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Original Article

Human papillomavirus (HPV) genotype distribution in women with cervical lesions in Southern Sichuan of China

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Abstract: This study investigated the prevalence and genotype distribution of human papillomavirus (HPV) in women with cervical pathological changes, including benign lesions, cervical intraepithelial neoplasia 1-3 (CIN 1-3), and invasive cervical cancer (ICC), in Southern Sichuan of China. The HPV subtypes were determined in cervical smear and paraffin-embedded tissues by using polymerase chain reaction (PCR) and tube array hybridization. The overall HPV infection rate was 53.66% (630/1174), of which the infection rate with the high risk HPV (HR-HPV) type (91.11%; 574/630) was significantly higher than that of low risk HPV (LR-HPV) type (34.13%; 215/630) ($p < 0.001$). The peak ages of patients with the HPV infection were at 20-29 and 50-70 years in age, and the infection rate during these two age-periods was significantly higher than that in the other age groups ($p < 0.01$, respectively). In addition, a significantly higher rate of LR-HPV infection or single-type infection was observed in people with age of 20-29, compared to the other age groups. The most frequently observed HR-HPV genotypes in all samples examined were HPV16, 58, 52, 33, 81, 39 and 18 in order, and the most frequent LR-HPV genotypes were HPV11, 6, 43, 54 and 40 in order. Moreover, the HPV infection rate in patients with cervical benign specimens, CIN 1, CIN 2, CIN 3, and invasive cervical carcinomas was 15.9%, 54.55%, 71.31%, 88.03%, and 91.30%, respectively. The ratio of single-type infection, two-type infection, three-type infection, and four or more-type infection was 87.43%, 6.83%, 2.19%, and 3.55%, respectively. The overall HPV infection rate in women who lived in the Southern Sichuan of China was generally high, while people with different ages showed different vulnerabilities to different HPV subtypes. HPV infection is positively associated with the cervical lesion severity, and therefore, this study has provided useful information for developing a local area-based strategy for the prevention, early-diagnosis, choice of vaccine, and treatment of this major sexually transmitted disease.

Keywords: Human papillomavirus, prevalence, genotype, cervical lesions, Southern Sichuan of China

Introduction

Cervical cancer is the second most common cancer in women worldwide [1], Several studies have indicated that persistent infection of certain types of HPV is a causal factor for the development of cervical cancer [1]. Based on their association with cervical cancer, HPVs have been classified in two groups, e.g. "high-risk types" such as HPV16 and 18 with a higher carcinogenic potential, and "low-risk types" such as HPV6 and 11 with a lower carcinogenic potential [2, 3]. To develop a prophylactic vaccine against these infections promises an effective strategy to prevent the onset of the related cervical lesions. There are two licensed HPV vaccines so far, Gardasil (HPV6/11/16/18) and Cervarix (HPV16/18), which were devel-

Table 1. HPV infection in all age groups

Age	N	HPV positive n# (%)	HR-HPV$^{\theta}$ n※ (%)	LR-HPV n※ (%)	Single-type HPV n# (%)
20-29	211	141 (66.82*)	110 (78.01)	76 (53.90)$	125 (59.24)β
30-39	387	182 (47.03)	173 (95.05)	49 (26.92)	145 (37.47)
40-49	439	222 (50.57)	218 (98.20)	62 (27.93)	188 (42.82)
50-70	137	85 (62.04&)	73 (85.88)	28 (32.94)	64 (46.72)
Total	1174	630 (53.66)	574 (91.11)	215 (34.13)	522 (44.46)

Some samples can be counted more than once because of multiple infections. n#: Total number of women who was infected with HPV. n※: Total number of times which each genotype was detected. *: Compared with 30-39 and 40-49 age groups X^2 = 21.544, p = 0.000; X^2 = 15.271, p = 0.000. &: Compared with 30-39 and 40-49 age groups X^2 = 9.128, p = 0.003; X^2 = 5.523, p = 0.019. θ: The linear trend test value was 13.098, p = 0.000. $: X^2 = 24.374, p = 0.000; X^2 = 24.686, p = 0.000; X^2 = 9.378, p = 0.002. β: X^2 = 26.142, p = 0.000; X^2 = 15.384, p = 0.000; X^2 = 5.253, p = 0.022.

