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Genotypic analysis of human papillomavirus in cervical exfoliated cells from women in Zigong

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Abstract

Background This study investigated the human papillomavirus (HPV) infection status among women in Zigong from January 2016 to August 2024 and provides a comprehensive statistical analysis of HPV infection characteristics. The findings aim to enhance cervical cancer screening, inform vaccination strategies, and improve HPV infection prevention measures.

Methods We conducted a retrospective analysis on 48,474 female patients who visited the gynecology department of Zigong Fourth People's Hospital from January 2016 to August 2024. Cervical exfoliated cell samples were collected from the patients, and the genotypes of 10 low-risk HPV (LR-HPV) and 17 high-risk HPV (HR-HPV) were detected by flow fluorescent hybridization technique. The study explored HPV infection rates, genotype distribution, number of infections, type of infections, and age distribution. The chi-squared (χ^2) test was employed to compare infection statuses between groups.

Results Among the 48,474 patients, 9749 tested positive for HPV, with an overall infection rate of 20.11%. The HPV infection rate increased gradually from 2016 to 2024 ($P < 0.001$). The infection rates of single, double, triple, and ≥ quadruple infections were 15.11%, 3.54%, 1.00%, and 0.46%, respectively. The infection rates were 4.41% for LR-HPV-only, 13.13% for HR-HPV-only, and 2.57% for mixed LR and HR-HPV. HR-HPV primarily consisted of HPV types 52, 16, 53, and 58, with infection rates of 3.94%, 2.71%, 2.43%, and 2.42%, respectively. LR-HPV primarily consisted of types 61 and 81, with infection rates of 1.64% and 1.49%, respectively. A significant age correlation in HPV infection was observed ($P < 0.001$), with two distinct peaks in infection rates.

Conclusions The HPV infection rate among women visiting the gynecology department in Zigong is high, predominantly involving HPV types 52, 16, 53, and 58. Therefore, strengthening HPV screening efforts and focusing on standardized genotype screening is crucial. Additionally, selecting HPV vaccines targeting prevalent genotypes and actively conducting HPV prevention and control work can reduce the incidence of HPV-related cervical cancer and other HPV-related diseases.

Keywords Human papillomavirus, Infection rate, Genotype, Cervical cancer, Zigong region

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Background

Cervical cancer is the fourth most common malignancy among women worldwide [1]. In 2022, there were approximately 660,000 new cases of cervical cancer globally, resulting in 350,000 deaths [1]. Cervical cancer screening is inadequate in low- and middle- income countries, which account for approximately 90% of cervical cancer deaths — a rate 18 times higher than in developed countries [2]. China accounted for 18.60% of the global cervical cancer cases and 15.40% of related deaths in 2018 [3]. Over 95% of cervical cancers are associated with human papillomavirus (HPV), with persistent infection being the most significant cause [4]. HPV is the most common sexually transmitted virus, and most sexually active individuals will be infected at some point in their lives.

HPV is a small, non-enveloped, circular DNA virus classified under the Papillomavirus genus of the Papovaviridae family [4]. Over 200 HPV genotypes have been identified, with significant differences in infection rates and carcinogenicity among different types [5]. All definitely or possibly carcinogenic HPV types belong to one branch of the α genus [6]. Based on their cancer risk, the International Agency for Research on Cancer (IARC) classified HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as carcinogenic to humans (Group 1), HPV 68 as probably carcinogenic to humans (Group 2 A), and HPV types 26, 53, 66, 67, 70, 73, 82 in the high-risk clade as possibly carcinogenic to humans (Group 2B) [7]. HPV types 6 and 11 are not classifiable as to their carcinogenicity to humans (Group 3) [7]. HPV types are also divided into low-risk (LR-HPV) and high-risk (HR-HPV) categories based on their potential to cause either benign or malignant lesions [8]. LR-HPV causes warty lesions and low-grade intraepithelial neoplasia, mainly including types 6, 11, 40, 42, 43, 44, 55, 61, 81, and 83 [9]. HR-HPV causes cervical cancer and precancerous lesions. Among them, LR-HPV types 6 and 11 cause more than 90% of genital warts, while HR-HPV types 16 and 18 cause more than 70% of cervical cancers [10]. Additionally, HR-HPV can also lead to vaginal cancer, penile cancer, anal cancer, oropharyngeal cancer, and head and neck cancers [11]. LR-HPV genotyping can be used for differential diagnosis of genital warts and other conditions such as pearly penile papules, sebaceous hyperplasia, flat warts, and pseudo-warts, providing clinical evidence for the treatment and evaluation of genital warts. HR-HPV genotyping can be used for early detection, risk stratification, and monitoring of treatment and surgical outcomes in cervical cancer.

