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Xiangyu Deng
Affiliated Hospital of Southwest Medical University
zhangang Xiao
Southwest Medical University
Mingxing Li
Southwest Medical University
Rong Huang
Affiliated Hospital of Southwest Medical University
Fukuan Du
Southwest Medical University
Xu Wu
Southwest Medical University
Yueshui Zhao
Southwest Medical University
Jing Shen
Southwest Medical University
Yu Chen
Southwest Medical University
Pingxiu Zhang
Congcui Hu
Qinglian wen
West China Hospital of Sichuan University
dan Li
lidan0918@163.com

Affiliated Hospital of Southwest Medical University

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A large-scale cross-sectional cervical screening study reveals HPV prevalence in western China

Xiangyu Deng [1,#], Zhangang Xiao [3,4,#], Mingxing Li [3,4], Rong Huang [1], Fukuan Du [3,4], Xu Wu [3,4], Yueshui Zhao [3,4], Jing Shen [3,4], Yu Chen [3,4], Pingxiu Zhang [5], Congcui Hu [6], Qinglian Wen [2,*], Dan Li [1,*]

[1] Department of Oncology, Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China.
[2] Department of Radiation Oncology, Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China.
[3] Cell Therapy & Cell Drugs of Luzhou Key Laboratory, Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou, Sichuan, China.
[4] South Sichuan Institute of Translational Medicine, Luzhou, Sichuan, China.
[5] Yanyuan County Maternal and Child Health and Family Planning Service Center, Xichang, Sichuan, China.
[6] Yanyuan County People's Hospital, Xichang, Sichuan, China.

[#]*These authors contributed to this work equally.*

*Corresponding authors. Qinglian Wen (wql73115@163.com)；Dan Li（lidan0918@163.com）

Abstract

Numerous epidemiological studies have shown that significant regional variations exist in the prevalence of human papillomavirus (HPV) infection and the distribution of HPV subtypes during cervical screening. It is particularly noteworthy that in regions with high-risk factors such as high fertility rates, early pregnancy, and early marriage, women may be at higher risk of HPV infection. Therefore, conducting large-scale studies on cervical screening—especially in these underserved areas—is of great significance. This study conducted a large-scale cross-sectional study on cervical screening in western China (Xichang, Sichuan) using a combination of thin-layer cytology (TCT) and HPV testing. Among the 3,522 women who met the inclusion criteria, 13.77% exhibited cervical or vaginal abnormalities (positive cytology or HPV test results). Colposcopy revealed a total of 58 cases of cervical intraepithelial neoplasia (CIN) and cervical cancer. Among the participants who underwent HPV testing as part of cervical screening, 112 (23.09%) were infected with HPV; of these, 20 (17.86%) were infected with HPV types 16/18, while 82.14% were infected with non-HPV 16/18 types. Contraceptive method, number of pregnancies, and number of deliveries had a significant impact on the risk of HPV infection. HPV infection, particularly infection with high-risk HPV types, was positively correlated with the progression of cervical CIN lesions. The results of this study provide an empirical basis for understanding the epidemiology of HPV in Yanyuan County, Xichang City, China.

Keywords: Human papillomavirus; cervical screening; cervical intraepithelial neoplasia; prevalence.

Abbreviations

ADC: adenocarcinoma; ASC-US, atypical squamous cells of undetermined significance; BV: bacterial vaginosis; CC: cervical cancer; CI: confidence interval; CIN: cervical intraepithelial neoplasia; FV: fungal vaginitis; HPV: human papillomavirus; SCC: squamous cell carcinoma; 16S rRNA: 16S ribosomal ribonucleic acid; TV: trichomonas vaginalis; TBS: The Bethesda System; TCT, Thinprep cytology testing.