oped to mainly use for two cancer-causing types, HPV16 and HPV18. Those vaccines also shows some beneficial effect on HPV-caused benign lesions, such as verruca acuminata (HPV6/11) [4]. Some studies have suggested that those vaccines may be used for some HPV 16-related types (31, 33, 35 and 52) and HPV 18-related types (39, 45, 59, 68 and 85). However, vaccination against HPV16/18 did not show an effect on HPV 58 [5, 6], while prophylactic HPV vaccination is estimated to reach an approximately 69.7% protective effect against ICC [7, 8]. On the other hand, the infection of different HPV genotypes differs significantly by age, racial, geography, economic status, and sexual behavior [1]. It is well known that HPV16 and HPV18 are the most predominant high-risk types found in cervical cancer tissue in countries around the world, while the prevalence of the other high-risk HPV types varies from one region to another [9]. The attribution of HPV types in China is different from most countries in the world, because HPV58 and 52 are the most commonly observed HPV types in China [7]. Furthermore, previous studies indicated that the prevalence and HPV-type distribution in cervical lesions is also different in different regions of China [10-12]. Therefore, it is important to establish the distribution patterns of HPV infection in terms of different genotypes in a given area, in order to develop a better strategy to prevent this devastating disease for women.

In the present study, we analyzed HPV genotypes from 1174 Chinese women with cervical lesions who lived in the Southern Sichuan of China, in order to map the distribution of HPV infection among women across an abroad age range. Our data may help to further evaluate the potential impact and cost effectiveness of HPV screening and vaccination in Southern Sichuan of China.

Material and methods

Tissues collection

In this study, 612 scraping samples and 562 paraffin-embedded tissue samples were collected and used to determine the infection rate of the HPV, and 562 paraffin-embedded samples were collected and used to determine the relationship between the HPV genotypes and the severity of cervical lesions. Patients were recruited for tissue collections between May 2011 and December 2013 from six local hospitals (the Affiliated Hospital of Luzhou Medical College, the People's Hospital of Luhua, the First People's Hospital of Neijiang City, the Fourth People's Hospital of Zigong City, the Luxian County People's Hospital and the Fushun County People's Hospital). The standard histological diagnosis was used to define the status of cervical pathological changes. Cell samples were collected by scraping the uterine endocervix with a cytobrush, and were stored in 1.5 ml sterile phosphate-buffer saline and kept at 4°C until DNA extraction (within 3 days). By using the GPMT W2600 genotyping test system, 24 HPV subtypes including 16 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 81) and 8 LR-HPV types (HPV6, 11, 40, 42, 43, 44, 45, 70), were identified. The instruments and reagent were provided by YaNeng Biotechnology Company. This study was approved by the Medical Ethics Committee of Luzhou Medical College (No.20130051).

Experimental procedures for HPV genotyping

DNA extraction in cervical smear: Transferred 1 ml cell suspension into a 1.5 ml EP tube and centrifuged for 5 min at 12,000 rpm, and then the supernatant was discarded. The pellet was washed with 1 ml cell washing solution by turning up-down for a couple of times, briefly spine down, and the washing solution was carefully

discarded. After air-dry for a couple of minutes, 200 μl fast extract solution was added into each tube, and the tubes were incubated in water at 95°C for 15 min. After centrifuge for 3 min at 12,000 rpm, the supernatant was used for PCR.

DNA extraction in paraffin-embedded tissue: The paraffin-embedded tissues were processed as our established protocols. Briefly, 1 or 2 tissue blocks (6 × 6 mm square) were made, and then were put into a 1.5 ml tube containing 200 μl split solution. After heated at 95°C for 10 min in water, centrifuged for 2 min at 12,000 rpm, and incubated at 4°C for 5 min, tissues were treat with 10 μl of proteinase K (200 μg/ml) for 3 hrs at 56°C. The proteinase K was inactivated by heating at 95°C for 10 min, and then the solution was centrifuge for 2 min at 12,000 rpm. The supernatants were used for PCR.

PCR reaction: A total of 90 μl of amplified mixture and 10 μl of DNA solution from each sample were used for PCR amplification and the cycling program consisted of pre-denature at 50°C for 2 min, denature at 94°C for 5 min, and then with 28-cycles of denature at 94°C for 30 seconds, annealing at 48°C for 45 seconds, and extension at 72°C for 45 seconds. Thereafter, 25 more cycles were repeated with conditions of denature at 94°C for 30 seconds, annealing at 65°C for 45 seconds at 65°C, and extension at 72°C for 20 seconds. Each experiment was conducted with separate positive and negative controls, which were HPV DNA and DNA from cells without HPV, respectively. In addition, beta-globin was used as an internal control. The amplified products were then denatured and hybridized with the HPV-specific probes blotted as lines on the genotyping strips.