Currently, cervical cancer screening methods mainly include visual inspection with acetic acid, cervical cytology (Pap smear and liquid-based cytology), HR-HPV nucleic acid testing (DNA and E6/E7 mRNA), and biomarker testing (DNA methylation and p16/Ki-67 dual staining) [12]. Among these, nucleic acid testing has the highest detection rate and positive predictive value for high-grade intraepithelial neoplasia and cervical cancer screening [2, 13]. E6/E7 mRNA can monitor the activity of the virus and avoid detecting transient infections. Therefore, E6/E7 mRNA testing has a similar sensitivity to DNA testing and higher specificity, but the current longitudinal research evidence is still limited [12]. HR-HPV DNA testing for screening healthy populations is more effective than cytology alone and is equivalent to combined cytology and HR-HPV DNA testing [14]. The World Health Organization (WHO) still recommends HR-HPV DNA testing as the primary screening method for cervical cancer in women over 30 years old, with regular screening every 5 to 10 years [15]. In China, HPV genotyping is a widely used DNA testing method, and many test kits are available. The detection principles and types of genotyping vary among different kits, but HR-HPV always includes types 16 and 18, and LR-HPV always includes types 6 and 11.

Global data show significant differences in the characteristics of HPV infection in cervical exfoliated cells among women in different countries [16]. China, with its vast territory and large population, has significant temporal and spatial differences in HPV epidemiological characteristics due to uneven economic development. Zigong, located in the southern region of Sichuan Province, has underdeveloped local economic conditions, low per capita income, poor patient awareness of medical consultation, and a lack of large-scale HPV statistical data. Investigating HPV prevalence in Zigong is vital for early diagnosis and prevention of cervical intraepithelial neoplasia, warty lesions, and cervical cancer in this population. This study retrospectively analyzed the HPV genotyping results of cervical exfoliated cells from 48,474 women attending the gynecology department of Zigong Fourth People's Hospital, and the results are reported as follows.

Methods

Study population

The study included female patients who visited the gynecology department of Zigong Fourth People's Hospital from January 2016 to August 2024 and underwent cervical exfoliated cell HPV genotyping for 27 types. A total of 48,474 patients were included, ranging in age from 13 to 93 years, with an average age of 43 years. This is a retrospective study, and all patients were anonymized, ensuring no identifiable information was disclosed; thus, informed consent was not required. Ethical approval was obtained from the Ethics Committee of Zigong Fourth People's Hospital.

Cervical sample collection

Clinicians used a speculum to expose the cervix of the patient, wiped away cervical secretions with a cotton swab, and inserted a cervical brush into the cervix. The brush was gently rotated clockwise 4–5 times, then slowly withdrawn and placed into a tube containing cell preservation solution (Shanghai Tellgen Biotech Co., Ltd). The brush was broken off at the handle's crease, leaving the brush head in the tube, and the cap was tightened. Samples were stored at room temperature and tested within seven days.

HPV DNA extraction, PCR amplification, and genotyping

DNA was extracted from the samples using a nucleic acid extraction kit (Shanghai Tellgen Biotech Co., Ltd). First, the sample bottle containing the cervical brush was shaken to mix, and 200 μL was transferred to a 1.5 mL EP tube, centrifuged at 14,000 r/min for 3 min. The supernatant was discarded, 200 μL of nucleic acid extraction reagent was added, mixed, and heated in a 100 °C metal bath for 15 min. The EP tube was centrifuged at 14,000 r/min for 5 min, and the resulting supernatant containing the DNA template was subjected to PCR amplification.

The HPV genotyping test kit (Shanghai Tellgen Biotech Co., Ltd) includes PCR amplification reagents and hybridization reagents. Multiplex PCR amplification was performed using universal primers, and genotyping was conducted using hybridization. The kit can genotype 27 types, with HR-HPV including 17 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 26, 53, and 82, and LR-HPV including 10 types: 6, 11, 40, 42, 43, 44, 55, 61, 81, and 83. The genotyping detection principle is based on flow fluorescent hybridization technique, including 27 classification microspheres and 1 quality control microsphere. Probes hybridized with HPV DNA are coated on the 27 classification microspheres, and a positive signal from the type-specific probe indicates a positive HPV type. The quality control microsphere is a probe hybridized with the human β-globin gene.

The PCR amplification reaction volume was 20.8 μL, including 10 μL of premix (containing dATP, ddTTP, dCTP, dGTP, magnesium chloride in Tris-HCl buffer), 5.0 μL of primer mix (containing multiple primers), 0.8 μL of DNA polymerase, and 5 μL of DNA template. The PCR amplification reaction was as follows: 95 °C for 5 min, 1 cycle; 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, 5 cycles; 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, 35 cycles; 72 °C for 3 min, 1 cycle.

A volume of 22 μL of microsphere hybridization solution was loaded into a microplate, and 3 μL of the amplification product was added, mixed, and sealed with a cover film. Denaturation was performed at 95 °C for 5 min, hybridization at 48 °C for 30 min, and incubation at 48 °C for 15 min. The cover film was removed, 75 μL of streptavidin-phycoerythrin solution was added, resealed, and incubated at 48 °C for another 15 min. The microplate was transferred to a multifunctional flow cytometer (Luminex 200, USA) for detection. When the β-globin signal was >150 and the HPV type-specific probe signal was >150, the corresponding HPV type was determined to be positive.