Introduction

Cervical cancer (CC) is one of the leading causes of cancer death in women [1], which is one of the most common infection-related neoplasm. Persistent infection with high-risk human papilloma virus (HPV) has been identified as a major risk factor for CC development. A cross-sectional multicenter CC and precancerous HPV genotyping study conducted in 19 hospitals in 7 geographic regions of China found a prevalence of HPV infection in squamous cell carcinoma (SCC) of 97.6%, adenocarcinoma (ADC) of 85.3%, and grade-2/3 cervical intraepithelial neoplasia (CIN 2/3) of 98.9% [2]. Follow-up studies have also confirmed the strong association between HPV infection and cervical precancer and cancer [3,4]. It is recognized that around 99.7% of CC cases were caused by persistent high-risk HPV infection [5]. Given the role of HPV in CC, combined Thinprep cytology testing (TCT) and HPV test for screening precancerous lesions and early CC has thus been suggested to improve the cure rate of CC lesions, and reduce the mortality rate of CC [6,7].

Numerous epidemiological studies on HPV infection conducted across China and in other countries [8-11] showed that large regional differences in the HPV infectious rate and distribution of HPV types have been recognized during CC screening have shown that there are significant regional variations in HPV infection rates and the distribution of HPV subtypes during cervical cancer screening.[12] It is particularly noteworthy that women may face a higher risk of human papillomavirus infection when high-risk factors such as high fertility rates, early pregnancy, and early marriage are present. Therefore, particularly in these regions, incorporating large-scale HPV testing into

cervical cancer screening is of great significance. Xichang City is located in southwestern China and has a population of over 4.8 million. To date, no epidemiological studies on HPV infection have been reported in this region. Consequently, a key objective of this cross-sectional study is to implement cervical cancer screening based on combined TCT/HPV testing among a subset of women in this region.

Methods

Protocol. This was a cross-sectional study from 2019 to 2020, and all participants signed a written informed consent. A total of 3,540 women aged 21-65 years were recruited for cervical screening at Yanyuan County People's Hospital and Yanyuan County Maternal and Child Health and Family Planning Service Center in Xichang City, China from March 2019 to August 2020. Subjects were aged 21-65 years, with a median age of 44 years. All patients were screened according to the 2019 ASCCP management consensus guidelines [13]. The main tests for cervical screening were professional gynecological examination, TCT, HPV test, colposcopy, and biopsy. Those who had menstruation, vaginal medication in the last week, post-total hysterectomy, and pregnancy were excluded for the test. The related information of all subjects (BMI, age, history of miscarriage, history of pregnancy, contraceptive method, history of menopause, etc.) was recorded. Because this study mainly included women subjects who rarely smoke, the smoking status was not considered. Moreover, all subjects did not receive any HPV related vaccines. Histological methods were used to classify patients into different groups. If no histological diagnosis was available (e.g., healthy

controls, low-grade lesions under cytological surveillance), cytological methods were used for classification. Finally, a statistical analysis of the basic profile and screening results of the participants was performed. The flow chart for screening protocol is displayed in Figure 1.

