Prevalence and distribution of human papillomavirus genotypes among women in northern Henan Province of China

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Research

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Abstract

Background

Human papillomavirus (HPV) infection can cause cervical and other cancers including cancer of vulva, vagina, penis, anus, or oropharynx. However, data concerning the prevalence and genotype distribution of HPV among women are limited in northern Henan Province of China. This study aimed to make an investigation on the current prevalence and genotype distribution of HPV among women and provided comprehensive data to guide HPV-based cervical cancer prevention in northern Henan Province.

Methods

A total of 15616 women aged 16 to 81 years who attended the department of gynecology of Xinxiang central hospital between January 2018 and December 2019 were enrolled in this study. HPV DNA was detected by PCR method followed by HPV type-specific hybridization. The overall prevalence, age-specific prevalence and genotype distribution of HPV were investigated.

Results

The overall HPV prevalence was 19.7% among women in northern Henan Province. Single, double and multiple HPV infections accounted for 13.7%, 4.3% and 1.8% of the total cases, respectively. HPV prevalence was 41.8% among women aged 16 to 19 years, 21.1% among women aged 20 to 29 years, 18.1% among women aged 30 to 39 years, 17.7% among women aged 40 to 49 years, 20.8% among women aged 50 to 59 years, 22.9% among women more than 60 years. HPV infection rates differed significantly across different age groups. Most infections were caused by high-risk HPV (HR-HPV) and single genotype HPV infection was the most common pattern. The most common HR-HPV genotype was HPV16, followed by HPV52, HPV58, HPV53 and HPV39. The most common low-risk HPV (LR-HPV) genotype was HPV6, followed by HPV61, HPV81, HPV54 and HPV11.

Conclusions

HPV infection is common among women in northern Henan Province. The highest infection prevalence was found in women less than 20 years old. The 9-valent HPV vaccine for routine vaccination is strongly recommended in northern Henan Province.

Introduction

Cervical cancer, ranked after breast cancer, colorectal cancer and lung cancer, is the fourth most common cancer among women worldwide, with approximately 530,000 new cases and 275,000 deaths every year [1, 2, 3]. According to current data, nearly 85% of the women deaths from cervical cancer occurred in developing or underdeveloped countries[1]. In China, cervical cancer ranked as the eighth most common killer for women and continued to be a public health problem affecting women health[4]. Persistent infection with HR-HPV is the major cause of cervical cancer. Globally, HPV infection has been involved in more than 99% of cervical cancer, including both cervical squamous cell carcinoma and cervical adenocarcinoma. HPV is a non-enveloped, double-stranded DNA virus, with a genome of approximately 8.0 kb. As of 9 March 2015, more than 200 different types of HPV, identified numerically, have been identified by the International HPV Reference Center[5]. Based on epidemiological and biological data,

twelve HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) have been classified as HR-HPV by the International Agency for Research on Cancer[6]. Eight HPV types (26, 53, 66, 67, 68, 70, 73 and 82), identified as single HPV infections in about 3% cervical cancer, are classified as probable/possible (p) HR-HPV due to lack of biological data[7]. It is extremely difficult to isolate and culture HPV in vitro. Additionally, not all patients infected with HPV have an obvious antibody response. Thus, HPV DNA detection by PCR becomes a non-invasive and sensitive method for the confirmation of an active cervical HPV infection. Since persistent infection with HR-HPV is a necessary cause for cervical cancer development, DNA detection and genotyping of HPV can be used as an essential method for the control and prevention of HPV-related disease.

Up to present, three licensed prophylactic vaccines, a bivalent vaccine against HPV16 and 18, a quadrivalent vaccine against HPV 6, 11, 16 and 18 and a 9-valent vaccine against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58, are considered as effective and safe for prevention HPV infection. However, the current vaccines offer protection only targeted at a few HPV genotypes. What's more, virus-type restricted protection is present between HPV genotypes. Since the prevalence of HPV infections in women shows geographical distribution between countries and regions[8, 9], a better understanding of HPV prevalence and genotype distribution would be necessary and valuable for evaluating the effect of the current HPV vaccination program for cervical cancer and developing the next generation of vaccines, particularly in specific regions and areas where they are most needed.