Statistical analysis

Statistical analysis was performed using SPSS Version 13.0 for Windows. Comparison significance difference between groups using chi-square test :The chi-square test and the linear trend test were used to determine the significance difference between infection rate of age groups, and the difference between the infection rate of various HPV genotypes and HPV co-infection was detect by chi-square test. A *P* value less than 0.05 were considered significant. Relative frequencies of HPV genotypes were estimated as percentages and their 95% confidence intervals were estimated by binomial distribution analysis.

Results

Relationship between HPV infection and age

As shown in **Table 1**, the total infection rate of HPV was 53.66% (630/1174). Of the infected samples, the HR-HPV rate was 91.11% (574/630), and LR-HPV rate was 34.13% (215/630). Therefore, there is a big portion of patients with infection with multiple HPV types. Interestingly, there was no significant difference in total infection rate between 20-29 and 50-70 age groups, while both of these age groups had a significantly higher infection rate than 30-39 and 40-49 age groups (X^2 = 21.544, $p = 0.000$; X^2 = 15.271, $p = 0.000$ and X^2 = 9.128, $p = 0.003$; X^2 = 5.523, $p = 0.019$, respectively). HR-HPV positive rate was high in every age group, the linear trend test value was 13.098 (p = 0.000, p < 0.01). Both LR-HPV positive rate and single-type HPV infection in the group of 20-29 were significantly higher than other age groups (X^2 = 24.374, $p = 0.000$; X^2 = 24.686, $p = 0.000$; X^2 = 9.378, $p = 0.002$ and X^2 = 26.142, $p = 0.000$; X^2 = 15.384, $p = 0.000$; X^2 = 5.253, $p = 0.022$, respectively).

Distribution of viral genotypes

As shown in **Tables 2** and **3**, the percentage of HPV positivity in the total of specimens studied was 65.12% (366/562).

In subtype distribution, HPV16 was the most common HR-HPV type (30.02%) followed, in order of decreasing frequency, by HPV58 (11.7%), HPV52 (8.83%), HPV33 (4.42%), HPV81 (4.19%), HPV39 (3.31%) and HPV18 (3.09%). HPV11 was the most common LR-HPV type (8.61%) followed, in order of decreasing frequency, by HPV6 (3.97%), HPV43 (2.87%), HPV54 (1.10%) and HPV40 (1.10%).

HPV16 was present in 37.16% of total lesions, HPV58 in 14.48%, HPV52 in 10.93%, HPV11 in 10.66%, HPV6 in 4.92% and HPV43 in 3.55% of them.

In all the samples from patients with cervical lesions included in 562 paraffin-embedded tis-

Table 2. Distribution of HPV genotypes found in the study according to the pathological diagnosis