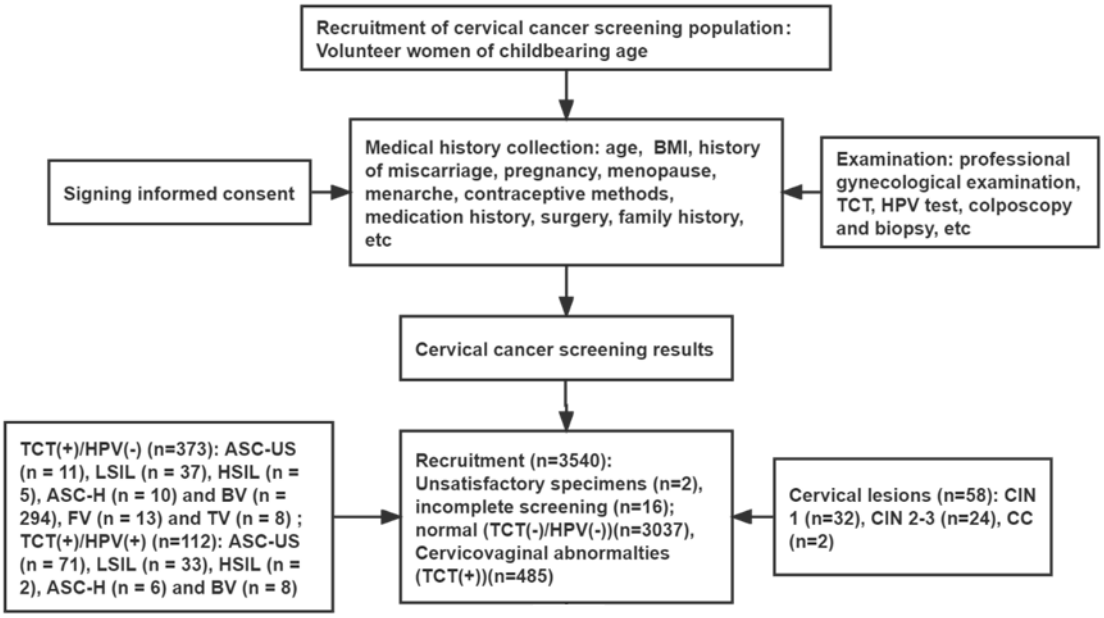


Figure 1. Flowchart of the recruitment and analysis process for cervical screening and follow-up biomarker study.

TCT. TCT method has been widely used to screen cervicovaginal abnormalities in combination with HPV test. In brief, a cervical brush was used to collect exfoliated cells from the junction of cervical squamous and columnar cells by rotating clockwise for 4-5 cycles, and cells were then washed in a vial containing preservative. Uniform thin-layer smears were prepared using a liquid-based cell smear kit (Guangzhou jinqirui Biotechnology Co., Ltd., China), fixed in 95% alcohol and stained. All samples were stained using standard Pap stain, which were then checked by a cytotechnologist and

reported by a cytopathologist based on The Bethesda System (TBS) 2014 criteria [14,15]. Negative results indicate negative for intraepithelial lesion or malignancy (NILM); while positive results include atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesions (LSIL), atypical squamous cells-cannot exclude high grade squamous intraepithelial lesions (ASC-H), atypical glandular cells (AGC), and high grade squamous intraepithelial lesion (HSIL). The definition of TCT negative (TCT(-)) is the normal epithelial cells in a smear, including laminated squamous epithelial cells and columnar epithelial cells, but with negative intraepithelial lesion or malignancy (NILM). Meanwhile, ASC-US and above (≥ASC, including the squamous cell abnormalities of ASC-US, ASC-H, LSIL, HSIL, and AGC are defined as TCT positive (TCT(+)). TCT(+) also reports microbiological findings, including trichomonas, fungi, actinomycetes, herpes simplex virus, and flora changes (suggestive of bacterial vaginosis).

HPV test. Samples were collected using a broom sampler inserted into the cervical canal. HPV DNA was extracted and detected using the HPV Genotyping Kit (Yaneng Biosciences, Shenzhen, China) according to the manufacturer's instruction [16]. The kit involves the use of *in vitro* PCR amplification and DNA reverse dot hybridization techniques, which can detect 23 common clinical HPV genotypes, including 6 low-risk HPV genotypes (6, 11, 42, 43, 81, 83) and 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82).

Colposcopy and biopsy. Patients with TCT=ASC-US/HPV+, or TCT > ASC-US results were subjected for further colposcopy. Cervical biopsy was performed if suspicious

lesions were found colposcopically. The results of the cervical biopsy were graded as normal, chronic cervicitis, CIN1, CIN2, CIN3 and invasive cancer. Those with TCT(-)/HPV(-) results entered the normal group (n = 3037), and others entered the cervicovaginal abnormalities group (n = 485) which had TCT(+)/HPV(-) (n = 373) or TCT(+)/HPV(+) (n = 112).

Statistical analysis. Statistical analysis was performed with GraphPad Prism 6.0 (U.S.), and significant differences in clinical characteristics were assessed with unpaired t-test or Fisher's exact test. All results were considered statistically significant at $p < 0.05$.

Ethics approval and consent to participate. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Clinical Trial Ethics Committee of Southwest Medical University Hospital (approval number: KY2019039).