As the data on HPV epidemiology and genotype distribution is lacking in northern Henan Province, which is located in the central China and has the largest population in China, it is impossible to correctly evaluate the prevalence of HPV infection and consequently it is difficulty to take preventive measures, such as the introduction of HPV vaccine in these areas. Therefore, the prevalence and genotypic distribution of HPV among women in Henan Province were identified in this study. The results of this research are of great importance for estimation of the awareness of HPV infection and the introduction of vaccination program in northern Henan Province of China.

Materials And Methods

Study population and specimen collection

Women aged between 16 and 81 attending regular gynecological outpatient clinic in Xinxiang central hospital between January 2018 and December 2019 in northern Henan Province were invited to join in this study. They were divided into six aged groups. The G1 group meant age $\leq$ 20; the G2 group meant $20 \leq \text{age} < 30$; the G3 group meant $30 \leq \text{age} < 40$; the G4 group meant $40 \leq \text{age} < 50$; the G5 group meant $50 \leq \text{age} < 60$; the G6 group meant age ≥ 60 . Women were excluded for: the presence of cervical cancer, pregnancy at the time of enrollment, previous HPV vaccination, without age information, hysterectomy or immunosuppression. Finally, a total of 15616 participants were included in this study for analysis.

Cervical specimens were collected by the trained clinicians in Xinxiang central hospital. Cervical cells were collected from the cervical canal by using plastic brush. The brush was placed into a 2mL vial of preservation solution (HybriBio limited Corp, Chaozhou, Guangdong, China) for HPV DNA detection. This study was performed strictly in accordance with the Declaration of Helsinki and approved by the Ethics Committee in Xinxiang Medical University. Informed consent was obtained from all participants before enrollment.

Dna Extraction, Pcr Amplification And Hpv Genotyping

HPV DNA was extracted from the cervical cells using a DNA extraction kit according to the manufacturer's manual (HybriBio limited Corp, Chaozhou, Guangdong, China). Briefly, the exfoliated cervical cells were first digested by proteinase K. Then the released DNA was obtained through absorption to magnetic glass particles, washed and purified from these particles with the use of the automated nucleic acid extraction instrument (HybriBio limited Corp, Chaozhou, Guangdong, China). A water blank was performed through all steps of DNA extraction as a contamination control. The concentration and purity (OD260/OD280 between 1.6 ~ 1.8) of DNA were determined by Nanodrop 2000 (ThermoFisher Scientific, CA, USA). The extracted DNA was detected immediately or stored at -20°C.

HPV DNA amplification and genotyping were conducted using a commercial HPV genotyping kit for 37 HPV types, including 17 HR-HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 82) and 20 LR-HPV types (6, 11, 34, 40, 42, 43, 44, 54, 55, 57, 61, 67, 69, 70, 71, 72, 73, 81, 83, 84). The kits have been authorized by the China Food and Drug Administration for clinical use. The experimental procedures, including PCR and flow-through hybridization, were followed the manufacturer's manual. The HPV L1 consensus biotinylated primer sets were used in the PCR assay. One µL of the DNA was used in the 25-µL PCR. PCR reaction was initiated denaturation at 95 °C for 9 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, with a final extension at 72 °C for 5 min.

HPV genotyping was performed by flow-through hybridization method. The flow-through hybridization was performed on a medical nucleic acid hybridization instrument prewarmed at 45°C prior to usage. A nylon membrane on which 37 HPV genotype-specific oligonucleotide probes secured was placed into the instrument. The biotinylated PCR product was denatured at 95 °C for 5 min and then chilled on ice for 2 min before hybridization. The PCR product was mixed with the hybridization solutions and the mixture was added into the sample wells to conduct with flow-through hybridization for 10 min. The nylon membrane was washed with the hybridization solution three times and the empty region was blocked without reaction. The hybridizing signal were detected with streptavidin alkaline phosphatase, binding to biotinylated PCR products, and its substrate NBT/BCIP (nitro-blue tetrazolium-5-bromo-4-chloro-3-indolylphosphate). The genotype result was detected by the position of the HPV-genotype probes on the membrane. The blue dot on the membrane, indicating a positive result, was judged by the naked eyes. Multiple dots showed multiple infections. Quality controls were carried out throughout the whole experiment, including PCR amplification and hybridization by using positive and negative controls provided by the kit.