Genotype found	Total lesions					Benign lesions					CIN 1				
	N	%*	95% CI	%**	95% CI	N	%*	95% CI	%**	95% CI	N	%*	95% CI	%**	95% CI
HR-HPVs															
16	136	37.16	32.21-42.11	30.02	25.80-34.24	1	7.14	0.18-33.84	5.26	0.13-25.92	16	20.51	11.55-29.47	15.534	8.54-22.53
58	53	14.48	10.88-18.09	11.70	8.74-14.66	0	0.00	0.00-0.00	0.00	0.00-0.00	14	17.95	9.43-26.47	13.592	6.97-20.21
52	40	10.93	7.73-14.13	8.83	6.22-11.44	0	0.00	0.00-0.00	0.00	0.00-0.00	11	14.10	6.38-21.83	10.68	4.71-16.64
33	20	5.46	3.14-7.79	4.42	2.52-6.31	0	0.00	0.00-0.00	0.00	0.00-0.00	5	6.41	2.10-14.37	4.85	1.59-10.95
81	19	5.19	2.92-7.46	4.19	2.35-6.04	1	7.14	0.18-33.84	5.26	0.13-25.92	8	10.26	3.52-16.99	7.77	2.60-12.94
39	15	4.10	2.07-6.13	3.31	1.66-4.96	0	0.00	0.00-0.00	0.00	0.00-0.00	3	3.85	0.81-10.86	2.91	1.68-8.29
18	14	3.83	1.86-5.79	3.09	1.50-4.68	0	0.00	0.00-0.00	0.00	0.00-0.00	3	3.85	0.81-10.86	2.91	1.68-8.29
51	13	3.55	1.66-5.45	2.87	1.33-4.41	0	0.00	0.00-0.00	0.00	0.00-0.00	5	6.41	2.10-14.37	4.85	1.59-10.95
66	12	3.28	1.45-5.10	2.65	1.17-4.13	0	0.00	0.00-0.00	0.00	0.00-0.00	5	6.41	2.10-14.37	4.85	1.59-10.95
35	11	3.01	1.26-4.75	2.43	1.01-3.85	0	0.00	0.00-0.00	0.00	0.00-0.00	4	5.13	1.42-12.62	3.88	1.07-9.60
45	8	2.19	0.69-3.68	1.77	0.55-2.98	0	0.00	0.00-0.00	0.00	0.00-0.00	1	1.28	0.03-6.93	0.97	0.02-5.29
53	8	2.19	0.69-3.68	1.77	0.55-2.98	0	0.00	0.00-0.00	0.00	0.00-0.00	1	1.28	0.03-6.93	0.97	0.02-5.29
56	7	1.91	0.51-3.32	1.55	0.41-2.68	0	0.00	0.00-0.00	0.00	0.00-0.00	1	1.28	0.03-6.93	0.97	0.02-5.29
31	6	1.64	0.34-2.94	1.32	0.27-2.38	0	0.00	0.00-0.00	0.00	0.00-0.00	2	2.56	0.31-8.97	1.94	0.24-6.85
59	4	1.09	0.02-2.16	0.88	0.01-1.10	0	0.00	0.00-0.00	0.00	0.00-0.00	3	3.85	0.81-10.86	2.91	1.68-8.29
68	4	1.09	0.02-2.16	0.88	0.01-1.10	0	0.00	0.00-0.00	0.00	0.00-0.00	3	3.85	0.81-10.86	2.91	1.68-8.29
LR-HPVs															
11	39	10.66	7.49-13.82	8.61	6.03-11.19	9	64.29	39.19-89.39	47.37	24.92-69.82	8	10.26	3.52-16.99	7.77	2.60-12.94
6	18	4.92	2.70-7.13	3.97	2.17-5.77	5	35.71	12.81-64.79	26.32	9.16-51.26	5	6.41	2.10-14.37	4.85	1.59-10.95
43	13	3.55	1.66-5.45	2.87	1.33-4.41	1	7.14	0.18-33.84	5.26	0.13-25.92	3	3.85	0.81-10.86	2.91	1.68-8.29
54	5	1.37	0.17-2.56	1.10	0.09-1.30	0	0.00	0.00-0.00	0.00	0.00-0.00	1	1.28	0.03-6.93	0.97	0.02-5.29
40	5	1.37	0.17-2.56	1.10	0.09-1.30	1	7.14	0.18-33.84	5.26	0.13-25.92	1	1.28	0.03-6.93	0.97	0.02-5.29
42	1	0.27	0.00-1.51	0.22	0.00-1.23	0	0.00	0.00-0.00	0.00	0.00-0.00	0	0.00	0.00-0.00	0.00	0.00-0.00
44	1	0.27	0.00-1.51	0.22	0.00-1.23	0	0.00	0.00-0.00	0.00	0.00-0.00	0	0.00	0.00-0.00	0.00	0.00-0.00
70	1	0.27	0.00-1.51	0.22	0.00-1.23	1	7.14	0.18-33.84	5.26	0.13-25.92	0	0.00	0.00-0.00	0.00	0.00-0.00

Some samples can be counted more than once because of multiple infections. N: Total number of times which each genotype was detected. %*: Percentages referred to the number of lesions infected by one or several genotypes (366 total lesions, 14 benign and 78 CIN 1). %**: Percentages referred to the total number of virus detected (453 viruses in the total of lesions, 19 in the benign lesions and 103 in CIN 1). 95% CI: 95% confidence intervals used for estimate percentages.

Table 3. Distribution of HPV genotypes found in the study according to the pathological diagnosis