Results

Among 3540 subjects, 2 unsatisfactory specimens and 16 incomplete screenings were excluded for cervical screening. As a result, 3522 subjects completed the cervical screening test. A total of 485 cases were detected with cervicovaginal abnormalities (TCT+), with a prevalence of 13.77% (Table 1). Among these TCT(+) cases who had HPV test, 112 (23.09%) were HPV positive while 373 were HPV negative. The TCT(+)/HPV(+) subjects included ASC-US (n = 71), LSIL (n = 33), HSIL (n = 2), ASC-H (n = 6) and BV (n = 8, simultaneously with ASC-US). The TCT(+)/HPV(-)

subjects were detected with ASC-US (n = 11), LSIL (n = 37), HSIL (n = 5), ASC-H (n = 10), BV (n = 294, 5 simultaneously with ASC-US), FV (n = 13) and TV (n = 8).

Table 1. Results of TCT and HPV test (n = 3522).

Subjects	Number of cases	Subjects	Number of cases
Normal (TCT-, HPV-)	3037		
TCT(+)/HPV(+)	112	TCT(+)/HPV(-)	373
ASC-US	71	ASC-US	11
LSIL	33	LSIL	37
HSIL	2	HSIL	5
ASC-H	6	ASC-H	10
BV*	8	BV**	294
Other	0	FV	13
		TV	8

Note: *These subjects with BV were identified as ASC-US. **These populations had 5 cases simultaneously identified with ASC-US. ASC-US, atypical squamous cells of undetermined significance; LSIL, low grade squamous intraepithelial lesions; ASC-H, atypical squamous cells-cannot exclude high grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial lesion; BV, Bacterial vaginitis; FV, Fungal vaginitis; TV, Trichomonas vaginalis.

Among the 112 HPV positives (Table 2), there were 17 cases of low-risk HPV (with 12 ASC-US and 5 LSIL), 86 high-risk HPV (with 51 ASC-US, 27 LSIL, 2 HSIL, and 6 ASC-H) and 9 mixed high/low-risk HPV (with 8 ASC-US, and 1 LSIL). Particularly, 20 cases (17.86%) were detected with the 16/18-type high-risk HPV, which included 12 ASC-US, 4 LSIL, 2 HSIL, and 2 ASC-H.

147 **Table 2. Cytology results based on different HPV types.**

HPV status	ASC-US	>ASC-US			BV	Total
		LSIL	HSIL	ASC-H		
Low-risk HPV	12	5	0	0	0	17
High-risk HPV	51	27	2	6	8	86
Low- and High-risk HPV	8	1	0	0	0	9
16/18-type HPV	12	4	1	2	1	20
Total	71	33	2	6	8	112

148 Note: ASC-US, atypical squamous cells of undetermined significance; LSIL, low grade
149 squamous intraepithelial lesions; ASC-H, atypical squamous cells-cannot exclude high
150 grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial
151 lesion; BV, Bacterial vaginitis.

152

153 Subsequently, we analyzed the correlation between HPV-infection and other
154 potential risk factors such as age, number of pregnancies, number of abortions, number
155 of childbirths, and type of contraception. The results are displayed in Table 3. It is
156 suggested that the method of contraception, number of pregnancies and number of
157 births had a significant impact on the risk of HPV infection. Women with four or more
158 pregnancies were at increased risk of HPV infection compared to those with fewer than
159 three pregnancies. Those who have one or more childbirths had high risk of HPV
160 infections compared to those who did not undergo childbirth, with the 2 or 3 childbirths
161 at the highest risk. Contraceptive method using Paragard (copper) IUD led to the
162 highest HPV infection risk of 51.79%, followed by the no contraception (34.82%).

Table 3. Comparison of clinical data of 3149 subjects (normal, n = 3037; HPV, n = 112) in cervical screening.

