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HPV specificity and multiple infections and association with cervical cytology in Chongqing, China: a cross-sectional study

Qinli Luo[1*], Haiyan Zhang[1], Xianghua Zeng[1], Na Han[1], Zhen Ma[1] and Hanyi Luo[1]

Abstract

Background It is important to assess the relationship between specific HPV genotype or multiple infection and cervical cytology. The protection provided by the HPV vaccine is type-specific, and the epidemiology feature of coinfections needs to be investigated. The aim is to provide baseline information for developing HPV vaccination and management of HPV-positive populations in the region.

Methods A total of 3649 HPV-positive women were collected from 25,572 women who underwent 15 HR-HPV genotypes and ThinPrep cytologic test (TCT) results. Logistic regression was used to determine the correlation between the risk of cytology abnormalities and specific HPV infection. We calculated odds ratios (ORs) to assess coinfection patterns for the common two-type HPV infections. chi-squared test was used to estimate the relationship between single or multiple HPV (divided into species groups) infection and cytology results.

Results The results showed there was a positive correlation between HPV16 (OR = 4.742; 95% CI 3.063–7.342) and HPV33 (OR = 4.361; 95% CI 2.307–8.243) infection and HSIL positive. There was a positive correlation between HPV66 (OR = 2.445; 95% CI 1.579–3.787), HPV51 (OR = 1.651; 95% CI 1.086–2.510) and HPV58(OR = 1.661; 95% CI 1.166–2.366) infection and LSIL. Multiple HPV infections with α9 species (OR = 1.995; 95% CI 1.101–3.616) were associated with a higher risk of high-grade intraepithelial lesions (HSIL) compared with single HPV infection. There were positive correlations between HPV66 and HPV56 (α6) (OR = 3.321; 95% CI 2.329–4.735) and HPV39 and HPV68 (α7). (OR = 1.677; 95% CI 1.127–2.495)**.** There were negative correlations between HPV52, 58, 16 and the other HPV gene subtypes.

Conclusion HPV33 may be equally managed with HPV16. The management of multiple infections with α9 may be strengthened. The 9-valent vaccine may provide better protection for the population in Chongqing currently. The development of future vaccines against HPV51 and HPV66 may be considered in this region.

Keywords High-risk HPV, TCT, Multiple infections, Vaccine

Introduction

Cervical cancer is the fourth most common cancer amongst women in the world, with approximately 604,000 new cases and 342,000 deaths in 2020 [1]. Persistent high-risk human papilloma virus (HR-HPV) infection is a key risk factor leading to precancerous intraepithelial lesions [2]. At present, more than 200 HPV genotypes have been identified, among which HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are

*Correspondence:
Qinli Luo
m13668488467@163.com
[1] Chongqing Cancer Multi-Omics Big Data Application Engineering Research Center, Chongqing University Cancer Hospital, 181 Hanyu Road, Shapingba District, Chongqing 400030, China

high-risk carcinogenic types [3]. Most of the carcinogenic or HR-HPV genotypes are α-papilloma virus [3].α9 clade (including HPV16, 58, 33, etc.) is the most important species, which was more likely to result in serious cervical lesions [4]. α7 is mainly associated with high-risk mucosal lesions, including HPV18, 45, 39, etc., and the others include α5 (HPV51, 82), α6 (HPV56, 66) etc. [5]. Trottier et al. [6] found that multiple HPV infections are associated with the highest risk of invasive cervical cancer compared with single infection, especially coinfection with α9 [7].

HPV16 and HPV18 are associated with 70% of cervical cancer [8]. However, other high-risk genotypes can also lead to cervical intraepithelial neoplasia and cervical cancer except HPV16/18 [3]. The influence of the different HPV genotypes on the development of cervical neoplasia remains and need further research [9].So it is necessary for optimizing specific genotype-based screening strategy and management of risk stratification due to the risk difference of different genotypes and multiple infection.