Genotype found	CIN 2					CIN 3					ICC				
	N	%*	95% CI	%**	95% CI	N	%*	95% CI	%**	95% CI	N	%*	95% CI	%**	95% CI
HR-HPVs															
16	23	26.44	17.17-35.70	21.70	13.85-29.55	47	45.63	36.01-55.25	36.72	28.37-45.07	49	58.33	47.79-68.88	50.52	40.57-60.47
58	8	9.20	3.12-15.27	7.55	2.52-12.58	14	13.59	6.97-20.21	10.94	5.53-16.34	17	20.24	11.65-28.83	17.53	9.96-25.09
52	11	12.64	5.66-19.63	10.38	4.57-16.18	12	11.65	5.45-17.85	9.38	4.33-14.42	6	7.14	1.64-12.65	6.19	1.39-10.98
33	7	8.05	2.33-13.76	6.60	1.88-11.33	6	5.83	1.30-10.35	4.69	1.03-8.35	2	2.38	0.31-8.94	2.06	0.25-7.26
81	3	3.45	0.72-9.80	2.83	0.59-8.04	5	4.85	1.59-10.95	3.91	1.27-8.87	2	2.38	0.31-8.94	2.06	0.25-7.26
39	7	8.05	2.33-13.76	6.60	1.88-11.33	4	3.88	1.07-9.60	3.13	0.87-7.79	1	1.19	0.03-6.95	1.03	0.03-7.26
18	6	6.90	1.57-12.22	5.66	1.26-10.06	2	1.94	0.24-6.85	1.56	0.19-5.52	3	3.57	0.81-10.82	3.09	0.64-8.77
51	3	3.45	0.72-9.80	2.83	0.59-8.04	4	3.88	1.07-9.60	3.13	0.87-7.79	1	1.19	0.03-6.95	1.03	0.03-7.26
66	4	4.60	1.27-11.30	3.77	1.04-9.36	1	0.97	0.02-5.29	0.78	0.02-4.29	2	2.38	0.31-8.94	2.06	0.25-7.26
35	4	4.60	1.27-11.30	3.77	1.04-9.36	2	1.94	0.24-6.85	1.56	0.19-5.52	1	1.19	0.03-6.95	1.03	0.03-7.26
45	3	3.45	0.72-9.80	2.83	0.59-8.04	2	1.94	0.24-6.85	1.56	0.19-5.52	2	2.38	0.31-8.94	2.06	0.25-7.26
53	4	4.60	1.27-11.30	3.77	1.04-9.36	2	1.94	0.24-6.85	1.56	0.19-5.52	1	1.19	0.03-6.95	1.03	0.03-7.26
56	4	4.60	1.27-11.30	3.77	1.04-9.36	1	0.97	0.02-5.29	0.78	0.02-4.29	1	1.19	0.03-6.95	1.03	0.03-7.26
31	3	3.45	0.72-9.80	2.83	0.59-8.04	0	0.00	0.00-0.00	0.00	0.00-0.00	1	1.19	0.03-6.95	1.03	0.03-7.26
59	1	1.15	0.03-6.23	0.94	0.02-5.15	0	0.00	0.00-0.00	0.00	0.00-0.00	0	0.00	0.00-0.00	0.00	0.00-0.00
68	0	0.00	0.00-0.00	0.00	0.00-0.00	1	0.97	0.02-5.29	0.78	0.02-4.29	0	0.00	0.00-0.00	0.00	0.00-0.00
LR-HPVs															
11	7	8.05	2.33-13.76	6.60	1.88-11.33	12	11.65	5.45-17.85	9.38	4.33-14.42	3	3.57	0.81-10.82	3.09	0.64-8.77
6	5	5.75	1.91-12.90	4.72	1.54-10.65	2	1.94	0.24-6.85	1.56	0.19-5.52	1	1.19	0.03-6.95	1.03	0.03-7.26
43	2	2.30	0.28-8.07	1.89	0.23-6.63	5	4.85	1.59-10.95	3.91	1.27-8.87	2	2.38	0.31-8.94	2.06	0.25-7.26
54	0	0.00	0.00-0.00	0.00	0.00-0.00	3	2.91	1.68-8.29	2.34	0.49-6.70	1	1.19	0.03-6.95	1.03	0.03-7.26
40	1	1.15	0.03-6.23	0.94	0.02-5.15	2	1.94	0.24-6.85	1.56	0.19-5.52	0	0.00	0.00-0.00	0.00	0.00-0.00
42	0	0.00	0.00-0.00	0.00	0.00-0.00	1	0.97	0.02-5.29	0.78	0.02-4.29	0	0.00	0.00-0.00	0.00	0.00-0.00
44	0	0.00	0.00-0.00	0.00	0.00-0.00	0	0.00	0.00-0.00	0.00	0.00-0.00	1	1.19	0.03-6.95	1.03	0.03-7.26
70	0	0.00	0.00-0.00	0.00	0.00-0.00	0	0.00	0.00-0.00	0.00	0.00-0.00	0	0.00	0.00-0.00	0.00	0.00-0.00