Statistical analysis

Statistical analyses were performed using SPSS version 29.0 software. All participants were grouped by age into the following categories: ≤20 years, 21–30 years, 31–40 years, 41–50 years, 51–60 years, and ≥61 years, to assess HPV infection status. Categorical data were expressed as percentages, and the chi-square (χ^2) test was employed. A $P<0.05$ was considered statistically significant.

Results

HPV genotype distribution in different years

Among the 48,474 patients included in the study, 9749 tested positive for HPV, yielding an overall infection rate of 20.11%. The HPV infection rate increased from 14.86% in 2016 to 26.18% in 2024, demonstrating a fluctuating upward trend. This difference was statistically significant (χ^2=439.871, $P<0.001$). In terms of specific genotypes, most HPV genotypes exhibited an increasing infection trend. However, LR-HPV types 6 and 11, as well as HR-HPV types 16 and 18, showed a slight decline. The 2024 data were analyzed up to August (Table 1).

HPV genotype distribution in different age group

LR-HPV infections were mainly of types 61, 81, and 44, with infection rates of 1.64% (793/48,474), 1.49% (722/48,474), and 1.17% (568/48,474), respectively. HR-HPV infections were primarily of types 52, 16, 53, and 58, with infection rates of 3.94% (1910/48,474), 2.71% (1314/48,474), 2.43% (1177/48,474), and 2.42% (1175/48,474), respectively. Additionally, LR-HPV types 61, 81, 43, 6, 55, 11, and 42, and HR-HPV types 52, 16, 53, 58, 39, 56, 51, 59, 18, 66, 33, 35, and 45 showed age-related bimodal distribution in infection rates (Table 2).

HPV infection types in different age group

Among the 48,474 patients, 13.13% (6367/48,474) were HR-HPV-only infected patients, 4.41% (2137/48,474) were LR-HPV-only infected patients, and 2.57% (1245/48,474) were mixed LR and HR-HPV infected patients. HR-HPV-only infection was the most common, with infection rates exceeding 10% across all age groups. The infection rates of LR-HPV-only, HR-HPV-only, mixed LR and HR-HPV, and the total infection rate showed a bimodal distribution pattern with age, and the differences were statistically significant (χ^2 = 68.288,

Table 1 HPV genotype distribution from January 2016 to August 2024 [n (%)]

HPV genotype	2016 *n* = 2691	2017 *n* = 4591	2018 *n* = 5785	2019 *n* = 6419	2020 *n* = 5641	2021 *n* = 6629	2022 *n* = 6731	2023 *n* = 6301	2024 *n* = 3686
LR-HPV									
61	17(0.63)	44(0.96)	56(0.97)	103(1.60)	75(1.33)	131(1.98)	147(2.18)	131(2.08)	89(2.41)
81	16(0.59)	38(0.83)	58(1.00)	61(0.95)	54(0.96)	115(1.73)	120(1.78)	126(2.00)	134(3.64)
44	18(0.67)	25(0.54)	29(0.50)	75(1.17)	78(1.38)	100(1.51)	108(1.60)	72(1.14)	63(1.71)
43	15(0.56)	40(0.87)	47(0.81)	44(0.69)	38(0.67)	69(1.04)	69(1.03)	74(1.17)	43(1.17)
6	36(1.34)	37(0.81)	32(0.55)	48(0.75)	38(0.67)	45(0.68)	67(1.00)	70(1.11)	38(1.03)
55	12(0.45)	16(0.35)	31(0.54)	32(0.50)	27(0.48)	60(0.91)	62(0.92)	42(0.67)	46(1.25)
11	27(1.00)	32(0.70)	50(0.86)	46(0.72)	23(0.41)	29(0.44)	36(0.53)	51(0.81)	13(0.35)
42	2(0.07)	5(0.11)	5(0.09)	9(0.14)	8(0.14)	18(0.27)	19(0.28)	29(0.46)	22(0.60)
83	6(0.22)	13(0.28)	11(0.19)	14(0.22)	12(0.21)	9(0.14)	13(0.19)	14(0.22)	10(0.27)
40	1(0.04)	5(0.11)	5(0.09)	6(0.09)	2(0.04)	3(0.05)	4(0.06)	5(0.08)	3(0.08)
HR-HPV									
52	54(2.01)	120(2.61)	163(2.82)	210(3.27)	186(3.30)	316(4.77)	360(5.35)	311(4.94)	190(5.15)
16	80(2.97)	122(2.66)	173(2.99)	140(2.18)	136(2.41)	189(2.85)	208(3.09)	170(2.70)	96(2.60)
58	56(2.08)	101(2.20)	132(2.28)	132(2.06)	113(2.00)	171(2.58)	175(2.60)	178(2.82)	117(3.17)
53	32(1.19)	88(1.92)	97(1.68)	146(2.27)	132(2.34)	173(2.61)	210(3.12)	185(2.94)	114(3.09)
39	20(0.74)	40(0.87)	66(1.14)	71(1.11)	37(0.66)	96(1.45)	95(1.41)	96(1.52)	57(1.55)
56	16(0.59)	37(0.81)	47(0.81)	58(0.90)	57(1.01)	91(1.37)	96(1.43)	110(1.75)	44(1.19)
51	19(0.71)	42(0.91)	52(0.90)	38(0.59)	41(0.73)	83(1.25)	91(1.35)	63(1.00)	49(1.33)
59	17(0.63)	27(0.59)	52(0.90)	44(0.69)	50(0.89)	56(0.84)	63(0.94)	64(1.02)	38(1.03)
18	19(0.71)	41(0.89)	47(0.81)	36(0.56)	47(0.83)	52(0.78)	77(1.14)	43(0.68)	24(0.65)
66	12(0.45)	17(0.37)	31(0.54)	45(0.70)	33(0.59)	55(0.83)	51(0.76)	60(0.95)	34(0.92)
33	17(0.63)	24(0.52)	33(0.57)	30(0.47)	28(0.50)	37(0.56)	33(0.49)	44(0.70)	25(0.68)
68	2(0.07)	6(0.13)	35(0.61)	30(0.47)	20(0.35)	56(0.84)	49(0.73)	54(0.86)	27(0.73)
35	12(0.45)	18(0.39)	23(0.40)	23(0.36)	15(0.27)	28(0.42)	19(0.28)	28(0.44)	16(0.43)
31	4(0.15)	19(0.41)	8(0.14)	15(0.23)	12(0.21)	28(0.42)	34(0.51)	25(0.40)	24(0.65)
45	5(0.19)	14(0.30)	7(0.12)	16(0.25)	13(0.23)	13(0.20)	20(0.30)	19(0.30)	20(0.54)
26	0(0.00)	2(0.04)	0(0.00)	1(0.02)	3(0.05)	1(0.02)	2(0.03)	2(0.03)	3(0.08)
Total	400(14.86)	714(15.55)	973(16.82)	1121(17.46)	962(17.05)	1463(22.07)	1628(24.19)	1523(24.17)	965(26.18)