Statistical analysis

All statistical analyses were conducted using SPSS 18.0 for Windows (SPSS Inc., Illios, USA). HPV prevalence and genotype distribution were analyzed. Single, double and multiple HPV infections were defined as infection with one, two and more than two genotypes of infections, respectively. HPV prevalence in designated groups and corresponding 95% confidence intervals (95% CI) were calculated. The chi square (χ^2) test was used to evaluate the significance of difference between different age groups. $P < 0.05$ was considered to be statistically significant.

Results

Overall and age-specific HPV prevalence

From January 2018 to December 2019, a total number of 15616 cervical specimens, collected for HPV DNA detection, were used for statistical analysis. There were 3081 specimens positive for any HPV DNA and the overall prevalence of HPV was 19.7% (95% CI 19.1%~20.4%). The 15616 participants were divided into six age groups and the HPV infection rate in each group was calculated as shown in Table 1. HPV infection lied in each age group,

however, the infection rates were not the same, and the difference had statistical significance ($P<0.05$) by using χ^2 test. The highest prevalence of HPV infection was found among women in G1 group with the infection rate of 41.8% (95% CI: 36.5%~47.2%), followed by an infection rate of 22.9% (95% CI: 20.2%~25.5%) in G6 group and an infection rate of 21.1% (95% CI: 20.2%~25.5%) in G2 group. HPV prevalence was 18.1% (95% CI: 17.0%~19.3%) among women in G3 group, 17.7% (95% CI: 16.6%~18.8%) among women in G4 group and 20.8 (95% CI: 19.2%~22.4%) among women in G5 group.

Distribution of single, double and multiple HPV infections

The single, double and multiple HPV infections in different age groups were shown in Table 2. Single, double and multiple HPV infections accounted for 13.7% (95% CI: 13.1%~14.2%), 4.3% (95% CI: 4.0%~4.6%) and 1.8% (95% CI: 1.6%~2.0%) of the 15616 cases, respectively. Single genotype HPV infection was the most common pattern, and it occurred more frequently than double and multiple HPV infections. There were statistically significant differences in the age groups distribution of single infections ($P<0.05$). The group with the highest infection rate was G1 group (28.0%, 95% CI: 36.5%~47.2%). The infection rates declined as the age increased, reached the lowest levels in G4 group, and rose again in G6 group. Statistically significant differences were observed in the age groups distribution of double infections ($P<0.05$). Starting from G1 group with the highest infection rate of 10.8%, the infection rate reduced to the lowest levels in G3 group, and then began to increase again from G4 group. Significant differences were also observed in the age groups distribution of multiple infections ($P<0.05$), with the highest infection rate of 3.1% in G1 group and the lowest infection of 1.3% in G4 group.

HPV genotype distribution

There were 36 different HPV genotypes, including 17 HR-HPV genotypes and 19 LR-HPV genotypes, identified in this study. The prevalence of 17 HR-HPV was demonstrated in Table 3. The most common HR-HPV identified was HPV16 (4.3%), followed by HPV52 (3.5%), HPV58 (2.0%), HPV53 (1.8%) and HPV39 (1.5%). To be noted, HPV18 was only the seventh most common HR-HPV genotype to be detected. For individuals with single HR-HPV infection, the genotypes that ranked top five were HPV16, HPV52, HPV58, HPV53 and HPV39. For individuals with double HPV infection, the HR-HPV that ranked top five were HPV16, HPV58, HPV52, HPV39 and HPV53. For individuals with multiple HPV infection, the HR-HPV that ranked top five were HPV52, HPV16, HPV58, HPV51 and HPV53. These data suggested that HPV16 infection was predominant in HPV-positive patients. The prevalence of 19 LR-HPV genotypes was demonstrated in Table 4. The most common LR-HPV identified was HPV6, followed by HPV61, HPV81, HPV54 and HPV11. For individuals with single LR-HPV infection, the most commonly detected genotype was HPV61, followed by HPV54, HPV6, HPV81 and HPV11. For double and multiple HPV-infected individuals, HPV6 and HPV61 were the two most common LR-HPV. The overall genotypes that ranked top ten were HPV16, HPV52, HPV58, HPV53, HPV39, HPV51, HPV6, HPV18, HPV61 and HPV81. The top six HPV genotypes were all HR-HPV, demonstrating the most infection were caused by HR-HPV in northern Henan province.