Some samples can be counted more than once because of multiple infections. N: Total number of times which each genotype was detected. %*: Percentages referred to the number of lesions infected by one or several genotypes (87 CIN 2, 103 CIN 2 and 84 ICC). %**: Percentages referred to the total number of virus detected (106 viruses in CIN 2, 128 CIN 3 and 97 ICC). 95% CI: 95% confidence intervals used for estimate percentages.

sue samples, 81.68% of HPVs detected were HR-HPVs, and 18.32% were LR-HPVs. HPV16 and/or 18 were founded in 33.11% of total HPV infections detected and HPV11 and 6 in 12.58% of them, which indicated that the HR-HPV types without vaccines were more frequently observed than carcinogenic viruses (HPV16 and/or HPV18), which have been developed vaccines; the LR-HPV types (HPV11 and/or HPV6) included in tetravalent vaccine were detected more frequently than other LR-HPV types.

Relationship between diagnoses and HPV genotypes

In benign lesions, HPV11 was the most common type (47.37%), and HPV6 was the second common type (26.32%). HPVs11 and/or 6 comprised 73.68% of total HPV infections detected. Therefore, LR-HPV types included in tetravalent vaccine were detected more frequently than viruses not included in this vaccine.

In CIN 1 cases, HPV16 was the most common type (15.53%) followed, in order of decreasing frequency, by HPV58 (13.59%), HPV52 (10.68%), HPV81 (7.77%), HPV11 (7.77%), HPV 33 (4.85%), HPV51 (4.85%), HPV66 (4.85%), HPV6 (4.85%), and HPV35 (3.88%).

HPV16 was present in 20.51% of CIN 1 lesions, HPV58 in 17.95%, HPV52 in 14.10%, HPV81 in 10.26% and HPV11 in 10.26% of them. HR-HPVs were detected in 82.52% of total HPVs in CIN 1 lesions and LR-HPVs in 17.48% of them. HPVs16 and/or 18 were present in 24.36% of lesions and HPVs6 and/or 11 in 16.67% of them.

Therefore, HR-HPV types that were not included in vaccines were detected more frequently (84.61%) than carcinogenic viruses included in vaccines (24.36%), while LR-HPV types included in vaccine were detected more frequently (16.67%) than other LR-HPVs not included in vaccine (6.41%).

In CIN 2 cases, HPV16 was the most common type (21.70%) followed, in order of decreasing frequency, by HPV52 (10.38%), HPV58 (7.55%), HPV33 (6.60%), HPV39 (6.60%), HPV11 (6.60%), HPV18 (5.66%) and HPV6 (4.72%).

HPV16 was present in 26.44% of CIN 2 lesions, HPV52 in 12.64%, HPV58 in 9.20%, HPV33 in 8.05%, HPV39 in 8.05% and HPV11 in 8.05% of them. HR-HPVs were detected in 85.85% of total HPVs in CIN 2 lesions and LR-HPVs in 14.15% of them. The infection rate of HR-HPVs was significant higher than that of LR-HPVs. HPVs16 and/or 18 were present in 33.33% of lesions and HPVs6 and/or 11 in 13.80% of them.

HR-HPV types that were not included in vaccines were detected more frequency (71.26%) than carcinogenic viruses included in vaccines (33.33%). LR-HPV types included in vaccine were detected more frequently (13.80%) than other LR-HPVs not included in vaccine (3.45%).

In CIN 3 cases, HPV16 was the most common type (36.72%) followed, in order of decreasing frequency, by HPV58 (10.94%), HPV52 (9.38%), HPV11 (9.38%) and HPV33 (4.69%).

HPV16 was present in 45.63% of CIN 3 lesions, HPV58 in 13.59%, HPV52 in 11.65%, HPV11 in 11.65% and HPV33 in 5.83% of them. HR-HPVs were detected in 80.47% of total HPVs in CIN 3 lesions and LR-HPVs in 19.53% of them. The infection rate of HR-HPVs was significant higher than that of LR-HPVs. HPVs16 and/or 18 were present in 47.57% of lesions and HPVs6 and/or 11 in 13.59% of them.

There was no significant difference between HR-HPV types not included in vaccines were detected (52.43%) and carcinogenic viruses included in vaccines (47.57%). It was same to LR-HPV types (10.68% vs. 13.59%).

In ICC cases, HPV16 was the most common type (50.52%) followed, in order of decreasing frequency, by HPV58 (17.53%), HPV52 (6.19%), HPV18 (3.09%) and HPV11 (3.09%).