$P < 0.001$; $\chi^2 = 130.549$, $P < 0.001$; $\chi^2 = 527.987$, $P < 0.001$; $\chi^2 = 462.631$, $P < 0.001$). (Table 3).

Distribution of single and multiple HPV infections

Among the 9749 HPV-positive patients, 75.12% (7323/9749) had a single infection, 17.62% (1718/9749) had double infections, 4.99% (486/9749) had triple infections, and 2.28% (222/9749) had ≥ quadruple infections. (Table 4).

Distribution of single and multiple infections in different age group

The rates of single, double, triple, and ≥ quadruple infections varied significantly across the defined age groups ($\chi^2 = 70.673$, $P < 0.001$; $\chi^2 = 275.935$, $P < 0.001$; $\chi^2 = 344.052$, $P < 0.001$; $\chi^2 = 313.490$, $P < 0.001$), showing a bimodal distribution pattern. The peak for single infections was at ≤ 20 years and 51–60 years, while the peaks for double, triple, and ≥ quadruple infections were at ≤ 20 years and ≥ 61 years. (Table 5).

Distribution of LR-HPV-only, HR-HPV-only, and mixed LR and HR-HPV in co-infections with different numbers

Among the 9749 HPV-infected patients, 21.92% (2137/9749) were LR-HPV-only infections, 65.31% (6367/9749) were HR-HPV-only infections, and 12.77% (1245/9749) were mixed LR and HR-HPV infections. The composition ratio of LR-HPV-only and HR-HPV-only was highest in single infections, at 26.56% and 73.44% respectively, and gradually decreased with an increase in the number of infections. Conversely, the composition ratio of mixed LR and HR-HPV was lowest in double infections and gradually increased with an increase in the number of infections. (Table 6).

HPV genotype distribution in single and multiple infections

Among patients with single infections, the most common HPV genotypes were HPV 52 (16.62%), HPV 16 (11.85%), HPV 58 (9.55%), and HPV 53 (9.41%). Among patients with multiple infections, the most common genotypes were HPV types 52 (28.57%), 53 (20.12%), 58 (19.62%), and 16 (18.38%). The most common genotype

Table 2 HPV genotype distribution in different age group [n (%)]