The WHO strategy for cervical cancer elimination: vaccination against HPV and early detection and treatment of cervical cancer [10]. In England, cervical cancer in women born since Sept 1, 1995 had been almost eliminated because of the HPV immunization programme [11]. So the HPV vaccine is the most efficient and critical prophylactic strategy in cervical cancer related to HPV genotypes. The prevalence of HPV infection and genotype distribution vary between countries and regions [12–14]. Meanwhile, it is essential to acquire information about HPV type-specific pathogenicity for cervical cancer. After all, the ultimate goal of HPV vaccination is to prevent cervical cancer.

Interactions between HPV types may have an impact on the overall effectiveness of an HPV vaccination program [15]. Okoye JO et al. [16] also pointed out there are some limitations in the efficacy of vaccines available to prevent cervical cancer because of multiple HPV infection. Therefore, the investigation of multiple infections is also very critical for the development of new multivalent vaccines.

Currently, there are five vaccines available in China: three imported vaccines (bivalent 2vHPV (Cervarix®, GSK), quadrivalent 4vHPV(Gardasil®, MSD) and 9-valent(Gardasil® 9, MSD), domestically produced bivalent vaccine (Cecolin®) and (Walrinax®). HPV vaccination is not included in the national immunization program in China. The majority of provinces require payment for HPV vaccination. The primary target group aged 9–14 years is less than 5% in China, which is far from the WHO 2030 targets [17]. Moreover, the HPV vaccine uptake was lower in the western regions corresponding to the eastern regions [18] which may due to lower-income and the more decrease socioeconomic status. Chongqing is one of the cities in Western China. Therefore, it is urgent to strengthen Chongqing's vaccine policy and develop new suitable vaccines suitable for Chongqing.

Our study analyzed the correlations between the specific HPV genotype and different cytological abnormality, evaluate the effects of multiple infections on cervical cytology compared to single infection, further analysis the co-infection pattern of several common HPV genotypes, provide a theoretical management of risk stratification for HPV-positive women and the choice of appropriate HPV vaccines and development.

Materials and Methods

Study population

This study is a cross-sectional, single-center study based on data from women who had HPV and TCT tests at the Health Examination and Oncology Screening Center in Chongqing University Cancer Hospital between January 1, 2016 to April 30, 2023. Subjects were women between the ages of 18 and 87 years old who actively taking part in cervical cancer screening. A total of 25,572 females who were screened for HR-HPV were enrolled. Eligible women were included who had a history of sexual activity, were not pregnant, and had no history of cervical cancer, had no severe immunodeficiency disease or other malignancies. 3649 HR-HPV-positive females were retrospectively analyzed. All participants were performed with the informed consent of the enrolled women. The study was approved by the Ethics Committee of Chongqing University Cancer Hospital (No. CZLS2023149-A).

Specimen collection and HPV genotyping

Cervical cellular samples were collected by a gynecologist with a specialized cervical brush. Specimens were stored at cell storaging solution by Yuanjiang Medical Supplies Co., Ltd. (Ningbo, China). The 15 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82) was performed according to the manufacturer's instructions with the HPV Genotyping Kit by China Shanghai ZJ Bio-Tech Co., Ltd. HPV-positive was identified using PCR. The kits used 15 types of HPV specific primers and fluorescent probes according to the basic of TaqMan fluorescent probe-based quantitative real-time PCR. Run the following program: 94℃*2 min, 93℃*10 s, 62℃*31 s, for 40 cycles. The reaction temperature of the single point fluorescence was 62℃. The PCR was implemented in a 40 μl reaction mixture.