HPV16 was present in 58.33% of ICC lesions, HPV58 in 20.24% and HPV52 in 7.14% of them. HR-HPVs were detected in 91.75% of total HPVs in ICC lesions and LR-HPVs in 8.25% of them. The infection rate of HR-HPVs was significant higher than that of LR-HPVs. HPVs16 and/or 18 were present in 61.90% of lesions and HPVs6 and/or 11 in 4.76% of them.

For the HR-HPV subtypes, a significantly higher infection rate of HPV16 was found in ICC (58.33%) and CIN 3 (45.63%) groups, compared to the groups of CIN 1 and CIN 2 separately ($p < 0.05$, respectively), while a signifi-

Table 4. The proportion of single and co-infection in tissue samples

Pathological diagnosis	N	HPV positive* rate (%)	Single-type infection (%)	2 HPV types infection (%)	3 HPV types infection (%)	≥ 4 HPV types infection (%)
Benign lesions	88	14 (15.91)	11 (78.57)	2 (14.29)	0 (0.00)	1 (7.14)
CIN 1	143	78 (54.55)	65 (83.33)	4 (5.13)	6 (7.69)	3 (3.85)
CIN 2	122	87 (71.31)	77 (88.51)	5 (5.75)	1 (1.15)	4 (4.60)
CIN 3	117	103 (88.03)	88 (85.44)	12 (11.65)	1 (0.97)	2 (1.94)
ICC	92	84 (91.30)	79 (94.05)	2 (2.38)	0 (0.00)	3 (3.57)
Total	562	366 (65.12)	320 (87.43)	25 (6.83#)	8 (2.19)	13 (3.55)

*: The linear trend test value was 13.098, $p = 0.000$. #: The linear trend test value was 4.897, $p = 0.027$.

cant difference in the frequency of HPV58, 52 and 18 was not identified among each cervical lesions group (not included benign lesions). Furthermore, the rates of HPV11 and 6, which included in tetravalent vaccine, were significantly higher in benign lesions (64.29% vs. 35.71%) than other lesions cases.

The distribution of HPV types and the analysis between pathological groups vs. HPV risk types is shown in **Tables 2** and **3**.

Relationship between diagnoses and HPV co-infection

Table 4 shows HPV infection rate and proportion of co-infection according to cervical pathology status. In this study, we detected 562 paraffin-embedded samples, included 320 single-infection cases and 46 co-infection cases. HPV infection rate was found in 84 of 92 ICC cases (91.30%), in 103 of 117 CIN 3 cases (88.03%), in 87 of 122 CIN 2 cases (71.31%), in 78 of 143 CIN 1 cases (54.55%), in 14 of 88 benign lesions (15.9%). The HPV infection rates for CIN 3 and ICC were significantly higher than other lesions groups ($p < 0.001$), the linear trend test value was 140.144 ($p = 0.001$, $p < 0.001$). There was no significant difference of total co-infection or double infection in each group ($p > 0.05$). Co-infection rate decreased with the increase number of HPV types (the linear trend test value was 4.897, $p = 0.027$).

Discussion

China has a large population, vast territory, and a big diversity in nationalities, socio-economic status, and cultural customs. Therefore, HPV infection type and age distribution are obviously different [1, 13-15]. To our best knowledge, no epidemiologic data on HPV genotypes in female population with Cervical Lesions from southern Sichuan Province of China has been reported so far. To characterize of HPV infection in southern Sichuan is therefore particularly important, via which we are able to provide a theoretical basis for the application of vaccines and other protective and therapeutic strategies for this devastating illness in this area.

We found that there are two peak ages of the HPV infection, e.g. from 20 to 29 and from 50 to 70 years old. The infection rate during these ages was significantly higher than that in the other age groups, suggesting a "U"-shaped infection curve. This is different from the results reported from northwestern of China (from 20-29 years) [11], or from Chengdu area in Sichuan of China, where HPV prevalence was lower among patients older than 49 years [16]. However, our results are consistent with other studies carried out in southern China, like Macao [17], Hong Kong [18], Guangdong [19] and Yunnan [20]. Our study suggested that HPV was often acquired soon after sexual initiation, and the high prevalence in 20-29 age-window might be sexual behaviors-related, whereas the high prevalence in 50-70 age-window would be due to an impaired immune response [21].