Discussion

China has a vast territory and a large population of more than 1.4 billion. The prevalence of HPV varied greatly in different provinces of China. There have been some studies reported the prevalence and genotype distribution of HPV in different provinces in China[10, 11, 12, 13]. However, there are few reports on HPV prevalence in Henan Province. The present study provided the prevalence and genotype data of HPV from women attending regular gynecological outpatient clinic from January 2018 to December 2019 in northern Henan province of China. Our study showed that the overall HPV infection rate was 19.7%, which was similar to the results from several surveys of HPV prevalence that

reported in other provinces of China[14, 15, 16]. The prevalence of HPV in northern Henan province was lower than that reported in Shandong Province (28.4%) and Fujian Province(38.3%)[17, 18], but higher than Yunnan Province (12.9%) and Shanxi Province (8.92%)[10, 19]. The different HPV prevalence in northern Henan Province was expected to be explained by the different economic conditions, different living habits and customs, cultural diversity, sampling strategy, as well as the HPV detection methods. According to the previous reports, the HPV infection rates ranged from 6.7–44.5% in China[20]. Although the HPV infection rates in northern Henan Province was not the highest in China, more attention should be paid to the women infected with HPV.

This present study provided age-specific HPV infection rates among women. Our results showed that women under 20 years, with the overall infection rate of 41.8%, had the highest infection rate in the six designed groups. The single HPV infection rate, the double HPV infection rate and the multiple HPV infection rate were also the highest in women under 20 years. There are two possible reasons for this phenomenon: one reason is that compared with other groups, the total number of women under 20 years participating in the survey is relatively small. Healthy women under 20 years undergoing normal medical examinations were rarely required to be tested for HPV. Most of them were outpatients and they come to hospital for some medical problems, leading to the high prevalence of HPV. Another reason is that the young women are sexually active and they don't know how to protect themselves, which makes them sensitive to HPV infection. The above two reasons led to the highest infection rate in G1 group. It was reported that young women infected with HPV were temporary, and the virus would be cleared by the immune system in most of the cases[21, 22]. Therefore, the infection rate of HPV will gradually decline with the age increased. In the present study, the prevalence of HPV declined gradually in the middle-aged groups, and slightly increased in G6 group (22.9%), which indicated that women over 60 years suffered severely from HPV infection. For women over 60 years, their immune system function gradually decreased, therefore, they become susceptible to HPV infection. If the HPV cannot be eliminated by the immune system and persist for several years, this will represent a risk factor for neoplasia development. HPV infection rates ranged from 17.7–21.1% among women in groups G2 to G5. Since HPV infection is a major etiological factor for the cervical cancer development, the high prevalence rate of HPV indicated the significant burden of cervical cancer in Henan Province. It is a pity that despite we can detect the HPV infection, there is no medical treatment to eliminate HPV infection. Future treatment that could eliminate HPV infection and prevent progression of cervical cancer is of great interest.

Our present study also provided the age-specific distribution of single, double and multiple HPV infection rates. Except the G1 group, the single, double and multiple HPV infection rates of the rest groups were very close. Up to now, there has not been reached an agreement that whether multiple HPV infections increase the risk of cervical cancer than single HPV infection. Some studies have reported that multiple HPV infections had a higher risk with the occurrence and development of cervical cancer than single HPV infections[23, 24]. However, other studies have found that single HPV infections had a greater risk of developing cervical cancer with respect to multiple infections[25, 26]. In our study, the prevalence of single HPV infections is high, accounted for 13.7% of the total cases, and HPV16 was the most commonly detected genotype in single HPV infection. HPV16 infection is the one with the highest oncogenic risk[26]. Thus, more attention should be paid to single HPV16 infection. The double HPV infection and multiple HPV infection accounted for 4.3% and 1.8% of the total cases, respectively. The investigation of double and multiple HPV is of great importance to study the prevalence of HPV and is also of great significance to develop multivalent HPV vaccine.