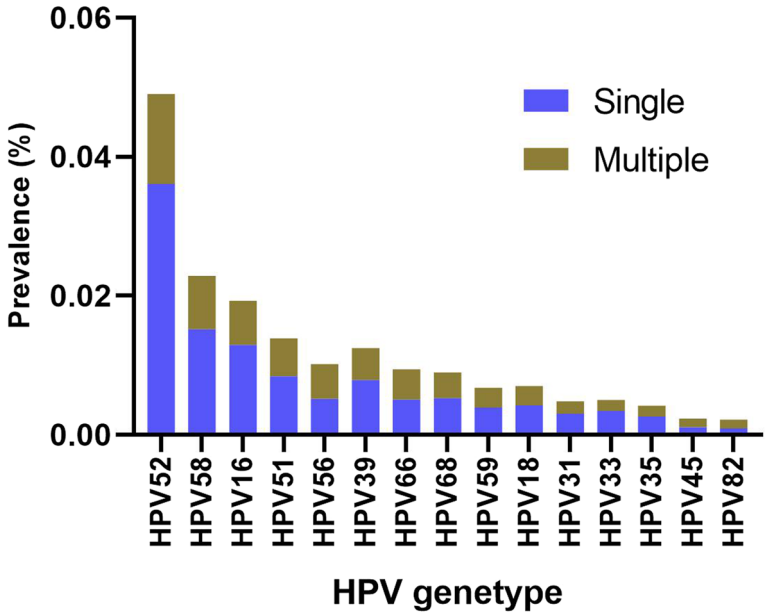

Fig. 1 Prevalence of single-type and multiple-type infection of each HPV subtype

Thinprep cytologic test

Cervical cytological Classifications were performed in conformity with the Bethesda system. The results were analyzed independently by two experienced pathologists, including negative intraepithelial lesions or malignancy (NILM), atypical squamous cell of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) (Due to the small number of cases, it will be incorporated into high-grade lesions), atypical glandular cells (AGC) (There were few cases and no correlation analysis was performed).HSIL in this study included HSIL and ASC-H in this study.

Statistical analysis

SPSS 26.0 was used for statistical analysis. Graphpad-prism9 software was used for graph drawing. The comparison between groups (single and multiple) was analyzed by chi-square test. Logistic regression was used to assess the correlation between HPV subtypes and cervical cytology. $P<0.05$ indicates that subtypes of HPV infection may be association with cervical cytology abnormalities (HSIL, LSIL, ASC-US). With these positive results of ASC-US, LSIL, and HSIL as the dependent variable (yes = 1, no = 0) and HPV infection as the independent variable, a logistic regression model was established after adjusting for age. Those relationships (positively associated (OR > 1): more likely to occur together and negatively associated (OR < 1): less likely to occur together) between those common 2 genotypes were analyzed by Logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) are presented. Taking HPV16, 52 and 58 infections as the dependent variable (yes = 1, no = 0) and the other several common HPV subtypes as the independent variable, the logistic regression model was established after adjusting for age.

Results

There were 715 multiple infections (563 cases were two HR-HPV infections, 152 cases were triple or more HR-HPV infections) among 3649 HR-HPV-positive females. Among all 15 HR-HPV genotypes, HPV52 (4.90%) was the most prevalent genotype, followed by HPV58 (2.29%), HPV16(1.93%), HPV51(1.39%), HPV39(1.25%), HPV56(1.02%), HPV66(0.09%) among total HPV infections. Here was the prevalence of the main multiple HPV infections: HPV52(1.30%), HPV58(0.77%), HPV16 (0.63%), HPV51(0.54%), HPV56(0.50%), HPV39(0.46%), HPV66(0.43%) Fig. 1.

The logistic regression between the HPV subtype and ASC-US, LSIL, and HSIL are shown in above Tables 1,2 and 3. The logistic regression model was established after adjusting for age.

The results showed that there was a significant correlation between HPV68, HPV52 infection and ASC-US ($P<0.05$) (Table 1). There was a positive correlation between HPV68 (OR = 1.856; 95% CI 1.206–2.855) and