Of the infected samples, the HR-HPV rate was 91.11%, the LR-HPV rate was 34.13%, there was a great portion of patients with multiple HPV types, but without correlation between co-infection and age. This is not consistent with the reports from Henan province of China [22] and Chaozhou of Guangdong province [23], which showed age-specific prevalence of multiple HPV. These results showed differences in the distribution of HPV infection rates and patterns among regions. On the other hand, in Southern Sichuan, HR-HPV positive rate was high in every age group, while both LR-HPV pos-

itive rate and single-type HPV infection in the group of 20-29 were significantly higher than other age groups, indicating the LR-HPV infection is more occurred among young women.

In the current study, HPV11 and 6 were the most and second common type (47.37% vs. 26.32%) and comprised 73.68% of total HPV infection detected. These two genotypes were significant more common than in CIN 1-3 and ICC lesions. These results indicated that LR-HPVs were likely the most important risk factor for benign lesions, which is consistent with their low carcinogenicity in previous studies [2, 3, 19].

In HR-HPV infection, HPV16 was the most frequent genotype in CIN 1-3 and ICC, similar to many other studies carried out in China [7, 16, 24], but not consistent with some reports that HPV16 was not the most common HPV type found in CIN 1 [12, 25]. As expected, HPV16 was significantly more frequent in group of ICC (58.33%) and CIN 3 (45.63%), increasing with the aggravation of cervical lesion severity (7.14% in benign lesions, 20.51% in CIN 1, 26.44% in CIN 2), indicated that HPV16 was the most risk factor for the occurring and progress of ICC. Our results were consistent with those reports from southern china [12], Sichuan province [24] and Chengdu [16].

HPV58 and 52 were the second and third most frequent genotype in CIN 1-3, and ICC lesions, in southern Sichuan, respectively. These results were similar to those in southern China, where HPV16, 52 and 58 accounted for the majority of high-grade lesions (CIN 2/3) [12], but were different from Chaozhou of China, where HPV 52 was the most common HR-HPV type, the HPV58 was the third most frequent genotype [19], and also Chengdu (HPV16, 58 and 18, in order) [16] and Fujian (HPV16, 18 and 31, in order) [25] of China. However, for a further analysis, we did not observe any significant difference in HPV58 and 52 among cervical lesions, excluding benign lesions. So we still cannot make a clear conclusion regarding the effect of them on cervical lesions.

The infection rate of HR-HPVs was significant higher than that of LR-HPVs in CIN 1-3 and ICC lesions, indicating that HR-HPVs is a risk factor for severe lesions. HPVs16/18 included in current available vaccines did not show a more frequent rate than HR-HPV types that are without vaccines available, while HPVs11/6 was detected more frequently than other low-types that are without tetravalent vaccine, indicating the current vaccines could not prevent the HR-HPVs infection effectively in this area (excepted HPV16). From above analysis, HPV16 is likely the most important risk factor for serious cervical lesions, while HPVs11 and 6 are for the benign lesions, indicating that the vaccine should include HPVs16, 11 and 6, at least in Sichuan. Our findings showed a relatively high prevalence of HPV58 and 52, supporting the hypothesis that the second-generation HPV vaccines, including HPV58 and 52, may offer higher protection for women in southern Sichuan of China, as Zhang L reported that in Wufeng County of China [14].

On the other hand, the HPV infection rate found in ICC and CIN 3 was significantly higher than that of benign lesions, CIN 1 and CIN 2, and the linear trend test value was 157.749 ($p < 0.001$), indicating that HPV infection rate was positively associated with levels of cervical pathological changes in Southern Sichuan. These results were in accordance with Zhejiang and Xinjiang province of China [26, 27]. The multiple infections had no clear effect on the development of cervical cancer, because there was no significant difference of total co-infection or double infection in each group ($p > 0.05$). This is different from some other area in China, where multiple-type infection was positively [28] or inversely [12] correlated with lesion severity. It was likely due to the number of co-infection in our area was not enough to exhibit the relationship between co-infection and lesion severity, a large number of case study should be continue conducted in the further.

In summary, the infection rate of HPV is significantly higher in women of age 20-29 and 50-70 with cervical lesions than other age groups in southern Sichuan of China. The most important high-risk genotypes are HPV16, 58 and 52, the low-risk genotypes are HPV11 and 6. These findings provide useful information for the development a better strategy to facilitate the cervical cancer prevention in this area, specifically those designed to use and increase HPV vaccine.

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Disclosure of conflict of interest

None.

Abbreviations

HPV, Human papillomavirus; HR-HPV, High risk of carcinogenesis HPV type; LR-HPV, Low risk of carcinogenesis HPV type; ICC, Invasive cervical carcinoma; CIN, Cervical intraepithelial neoplasia; PCR, Polymerase chain reaction.

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