HPV Genotype	≤ 20 *n* = 436	21–30 *n* = 6522	31–40 *n* = 12,378	41–50 *n* = 17,656	51–60 *n* = 8526	≥ 61 *n* = 2956	Total *n* = 48,474
LR-HPV							
61	12(2.75)	92(1.41)	160(1.29)	236(1.34)	211(2.47)	82(2.77)	793(1.64)
81	10(2.29)	78(1.20)	120(0.97)	220(1.25)	220(2.58)	74(2.50)	722(1.49)
44	4(0.92)	61(0.94)	111(0.90)	184(1.04)	163(1.91)	45(1.52)	568(1.17)
43	9(2.06)	81(1.24)	87(0.70)	115(0.65)	108(1.27)	39(1.32)	439(0.91)
6	36(8.26)	97(1.49)	65(0.53)	102(0.58)	65(0.76)	46(1.56)	411(0.85)
55	6(1.38)	34(0.52)	59(0.48)	99(0.56)	79(0.93)	51(1.73)	328(0.68)
11	28(6.42)	66(1.01)	37(0.30)	63(0.36)	60(0.70)	53(1.79)	307(0.63)
42	1(0.23)	10(0.15)	23(0.19)	38(0.22)	27(0.32)	18(0.61)	117(0.24)
83	0(0.00)	4(0.06)	21(0.17)	38(0.22)	24(0.28)	15(0.51)	102(0.21)
40	0(0.00)	10(0.15)	6(0.05)	5(0.03)	8(0.09)	5(0.17)	34(0.07)
HR-HPV							
52	23(5.28)	268(4.11)	462(3.73)	558(3.16)	431(5.06)	168(5.68)	1910(3.94)
16	34(7.80)	205(3.14)	255(2.06)	400(2.27)	264(3.10)	156(5.28)	1314(2.71)
53	15(3.44)	144(2.21)	249(2.01)	338(1.91)	308(3.61)	123(4.16)	1177(2.43)
58	20(4.59)	171(2.62)	255(2.06)	312(1.77)	258(3.03)	159(5.38)	1175(2.42)
39	10(2.29)	100(1.53)	115(0.93)	180(1.02)	116(1.36)	57(1.93)	578(1.19)
56	10(2.29)	81(1.24)	107(0.86)	139(0.79)	138(1.62)	81(2.74)	556(1.15)
51	6(1.38)	104(1.59)	96(0.78)	119(0.67)	106(1.24)	47(1.59)	478(0.99)
59	12(2.75)	77(1.18)	84(0.68)	120(0.68)	84(0.99)	34(1.15)	411(0.85)
18	15(3.44)	55(0.84)	97(0.78)	99(0.56)	68(0.80)	52(1.76)	386(0.80)
66	7(1.61)	63(0.97)	68(0.55)	71(0.40)	88(1.03)	41(1.39)	338(0.70)
68	1(0.23)	25(0.38)	57(0.46)	97(0.55)	69(0.81)	30(1.01)	279(0.58)
33	3(0.69)	29(0.44)	53(0.43)	94(0.53)	60(0.70)	32(1.08)	271(0.56)
35	1(0.23)	25(0.38)	31(0.25)	54(0.31)	43(0.50)	28(0.95)	182(0.38)
31	3(0.69)	11(0.17)	45(0.36)	55(0.31)	35(0.41)	20(0.68)	169(0.35)
45	1(0.23)	17(0.26)	27(0.22)	34(0.19)	36(0.42)	12(0.41)	127(0.26)
26	0(0.00)	0(0.00)	3(0.02)	3(0.02)	4(0.05)	4(0.14)	14(0.03)

Table 3 HPV infection rates in different age group [n (%)]

Age	LR-HPV-only	HR-HPV-only	Mixed LR and HR-HPV	Total
≤ 20	36(8.26)	62(14.22)	48(11.01)	146(33.49)
21–30	313(4.80)	934(14.32)	174(2.67)	1421(21.79)
31–40	457(3.69)	1567(12.66)	175(1.41)	2199(17.77)
41–50	709(4.02)	2016(11.42)	293(1.66)	3018(17.09)
51–60	473(5.55)	1268(14.87)	363(4.26)	2104(24.68)
≥ 61	149(5.04)	520(17.59)	192(6.50)	861(29.13)
Total	2137(4.41)	6367(13.13)	1245(2.57)	9749(20.11)

Table 4 Distribution of single and multiple HPV infections among HPV-positive patients

Infection Type	Total	Proportion (%)	Positive rate (%)
Single infection	7323	75.12	15.11
LR-HPV	1945	19.95	4.01
HR-HPV	5378	55.16	11.09
Multiple infections	2426	24.88	5.00
Double genotypes	1718	17.62	3.54
Triple genotypes	486	4.99	1.00
≥Quadruple genotypes	222	2.28	0.46
Total	9749	100.00	20.11

Table 5 Distribution of single and multiple infections in different age group [n(%)]

Age	Single genotype	Double genotypes	Triple genotypes	≥Quadruple genotypes
≤ 20	78(17.89)	32(7.34)	23(5.28)	13(2.98)
21–30	1046(16.04)	281(4.31)	78(1.20)	16(0.25)
31–40	1811(14.63)	309(2.50)	59(0.48)	20(0.16)
41–50	2426(13.74)	475(2.69)	81(0.46)	36(0.20)
51–60	1470(17.24)	399(4.68)	158(1.85)	77(0.90)
≥ 61	492(16.64)	222(7.51)	87(2.94)	60(2.03)
Total	7323(15.11)	1718(3.54)	486(1.00)	222(0.46)

combinations in multiple infections were HPV 52 + HPV 53 ($n = 106$), HPV 52 + HPV 58 ($n = 105$), HPV 52 + HPV 16 ($n = 84$), and HPV 52 + HPV 81 ($n = 80$). (Fig. 1).