Table 1 Logistic regression analyses of HPV infection and ASC-US

HPV Type	*B*	*SE*	*Wald*χ^2	*P*	*OR (95% CI)* [a]
HPV82	-	-	-	-	NA
HPV45	-0.155	0.599	0.067	0.796	0.857(0.265 ~ 2.769)
HPV35	-0.860	0.590	2.122	0.145	0.423(0.133 ~ 1.346)
HPV33	-0.117	0.371	0.099	0.753	0.89(0.43 ~ 1.842)
HPV31	-0.332	0.424	0.611	0.435	0.718(0.312 ~ 1.649)
HPV59	-0.135	0.319	0.179	0.672	0.874(0.468 ~ 1.632)
HPV18	0.171	0.287	0.355	0.551	1.186(0.676 ~ 2.081)
HPV68	0.618	0.220	7.914	0.005	1.856(1.206 ~ 2.855)
HPV66	-0.255	0.293	0.758	0.384	0.775(0.436 ~ 1.376)
HPV56	0.153	0.243	0.398	0.528	1.166(0.724 ~ 1.876)
HPV39	-0.270	0.259	1.088	0.297	0.764(0.46 ~ 1.268)
HPV51	0.032	0.219	0.021	0.886	1.032(0.672 ~ 1.586)
HPV16	-0.192	0.204	0.889	0.346	0.825(0.553 ~ 1.231)
HPV58	0.066	0.178	0.138	0.711	1.068(0.754 ~ 1.513)
HPV52	0.403	0.134	9.102	0.003	1.496(1.152 ~ 1.944)

[a] The analysis was adjusted for age

Table 2 Logistic regression analyses of HPV infection and LSIL

HPV Type	*B*	*SE*	*Wald*χ^2	*P*	*OR(95% CI)*[a]
HPV82	-0.330	0.725	0.207	0.649	0.719(0.174 ~ 2.979)
HPV45	-0.990	1.013	0.955	0.329	0.372(0.051 ~ 2.707)
HPV35	-1.672	1.008	2.750	0.097	0.188(0.026 ~ 1.355)
HPV33	0.457	0.339	1.824	0.177	1.580(0.814 ~ 3.067)
HPV31	-0.027	0.426	0.004	0.950	0.973(0.422 ~ 2.244)
HPV59	-0.694	0.460	2.275	0.131	0.499(0.203 ~ 1.231)
HPV18	0.314	0.309	1.032	0.310	1.369(0.747 ~ 2.509)
HPV68	0.435	0.264	2.705	0.100	1.545(0.920 ~ 2.594)
HPV66	0.894	0.223	16.048	0.000	2.445(1.579 ~ 3.787)
HPV56	0.216	0.270	0.638	0.424	1.241(0.731 ~ 2.106)
HPV39	-0.440	0.317	1.927	0.165	0.644(0.346 ~ 1.199)
HPV51	0.501	0.214	5.511	0.019	1.651(1.086 ~ 2.510)
HPV16	0.055	0.214	0.065	0.798	1.056(0.694 ~ 1.608)
HPV58	0.507	0.181	7.893	0.005	1.661(1.166 ~ 2.366)
HPV52	-0.270	0.165	2.674	0.102	0.763(0.552 ~ 1.055)

[a] The analysis was adjusted for age

HPV52 (OR = 1.496; 95% CI 1.152–1.944) infection and ASC-US (Fig. 2a).

The logistic regression results of HPV subtype infection and LSIL are shown. The results showed that there was a significant correlation between HPV66, HPV51 and HPV58 infection and LSIL ($P < 0.05$) (Table 2). The results showed there was a significant positive correlation between HPV66 (OR = 2.445; 95% CI 1.579–3.787), HPV51 (OR = 1.651; 95% CI 1.086–2.510) and HPV58 (OR = 1.661; 95% CI 1.166–2.366) and LSIL. (Fig. 2b).