It is important to know the HPV genotypes distribution since data concerning the distribution of HPV genotypes is concerning with the vaccine development. Persistent infection with HR-HPV is the primary etiological factor for cervical cancer. There is up to 70% of cervical cancers caused by HPV16 and HPV18, with the remainder of cervical cancers caused by other HR-HPV genotypes. In the present study, the HPV16, HPV52, HPV58, HPV53 and HPV39 were the top five HR-HPV genotypes. HPV16 ranked first in our study and the infection rate reached up to 4.3%. HPV52 and

HPV58 ranked second and third respectively. Compared with HPV16, both HPV52 and HPV58 had a lower correlation with cervical cancer. The top three genotypes distribution pattern is consistently with the data from several previous Chinese population-based HPV investigations [17, 27, 28]. It was reported HPV18 ranked second in Chinese women between 1991 and 2016[4]. In our report, HPV18 with an infection rate of 1.1%, ranked seven in the HR-HPV genotypes in the study, which was consistent with the recent results in several other regions in China[17, 19, 29].

What's more, the study also investigated the distribution of LR-HPV. Our study showed that the top five most common LR-HPV genotypes were HPV6, HPV61, HPV81, HPV54 and HPV11. For the LR-HPV types, cervical cancer association is very rate in the general women population. LR-HPV mainly cause genital warts, common warts, flats warts and many other skin lesions[30], which are usually asymptomatic but are sometimes accompanied by itching, burning or bleeding, leading to psychosocial disturbances. To date, the 9-valent HPV vaccine is used in the prevention of infection with HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV45, HPV52 and HPV58. Our results support the recommendation of the 9-valent HPV vaccine for routine vaccination in northern Henan Province. Meanwhile, vaccine against HPV53, HPV39 and HPV51 should also be developed for the women in this area.

The present investigation provided the recent data on the prevalence and genotype distribution of HPV in northern Henan Province, however, limitations existed. One limitation is that the relationship between the incidence of cervical cancer and the different HPV genotypes infection were not collected in this study. Another limitation is that the viral load in the enrolled individuals were not detected. It has been reported that both HPV genotype and viral load in combination show promise for predicting the development of cervical cancer in HPV positive women[24]. In the future, the role of different HPV genotypes infection and viral load on the risk of cervical cancer will be studied.

Conclusions

In conclusion, the epidemiology and genotype distribution of HPV were investigated in northern Henan Province. A high prevalence of HPV was confirmed in our study and most infections were caused by HR-HPV, which indicated the a high cervical cancer incidence in the region. HPV16, HPV52 and HPV58 were the dominant HR-HPV which were prevalent in northern Henan Province. The results in our study provide important information for cervical cancer screening and vaccination in women in northern Henan Province of China.

Abbreviations

HPV: Human papillomavirus; HR-HPV: High-risk HPV; LR-HPV: Low-risk HPV; 95% CI: 95% confidence intervals

Declarations

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Authors' contributions

XPW, RLS, MYW and LJZ designed and supervised the research. XPW, YS, XFW and GYW acquisition of data. RLS, MYW and LJZ analyzed and interpreted the data. XPW prepared the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data were collected from Xinxiang central hospital in Xinxiang city. We are grateful for their generous help. The data can be free shared. The materials were purchased from HybriBio Biotechnology Limited Corp. Any addition information may be obtained from the corresponding author on a reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee in Xinxiang Medical University. Informed consent was obtained from all participants before enrollment.

Consent for publication

Not applicable. This manuscript do not contain any individual person's data in any form (including individual details, images or videos).

Competing interests

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. The authors declare that they have no competing interests.