Discussion

Recent studies indicate that the carcinogenic potential of 17 HPV genotypes is supported, including four Group 2B genotypes (HPV 73, 26, 69, and 82) in addition to the 13 genotypes previously classified by IARC as Group 1/2A [5]. In HPV-related cervical cancers, HPV types 16, 18, 45, 33, 58, 31, and 52 account for 61.7%, 15.3%, 4.8%,

Table 6 Distribution of LR-HPV-only, HR-HPV-only, and mixed LR and HR-HPV in co-infections with different numbers [n (%)]

Number of co-infections	LR-HPV-only	HR-HPV-only	Mixed LR and HR-HPV	Total
Single genotype	1945(26.56)	5378(73.44)	/	7323
Double genotypes	176(10.24)	799(46.51)	743(43.25)	1718
Triple genotypes	14(2.88)	147(30.25)	325(66.87)	486
≥Quadruple genotypes	2(0.90)	43(19.37)	177(79.73)	222
Total	2137(21.92)	6367(65.31)	1245(12.77)	9749

3.8%, 3.5%, 2.8%, and 2.8% of cases, respectively; HPV types 35, 59, 39, 56, 51, 68, 73, 26, 69, and 82 collectively account for 5.3% [5]. Therefore, identifying the specific types of HPV infection in patients can more accurately assess their condition and disease risk, and provide richer information on the prevalence of each genotype. This study analyzed 27 HPV genotypes in cervical exfoliated cells from women attending the gynecology department of Zigong Fourth People's Hospital from January 2016 to August 2024. These genotypes cover almost all pathogenic HPV types, providing strong evidence for the epidemiological situation of HPV in Zigong and potentially influencing local HPV vaccination, cervical cancer screening, and public health policies.

The overall HPV infection rate in Zigong is 20.11%, similar to Xinxiang (19.7%) [17] and Guangzhou (21.66%) [18], lower than Shanghai (45.57%) [19] and Jinan (28.4%) [20], and higher than Shenzhen (17.83%) [21] and Hengyang (10.16%) [22]. HR-HPV (13.13%) is the main type of infection, three times that of LR-HPV (4.41%), similar to Guangxi (14.36%) [23], lower than Shandong (18.1%) [20], and higher than Qujing (10.87%) [9]. Therefore, there are significant differences in HPV infection rates across different regions in China. These differences may be due to factors such as economic development level, population structure differences, public health policies, education levels, and HPV vaccination.

From 2016 to 2019, there was a significant increase in the number of individuals participating in HPV screening in the Zigong region. Subsequently, with the exception of 2020, which saw a decline in participation, the numbers remained relatively stable in the following years. The decrease in 2020 may be attributed to the onset of the COVID-19 pandemic, during which stringent lockdowns and quarantine measures were implemented across various regions. From 2016 to 2024, the HPV infection rate in the Zigong region exhibited a marked increase, rising from 14.86% to 26.18%. This trend could be influenced by several factors, including economic development, shifts in sexual behaviors, the implementation of "Two Cancer" screenings (a free government-sponsored program in China targeting cervical and breast cancer),