Table 3 Logistic regression analyses of HPV infection and HSIL

HPV Type	*B*	*SE*	*Wald*χ^2	*P*	*OR(95% CI)*[a]
HPV82	-	-	-	-	NA
HPV45	-	-	-	-	NA
HPV35	-0.970	1.011	0.920	0.338	0.379(0.052 ~ 2.752)
HPV33	1.473	0.325	20.547	0.000	4.361(2.307 ~ 8.243)
HPV31	-0.388	0.721	0.289	0.591	0.678(0.165 ~ 2.790)
HPV59	-0.373	0.593	0.395	0.530	0.689(0.216 ~ 2.203)
HPV18	-0.096	0.518	0.034	0.853	0.909(0.329 ~ 2.506)
HPV68	-0.676	0.592	1.306	0.253	0.509(0.160 ~ 1.622)
HPV66	0.040	0.428	0.009	0.925	1.041(0.450 ~ 2.410)
HPV56	-0.490	0.516	0.900	0.343	0.613(0.223 ~ 1.685)
HPV39	-1.435	0.718	3.998	0.046	0.238(0.058 ~ 0.972)
HPV51	-0.580	0.464	1.564	0.211	0.560(0.225 ~ 1.390)
HPV16	1.556	0.223	48.714	0.000	4.742(3.063 ~ 7.342)
HPV58	0.147	0.281	0.275	0.600	1.159(0.668 ~ 2.008)
HPV52	-0.132	0.232	0.323	0.570	0.877(0.557 ~ 1.380)

[a] The analysis was adjusted for age

The logistic regression results of HPV subtype infection and HSIL are shown. The results showed that there was a correlation between HPV16, HPV33 and HPV39 infection and HSIL, and this difference reached statistical significance ($P < 0.05$) (Table 3). The results showed there was a significant positive correlation between HPV16(OR = 4.742; 95% CI 3.063–7.342) and HPV33 (OR = 4.361; 95% CI 2.307–8.243) infection and HSIL. There was a significant negative correlation between HPV39 (OR = 0.238; 95% CI 0.058–0.972) infection and HSIL (Fig. 2c).

Multiple HPV infections had an increased risk of LSIL (OR = 1.959; 95% CI 1.417–2.708) and HSIL (OR = 1.979; 95% CI 1.253–3.127) and it is statistically significant (LSIL: $P < 0.001$, HSIL: $P < 0.01$). The multiple HPV infections with clade α9 (more than 1 type belonging to clade α9) significant increased HSIL risk (OR = 1.995; 95% CI 1.101–3.616; $P < 0.05$). Women with multiple infections with clade α7, α6, and α5 are not at significant increased risk of HSIL compared to single infections. There were no differences for ASC-US and LSIL between multiple infections with clade α9, α7, α6, α5 and single infections ($P > 0.05$). The proportion of NILM with a single infection of α9 and α7 was significantly higher than that with multiple infections, and the differences were statistically significant ($P < 0.05$). There was no significant difference in α6 ($P > 0.05$) (Table 4).

Taking HPV16, 52 and 58 infections as the dependent variable (yes = 1, no = 0) and the other several common HPV subtypes as the independent variable, the logistic regression model was established after adjusting for age. The results showed that HPV52, HPV58, HPV51, HPV39,

