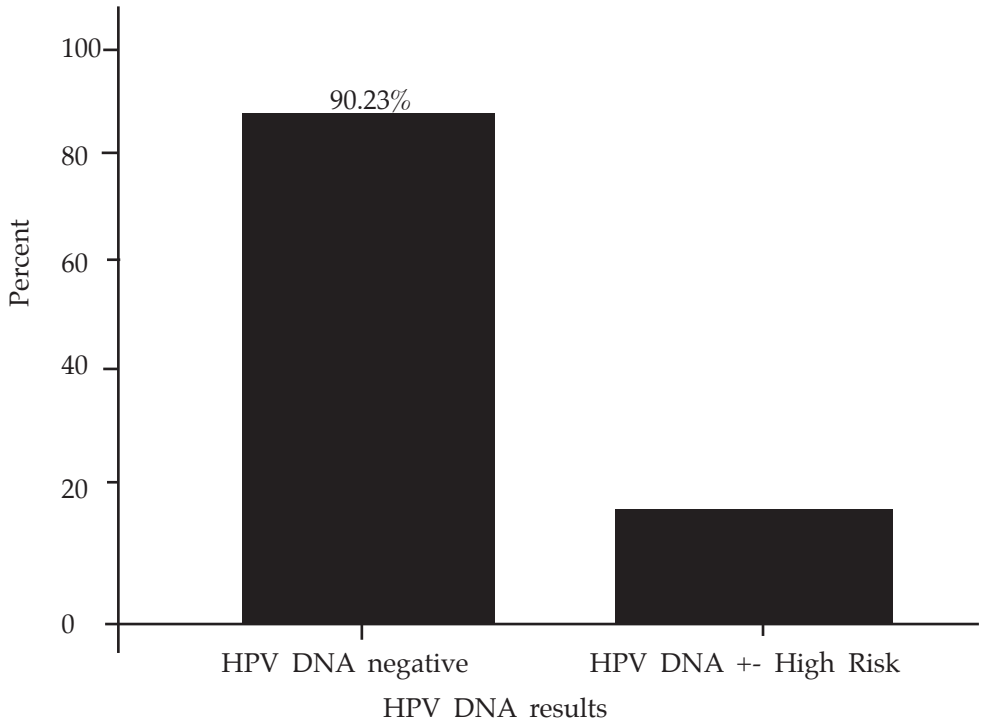
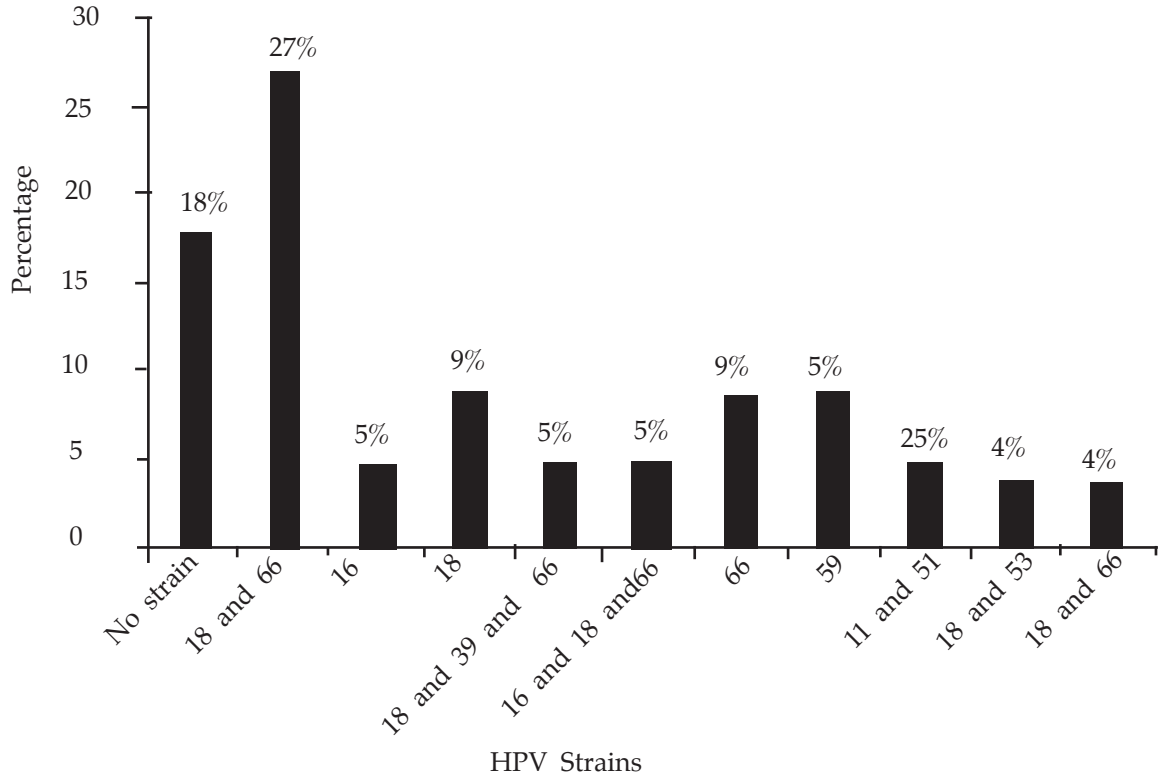


Figure 2
Distribution of the HPV strains



Most of the participants, who were positive, had multiple serotypes, with 27% infected with HPV strain type 18 and 66. There were no low risk strains isolated and 18% of the respondents who tested positive for HPV DNA had uncharacterised strains. Infection with mixed strains was also found.