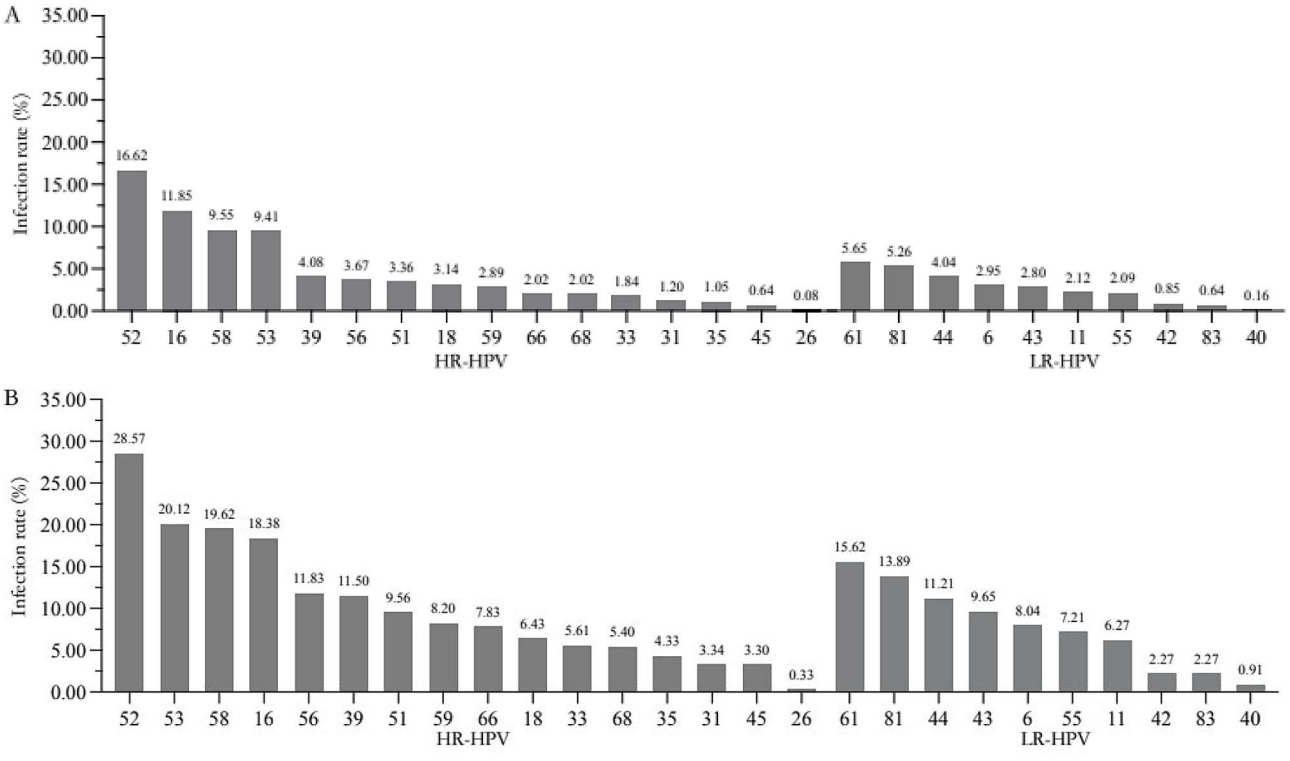

Fig. 1 Distribution of HPV genotypes in single infections (**A**) and multiple infections (**B**)

and advancements in the sensitivity and performance of diagnostic reagents. Most HPV genotypes showed a consistent upward trend with the overall infection rate. However, the infection rates of the most concerning HPV types 6, 11, 16, and 18 showed a slight downward trend, possibly due to the promotion and vaccination of HPV vaccines. Since 2017, bivalent, quadrivalent, and nine-valent vaccines have been licensed in mainland China, and domestic vaccines against HPV16 and 18 were also approved in 2019 [24]. A 10-year multicenter prospective study showed that women vaccinated with the quadrivalent HPV vaccine had significantly lower infection rates of HPV types 6, 11, 16, and 18 compared to unvaccinated groups, with similar protective efficacy for single, double, and triple doses [25]. Additionally, according to the Chinese Centre for Disease Control and Prevention (CDC), the incidence of genital warts in China slightly decreased from 7.26/100,000 to 7.19/100,000 from 2018 to 2023 [26], consistent with the slight downward trend in HPV 6 and 11 infection rates.

Differences in epidemiological changes between different types may be due to differences in infectivity. HPV prevention and control policies should change with epidemiological changes, such as increasing attention to types with a significant upward trend in infection rates and higher carcinogenicity (e.g., HR-HPV types 52 and 58) in cervical cancer diagnosis and treatment. A global systematic study showed that the carcinogenic potential of HPV types 53 and 66 is extremely low, with cervical cancer caused by them not exceeding 0.2% [5]. The infection rates of HPV types 53 and 66 in Zigong are high (collective prevalence of over 3%) and show a clear upward trend. Therefore, using them for cervical cancer screening may lead to patient anxiety, over-management (follow-up, biopsy, and treatment), and waste of medical resources [5]. Nevertheless, HPV types 53 and 66 can cause low-grade squamous intraepithelial lesions (LSIL) [27], with high infection rates in LSIL, at 7.8% and 6.6% respectively [16]. However, these LSIL patients rarely progress to high-grade squamous intraepithelial lesions (HSIL) and cervical cancer [28, 29]. Thus, excluding HPV types 53 and 66 from HR-HPV in future test kits warrants consideration. Additionally, a study by Li N et al. [30] showed that the HPV infection rate in Chengdu decreased from 22.71% to 21.72% from 2014 to 2021. Therefore, significant differences in HPV infection rates and trends may also exist in different regions within the same province.

Most HPV genotypes, HR-HPV-only, LR-HPV-only, mixed LR and HR-HPV, single infections, multiple infections, and total infection rates in Zigong show a bimodal distribution pattern, with the highest infection rates at ≤20 years or 20–30 years, gradually decreasing with age, and then rising again until reaching a second peak at 51–60 years or ≥61 years. Multiple studies have also confirmed that HPV infection rates show a bimodal distribution pattern, with young and elderly women being the two stages with the highest infection rates [9, 17–23, 30–32]. High infection rates in young women are due to active sexual behavior, multiple sexual partners, and insufficient awareness of safe sexual practices, followed by natural clearance of HPV in most women, leading to a gradual decrease in infection rates. HPV infections are usually naturally cleared by the immune system within a year, with less than 10% of the population progressing from new infection to persistent infection to precancerous lesions to cancer [33]. Subsequently, with age, declining immune function and changes in living conditions can lead to a rise in HPV infection rates in middle age. The average age of cervical cancer diagnosis in China is 48 years, cases are rare before the age of 20, with the incidence peaking between 40 and 60 years, and showing a declining trend after 60 years of age [34]. Therefore, the peak age of cervical cancer incidence does not coincide with the peak age of HPV infection, possibly due to the strong virus clearance ability in young women and the 10–20 years natural evolution period from persistent HPV infection to cervical cancer. Therefore, sex education should be strengthened, protective measures should be encouraged during sexual behavior, and HPV screening should be recommended for sexually active women of all ages.