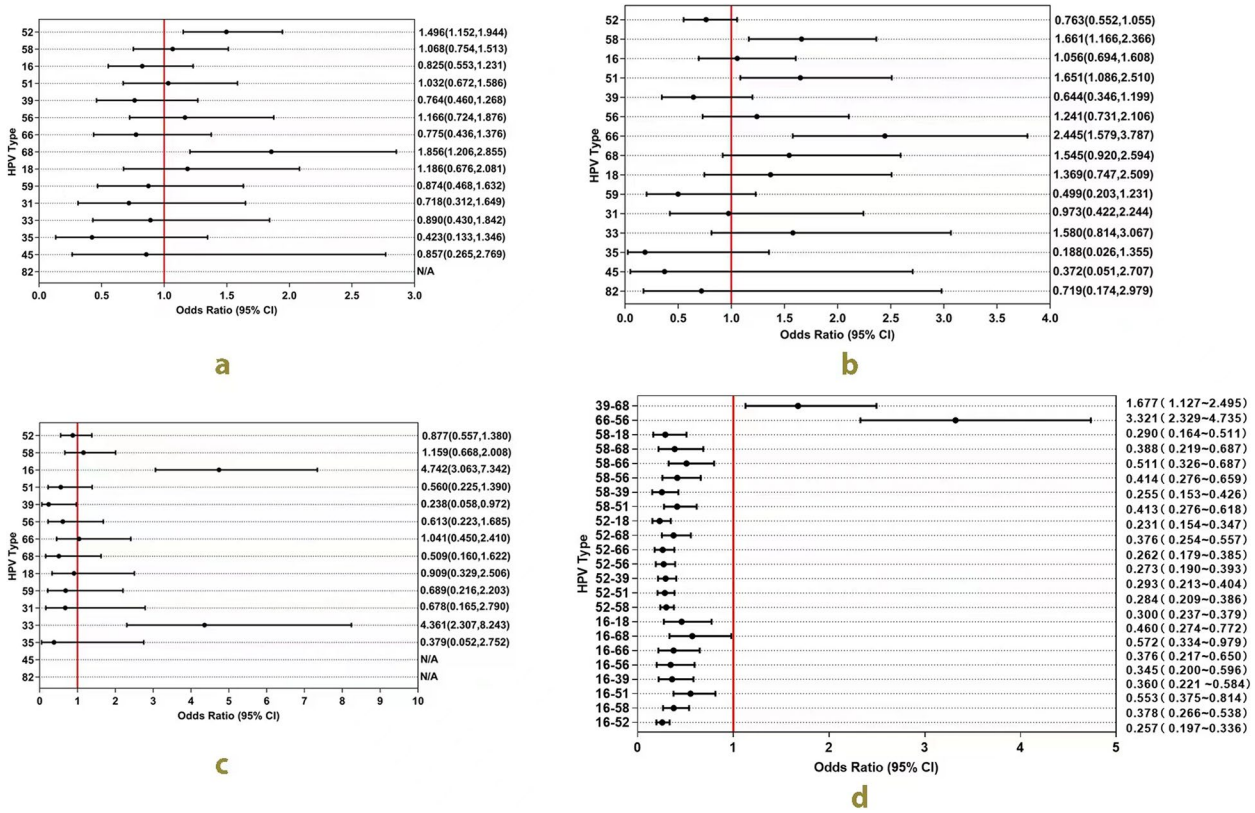

Fig. 2 **a** Odds ratios and 95% Confidence Intervals (CI) of HPV infection and ASCUS. **b** Odds ratios and 95% Confidence Intervals (CI) of HPV infection and LSIL. **c** Odds ratios and 95% Confidence Intervals (CI) of HPV infection and HSIL **d** Odds ratios and 95% Confidence Intervals (CI) for the common two-type HPV infections

HPV56, HPV66, HPV68, HPV18 infection and HPV16 infection were negatively correlated (All OR < 1). There were negative correlations between HPV58, HPV51, HPV39, HPV56, HPV66, HPV68, HPV18 infection and HPV52 infection (All OR < 1). There were negative correlations between HPV51, HPV39, HPV56, HPV66, HPV68, HPV18 infection and HPV58 infection (All OR < 1). As co-infection with HPV66 and HPV56, HPV39 and HPV68 were common, we further analyzed the association. There were positive correlations between HPV66 and HPV56 (OR, 3.321, 95% CI 2.329–4.735), HPV39 and HPV68 (OR, 1.677, 95% CI 1.127–2.495) (Fig. 2d).

Discussion

Obtaining special epidemiological data about HPV infections was very important for cervical cancer prevention through HPV DNA testing and vaccination with regional-specific HPV preventive vaccines. In this study, the most popular genotypes were HPV52, HPV58 and HPV16, which was not consistent with other counties and other parts of our country [19, 20]. The distribution of HPV genotypes varies with geographic location and ethnic groups [21].