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Tables

Table 1 The prevalence of HPV among women in different groups

Group	Age, y	Sample size	Positive No.	Prevalence, % (95% CI)	P Value
G1	<20	325	136	41.8%36.5-47.2%	<0.05
G2	20-29	2874	605	21.1%19.6-22.5%	
G3	30-39	4222	765	18.1%17.0-19.3%	
G4	40-49	4829	856	17.7%16.6-18.8%	
G5	50-59	2417	502	20.8%19.2-22.4%	
G6	>60	949	217	22.9%20.2-25.5%	
Total		15616	3081	19.7%19.1-20.4%	

Table 2 Single, double and multiple HPV infections in different groups

Group	Sample size	Single infection		Double infection		Multiple infection	
		Positive	Prevalence, %	Positive	Prevalence, %	Positive	Prevalence, %
		No.	(95% CI)	No.	(95% CI)	No.	(95% CI)
G1	325	91	28.0[23.1-32.9]	35	10.8[7.4-14.2]	10	3.1[1.2-5.0]
G2	2874	396	13.8[12.5-15.0]	151	5.3[4.4-6.1]	58	2.0[1.5-2.5]
G3	4222	562	13.3[12.3-14.3]	142	3.4[2.8-3.9]	61	1.4[1.1-1.8]
G4	4829	611	12.7[11.7-13.6]	181	3.7[3.2-4.3]	64	1.3[1.0-1.6]
G5	2417	331	13.7[12.3-15.1]	115	4.8[3.9-5.6]	56	2.3[1.7-2.9]
G6	949	143	15.1[12.8-17.3]	43	4.5[3.2-5.9]	31	3.3[2.1-4.4]
Total	15616	2134	13.7[13.1-14.2]	667	4.3[4.0-4.6]	280	1.8[1.6-2.0]
P Value		<0.05		<0.05		<0.05	

Table 4 Distribution of LR-HPV genotypes in study participants

HPV type	Single infection		Double infection		Multiple infection		Total infection
	Positive No.	Prevalence, % (95% CI)	Positive No.	Prevalence, % (95% CI)	Positive No.	Prevalence, % (95% CI)	Prevalence, % (95% CI)
HPV6	78	0.50.4-0.60	88	0.60.4-0.70	52	0.30.2-0.40	1.40.1.2-1.60
HPV61	111	0.70.6-0.80	27	0.20.1-0.20	30	0.20.1-0.30	1.10.9-1.20
HPV81	70	0.40.3-0.60	55	0.40.3-0.40	43	0.30.2-0.40	1.10.9-1.20
HPV54	79	0.50.4-0.60	51	0.30.2-0.40	32	0.20.1-0.30	1.00.9-1.20
HPV11	49	0.30.2-0.40	35	0.20.1-0.30	32	0.20.1-0.30	0.70.6-0.90
HPV40	48	0.30.2-0.40	23	0.10.1-0.20	9	0.10.0-0.10	0.50.4-0.60
HPV84	27	0.20.1-0.20	26	0.20.1-0.20	25	0.20.1-0.20	0.50.4-0.60
HPV34	27	0.20.1-0.20	14	0.10.0-0.10	15	0.10.0-0.10	0.40.3-0.50
HPV70	16	0.10.1-0.20	16	0.10.1-0.20	10	0.10.0-0.10	0.30.2-0.40
HPV44	20	0.10.1-0.20	12	0.10.0-0.10	9	0.10.0-0.10	0.30.2-0.30
HPV42	21	0.10.1-0.20	9	0.10.0-0.10	8	0.10.0-0.10	0.20.2-0.30
HPV55	10	0.10.0-0.10	16	0.10.1-0.20	3	0.00.0-0.00	0.20.1-0.30
HPV73	12	0.10.0-0.10	5	0.00.0-0.10	11	0.10.0-0.10	0.20.1-0.20
HPV43	4	0.00.0-0.10	8	0.10.0-0.10	9	0.10.0-0.10	0.10.1-0.20
HPV69	8	0.10.0-0.10	6	0.00.0-0.10	4	0.00.0-0.10	0.10.1-0.20
HPV57	4	0.00.0-0.10	9	0.10.0-0.10	0	0.00.0-0.00	0.10.0-0.10
HPV83	5	0.00.0-0.10	3	0.00.0-0.00	3	0.00.0-0.00	0.10.0-0.10
HPV67	3	0.00.0-0.00	1	0.00.0-0.00	4	0.00.0-0.10	0.10.0-0.10
HPV71	5	0.00.0-0.10	1	0.00.0-0.00	2	0.00.0-0.00	0.10.0-0.10

Due to technical limitations, Table 3 is only available as a download in the Supplemental Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table3DistributionofHR.docx](#)