Table 2
Association between sexual behaviour and HPV status

	HPV negative n, (%)	HPV positive n, (%)	P-value
Age at first sexual intercourse			0.273
<10 years	2(1)	1(4)	
10-15 years	31(14)	2(9)	
>15 years	189(85)	20(87)	
Missing/Unknown	9	2	
Number of lifetime partners		0.882	
<2	138(63)	14(67)	
3-5	60(27)	6(28)	
>5	22(10)	1(5)	
Missing/Unknown	11	4	
Sexual partners in the past six months			>0.999
2	189(88)	20(91)	
3-5	21(10)	2(9)	
>5	4(2)	0	
Missing/Unknown	17	3	
Frequency of intercourse per week			
≤1	147(69)	17(89)	0.164
2-3	42(19)	1(5.5)	
>3	25(12)	1(5.5)	
Missing/Unknown	17	6	
Practice douching after sex			0.446
Yes	46(22)	6(32)	
No	161(78)	13(68)	
Missing/Unknown	24	6	
Ever treated for an STI			0.951
Yes	31(14)	3(12.5)	
No	139(62)	16(67)	
Not sure (Discharge)	55(24)	5(20.5)	
Missing/Unknown	6	1	
Does current partner have another partner			0.574
Yes	14(7)	3(13)	
No	100(47)	10(43.5)	
Do not know	99(46)	10(43.5)	
Missing/Unknown	18	2	
Is your partner circumcised			0.810
Yes	178(84.4)	20(87)	
No	26(12.3)	3(13)	
Did not know	7(3.3)	0	
Missing/Unknown	20	2	

Association between sexual behaviour and HPV status was not statistically different between study participants who were HPV negative and HPV positive. The factors were: age at first sexual intercourse, number of lifetime partners, sexual partners last six months, frequency of intercourse per week, practice douching after sex, ever treated for an STI, if current partner has another partner, if partner was circumcised (Table 2). Of those who tested positive only two reported having had more than one sexual partner while in six there was no response on the number of sexual partners, this number is small and a conclusion of increased vulnerability may not be made from it.

DISCUSSION

Two hundred and sixty four adolescent girls were enrolled in this study to determine among other things the DNA genotype in those who were positively identified to have HPV infection, using the PCR test. The study was carried out at the KNH Youth clinic. This is a youth friendly clinic which was set up to specifically target the youth where their needs would be addressed by those trained to handle this age group.

The prevalence of cervical HPV infection as defined by a positive HPV DNA test was found to have been 9 % among the adolescent girls. This prevalence is lower than the World Health Organisation estimates for HPV infection in the African countries of 22 % (5) and also compared to the findings of a study in Kenyans done among a high risk population of men around Lake Victoria of 53 % (6). Similar study done in Gambia, West Africa has shown that the prevalence of cervical HPV infection among girls aged 15-24 years was 15 %(36). The mean age in our study was 22.6 years, this compares favourably with the findings of the study in Gambia.

The findings of the low prevalence in this study though encouraging is still of concern considering the possible complications of HPV infections since more than 90% of adolescent girls aged between 12 and 26 years are at risk of HPV infection. The mean age of the youth with positive HPV infection was 22.6 years. This is the age when there is increased sexual activity and peer pressure plays a big role in influencing sexual behaviour. Much higher HPV prevalence figures have been reported in some unselected studies from Eastern and Southern Africa, ranging from 34% in rural Zimbabwe (36) to 44% in urban Kenya population who were already considered as a high risk group (37).

In this study a total of eight different subtypes were detected using the PCR method. Twenty five samples (9.8%) were HPV-DNA-positive by PCR. A total of eight different HPV sub-types were detected. Of the positive samples, (16%) were positive for a single HPV type, 17 (68%) were samples containing more than one HPV type (mixed: 32%, two types and 16%, three types). The most prevalent HPV types were HR types 18 and 66. An uncharacterised type was found in 16% of the HPV-positive samples. In this study HPV type 16 was not among the most frequent HPV sub-type.

This is an important finding since there was no low risk type of HPV isolated from the positive group and the number of mixed sub-types isolated was higher than the individual sub-types. This would, therefore, call for more work to be done on the role the other sub-types of HPV especially 66 other than the types 16 and 18 would be contributing towards the development of invasive cancers in our region. There is also the need to compare the subtypes seen in the adolescent and adult if there is similarity or if age may have a factor in the sub-type of HPV infection seen in our region.

The primary prevention of HPV infection is the use of the available vaccines of which two are available in the market Cervarix and Gardasil, studies done in Nairobi in 2010 looking into the vaccine administration acceptance by parents showed that only 58% were willing to have their daughters vaccinated and their main concern was the lack of adequate information on the vaccine use. This then calls for increased advocacy to the population especially through the schools (27). Majority of those seen in the KNH youth clinic are still in schools and, therefore, the need for increased awareness of HPV vaccine use for the prevention of invasive cervical cancer in this group is important.

In conclusion, the prevalence of HPV among adolescents being cared for at KNH was 9.8% and the most prevalent sub-types were the HR HPV types 18 and 66. A national study should be conducted to determine the national prevalence of HPV infection. There is need of advocacy on HPV infection prevention through Vaccine use.

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