The correlation between specific HPV and cervical cell abnormalities may get guidance for preventative strategies and HPV vaccination programs. In our study, the logistic regression analysis was performed to determine whether there was a correlation between the occurrence of different cytological results and specific HPV subtypes. We found that HPV16 and HPV33 were positively correlated with HSIL. Luo Q et al. [10] Pointed out that HPV16 and HPV33 may be the most oncogenic genotypes. According to the principle of "equal risk equal management" [22], HPV33 may be equally managed with HPV16. Women infected by HPV33 might be early referral to colposcopy [23]. In addition, there was a positive correlation between HPV66, HPV51 and HPV58 infection and LSIL. In United State, HPV58 were associated with a relatively higher risk of cervical lesions in addition to HPV16, HPV18 and HPV33 [24]. The study found HPV58 was the most carcinogenic subtype except HPV16 [25]. In our study HPV66 was also commonly seen in multiple infections and was positively correlated

Table 4 The relationship between different HPV clade and TCT results

Type	No	NLIM + No(%)	OR(95% CI)[a]	χ^2	*P*	ASCUS + No(%)	OR(95% CI)[a]	χ^2	*P*
HPV				28.312	< 0.001			3.028	0.082
Single	2946	2562(87.0)	1.000			189(6.4)	1.000		
Multiple	703	556(79.1)	0.555(0.449–0.687)			58(8.3)	1.334(0.982–1.814)		
α9 species				6.656	0.010			1.454	0.228
Single	2199	1864(84.8)	1.000			150(6.8)	1.000		
Multiple	235	184(78.3)	0.624(0.447–0.870)			21(8.9)	1.402(0.867–2.265)		
α7 species				7.208	0.007			3.331	0.068
Single	810	714(88.1)	1.000			52(6.4)	1.000		
Multiple	75	58(77.3)	0.441(0.246–0.793)			9(12.0)	2.047(0.963–4.349)		
α6 species				0.503	0.478			0.972	0.324
Single	412	345(83.7)	1.000			31(7.5)	1.000		
Multiple	44	35(79.5)	0.752(0.345–1.639)			1(2.3)	0.284(0.038–2.131		
α5 species				0.000	1.000			0.000	1.000
Single	401	339(84.5)	NA			25(6.2)	NA		
Multiple	3	3(100.0)				0(0.0)			
Type	No	LSIL + No(%)	OR(95% CI)[a]	χ^2	*P*	HSIL + No(%)	OR(95% CI)[a]	χ^2	*P*
HPV				15.941	< 0.001			9.135	0.003
Single	2946	130(4.4)	1.000			60(2.0)	1.000		
Multiple	703	57(8.1)	1.959(1.417–2.708)			28(4.0)	1.979(1.253–3.127)		
α9 species				0.831	0.362			5.591	0.018
Single	2199	110(5.0)	1.000			67(3.0)	1.000		
Multiple	235	15(6.4)	1.346(0.770–2.354)			14(6.0)	1.995(1.101–3.616)		
α7 species				1.666	0.197			0.000	1.000
Single	810	33(4.1)	1.000			10(1.2)	1.000		
Multiple	75	6(8.0)	2.135(0.861–5.296)			1(1.3)	1.071(0.135–8.504)		
α6 species				3.462	0.063			0.000	1.000
Single	412	28(6.8)	1.000			8(1.9)	1.000		
Multiple	44	7(15.9)	2.638(1.076–6.468)			1(2.3)	1.177(0.143–9.647)		
α5 species				0.000	1.000			0.000	1.000
Single	401	30(7.5)	NA			5(1.2)	NA		
Multiple	3	0(0.0)				0(0.0)			

Multiple: two and more than two multiple infections

NA Not applicable

[a] The analysis was adjusted for age

with LSIL lesions. The research found that HPV66 had the higher prevalence in premalignant lesions [26]. HPV51 may play the contributory role to cervical cancer. The prevalence of HPV51 was higher in cervical lesions not only in Chongqing, but also in the other areas [27]. More attention should be paid to HPV 51 infection in the post-vaccine era in the area. Therefore, it seems that these three HPV infections (HPV66, HPV51 and HPV58) may also need to be managed intensively. There was a positive correlation between HPV68 and HPV52 infection and ASC-US (OR > 1). Although HPV52 was the most prevalent type in Chongqing, it was not associated with HSIL and LSIL in this study.