The distribution of HPV genotypes has strong regional characteristics. HPV types 16, 18, 31, and 45 are the most common genotypes among cervical cancer patients in the United States, while HPV types 16, 18, 33, and 31 are the most common in Europe [16]. Investigating the distribution of HPV genotypes in different regions is important for selecting HPV screening kits and developing HPV vaccines [35]. This study shows that HR-HPV in Zigong is mainly infected with HPV types 52 (3.94%), 16 (2.71%), 53 (2.43%), and 58 (2.42%), similar to the HR-HPV genotype distribution in multiple regions in China, with the common feature of having HPV types 52, 16, and 58 among the top four infections [9, 17–23, 30–32]. LR-HPV is mainly infected with HPV types 61 (1.64%), 81 (1.49%), and 44 (1.17%), while the common feature of LR-HPV infections in multiple regions in China is having HPV types 6 and 11 among the top three [9, 17–23, 30, 31]. Notably, the infection rates of HPV types 6 and 11 are only about half that of HPV type 61, but their infection rates are very high in women ≤20 years, being three times and two times that of HPV type 61, respectively. In fact, genital warts are indeed more prevalent among younger populations with more active sexual behavior [36]. The bivalent HPV vaccine covers HPV types 16 and 18, the quadrivalent covers HPV types 6, 11, 16, and 18, and the nine-valent covers HPV types 6, 11, 16, 18, 52,

58, 31, 33, and 45. Therefore, women should be encouraged to choose the nine-valent HPV vaccine whenever possible to effectively prevent HPV types 52 and 58. For genotypes with lower infection rates, such as HPV types 40 and 26, we can consider further follow-up studies to determine whether HPV screening in Zigong can exclude these two genotypes from the test kits, reducing the economic burden on patients and saving medical resources.

HPV multiple infections can increase the risk of persistent HPV infection, cervical disease progression, and cervical cancer occurrence [37–40], so investigating the proportion of HPV multiple infections is of great significance. The proportion of multiple HPV infections in Zigong is 24.88% of the total infections, similar to Zhejiang (22.48%) [31] and Shenzhen (25.72%) [21], higher than Hengyang (18.06%) [22] and Wenzhou (16.65%) [41], and lower than Shandong Province (37.2%) [20] and Shanghai (28.79%) [19]. As the number of infections increases, the composition ratio of LR-HPV-only or HR-HPV-only infections gradually decreases, while the composition ratio of mixed LR and HR-HPV infections gradually increases. Therefore, there may be a potential synergistic effect between high-risk and low-risk types to promote multiple infections, but this needs further research to confirm. HPV types 52, 53, 58, and 16 are the most common genotypes in multiple infections. Double infections are the most common multiple infections, accounting for 70.81%. The most common double infection combinations are HPV 52 + HPV 53, HPV 52 + HPV 58, HPV 52 + HPV 16, and HPV 52 + HPV 81, all of which are HR + HR-HPV infection patterns.

This study provides a comprehensive analysis of HPV infection rates and genotype distributions in Zigong, Sichuan Province, over an extended period and with a large sample size. However, certain limitations remain. First, most patients undergoing HPV screening only had HPV testing alone, with few undergoing combined cytology testing. Therefore, this study did not link HPV infection with cervical cytology results. Second, the study subjects lacked more detailed background information such as culture, economy, and number of sexual partners. Therefore, it is not possible to determine the impact of different backgrounds on the prevalence of HPV infection. Third, there is a lack of data on HPV vaccination among the study subjects, making it impossible to link changes in infection rates of HPV types 6, 11, 16, and 18 with vaccination.

Conclusion

Based on the analysis of HPV infections in cervical exfoliated cells from 48,474 gynecology patients in Zigong Fourth People's Hospital, we conclude the following:

①From 2016 to 2024, the overall HPV infection rate showed an increasing trend, while infection rates for HPV types 6, 11, 16, and 18 exhibited a slight decline;

②Most HPV genotypes, HR-HPV-only, LR-HPV-only, mixed LR and HR-HPV, single infections, multiple infections, and the total infection rate exhibited a bimodal distribution, with peaks in young and elderly women;

③Among HPV-positive patients, HR-HPV-only infections were predominant, followed by LR-HPV-only and mixed LR and HR-HPV infections.

④Single infections remain the predominant form of HPV infection, with HPV types 52, 16, 53, and 58 being the most common genotypes.

Abbreviations

HPV	Human Papillomavirus
LR-HPV	Low-risk Human Papillomavirus
HR-HPV	High-Risk Human Papillomavirus
CDC	Centre for Disease Control and Prevention
LSIL	Low-Grade Squamous Intraepithelial Lesion
HSIL	High-Grade Squamous Intraepithelial Lesion

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Author contributions

X.M: Methodology, Data cleaning and analysis, Investigation and Writing original draft. C.W, T.W: Investigation, Date collection, Validation and Formal analysis. X.Y, L.S: Supervision, Review and Editing.

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Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The study process was approved by the Medical Research Ethics Committee in Zigong Fourth People's Hospital. All patients are completely anonymous and free from any personal privacy.

Consent for publication

The authors agree to publication.

Competing interests

The authors declare no competing interests.

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