Besides specific HPV genotypes, multiple infection may be one of the high-risk factors for cervical cancer [28]. We also found that multiple HPV infection was associated with an increased risk of LSIL and HSIL compared with single infection, which was in line with the study [29]. In this study, the effects of multiple infection and single infection on cervical lesions in different HPV populations (including α9, α5, α6, α7) were further investigated, and it was found that coinfection with multiple a9 species was associated with an increased risk of

HSIL. Previous studies have found that co-infection with α9 increases the risk of cervical disease [30, 31]. Other studies have indicated that multiple α7 subtype infections have an increased risk of cervical cancer [32]. In our study, multiple subtype infections with the α7 clade were found to be more likely to develop LSIL, but did not increase the risk of HSIL. Although multiple infections increase the risk of HSIL, it is likely to be largely due to multiple infections with α9 (HPV co-infection with α9 was the most). Therefore, the management of multiple infections with α9 may be strengthened.

In this study, we further investigated the association between the most common multiple HR-HPV. We found HPV66 and HPV56 (α6), HPV39 and HPV68 (α7) had the positive relationship, which is consistent with the relevant study in the United States [33]. This may be related to conserved amino acid sequences or structural similarity of shared neutralizing epitopes [34]. However, It was found negative interactions between the common α9 (HPV16, 52, and58) and other HPV subtypes (HPV51、HPV39、HPV56、HPV66、HPV68、HPV18) in this study. Dickson EL [35] also found that α9 appeared to be the only species with a strong negative association.

To a large extent, the protection provided by the HPV vaccine is type-specific. There may be more efficiency on the 9-valent vaccine against cervical lesions (against the top three prevalent HPV52, 58, and 16 as well as the more carcinogenic HPV 33, although its prevalence was not high) in Chongqing. Therefore, the 9-valent vaccine may provide better protection for the population of the vaccines currently available in Chongqing. But there is some challenge such as high-cost and insufficient vaccine supply about the 9-valent vaccine. Moreover the 9-valent HPV vaccine does not contain approximately 10% of HPV genotypes associated with high-grade lesions [36]. Our study found that the 9-valent HPV does not encompass the other HPV genotypes which lead to abnormal cytology in Chongqing. Therefore, it is necessary to develop new HPV vaccines to deal with related problems in China. Some HPV vaccines [37] (qvHPV vaccine, several 9vHPV vaccines, an 11vHPV vaccine, and a 14vHPV vaccine) are under development in China, and the successful marketing of locally developed HPV vaccines may help solve the supply problem and improve the vaccination rate. Considering the higher prevalence and pathogenicity of HPV51 and HPV66, the development of vaccines against HPV51 and HPV66 may be intensified in this region.

In the long term, high HPV vaccination coverage may affect the distribution of HPV infection types and the risk of type replacement may increase if HPV vaccination fails to induce strong cross-protection [38]. In this study, HPV16, HPV52, and HPV58 were negative associated with other common high-risk genotypes, which may increase the possibility of type substitution after HPV16 vaccination. A German study [39] found a decrease in HPV16, 18, and 31 infection rates and an increase in non-vaccine HPV infections such as HPV51, 53, 56, and 66. The cross-protection might associate with the phylogenetic distance from vaccine types [40]. From the perspective of vaccine substitution, the local vaccines in Chongqing also seemed to include HPV51 and HPV66. The other researches also recommended that HPV66 or HPV51 should be included in future HPV vaccine formulations [41–43].

This study was a single-center study. The data was based on a relatively small sample size. The multicenter and prospective studies in different geographic regions are still needed to be conducted to assure the results in the future.

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Authors' contributions
QLL designed the experiments. HYZ and XHZ performed the experiments. NH and ZM collected and analyzed the data. NH, ZM and HYL drafted manuscript. All authors read and approved the final manuscript.

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None.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All patients were informed and signed informed consent voluntarily. This study was approved by the ethics committee of Chongqing University Cancer Hospitaland complied with the guidelines outlined in the declaration of Helsinki were followed. The written consent was received from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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