Characteristics	Normal (n = 3037)	HPV (n = 112)	Total (n = 3149)	P value
Age, Years Mean (SD, range)	44.74(6.483,21-65)	44.25(7.043,26-65)	44.72(6.504,21-65)	0.2701
No. of Pregnancy (%)				**0.0198**
0	8(0.26)	0(0)	8(0.25)	
1	59(1.94)	5(4.46)	64(2.03)	
2	651(21.44)	30(26.79)	681(21.63)	
3	921(30.32)	26(23.21)	947(30.07)	
4	682(22.46)	32(28.57)	714(22.67)	
> 4	597(19.66)	12(10.71)	609(19.34)	
unknown	119(3.92)	7(6.25)	126(4.00)	
No. of abortion (%)				0.1588
0	1056(34.77)	40(35.71)	1096(34.8)	
1	846(27.86)	29(25.89)	875(27.79)	
2	702(23.11)	32(28.57)	734(23.31)	
3	198(6.52)	3(2.68)	201(6.38)	
≥4	115(3.79)	1(0.89)	116(3.68)	
unknown	120(3.95)	7(6.25)	127(4.03)	
No. of childbirth (%)				**0.0012**
0	10(0.33)	0(0)	10(0.32)	
1	135(4.45)	14(12.5)	149(4.73)	
2	1746(57.49)	65(58.04)	1811(57.51)	
3	913(30.06)	24(21.43)	937(29.76)	
≥4	115(3.78)	4(3.57)	119(3.78)	
unknown	118(3.89)	5(4.46)	123(3.91)	
Type of contraception (%)				**<0.0001**
No contraception	1329(43.76)	39(34.82)	1368(43.44)	
IUD	1455(47.91)	58(51.79)	1513(48.5)	
condoms	125(4.12)	6(5.36)	131(4.16)	
Ligation	15(0.49)	3(2.68)	18(0.57)	
contraceptive	1(0.03)	1(0.89)	2(0.06)	
unknown	112(3.69)	5(4.46)	117(3.72)	
HPV status (%)				
high-risk		88(78.57)	88(78.57)	
high-risk and Low-risk		9(8.04)	9(8.04)	
Low-risk		15(13.39)	15(13.39)	

Note: Normal group included those detected with TCT(-)/HPV(-). HPV group included those detected with HPV infections.

After the cytology and HPV tests, a total of 119 subjects who had >ASC-US or =ASC-US/HPV(+) were sent for colposcopy. As a result, 58 cases were detected with ≥CIN1 (32 CIN1, 24 CIN2/3, and 2 CC), while others were considered to be at risk of a cervical lesion (Risk population). The correlation analysis between HPV infection and cervical status is shown in Table 4. Ratio of HPV infection increased along with increased disease severity (Risk population, CIN1, and CIN2/3), while high-risk HPV infection was proportional to disease severity, where 16/18-type HPV ratio also increased with increasing disease severity. It is evident that HPV infection, especially the high-risk HPVs, is positively associated with cervical lesion progression. The two CC cases did not show the same trend.

Table 4. Cytology results based on different HPV types.

Subjects	Total	HPV (%)	High-risk HPV (%)	Low-risk HPV (%)	16/18-type HPV (%)
CC	2	1(50.00)	1(50.00)	0	0
CIN2-3	24	15(62.50)	15(62.50)	0	6(25.00)
CIN1	32	17(53.13)	16(50.00)	1(3.13)	3(9.38)
Other risk population*	61	27(44.26)	23(37.70)	4(6.56)	2(3.28)

Note: *indicating the subjects who underwent colposcopy but did not have CIN or CC lesions.

Discussion

HPV prevalence statistics in recent years has been reported in China, showing a prevalence of 15.9% in Shenzhen [17]; 21.1% in Hani women, 12.6% and Han women in Yunnan [18]; 8.42% in Uyghur women in Xinjiang [19], 18.34% in southeastern China [20], and 9.5% in Shanghai [21]. The prevalence of HPV in Sichuan, particularly in the poor regions, has not yet been investigated. In the present study, we performed a large-scale cross-sectional study to investigate the HPV infection status in western Sichuan, China. It was reported for the first time that the HPV infections in Xichang, Sichuan was at a relative high level of 23.09%. The results from our study added knowledge into the HPV infection in western China.

It is found that HPV infection, especially the high-risk HPVs, is positively associated with cervical lesion progression. But, the two CC cases did not show the same trend, which may be due to the limited number of samples.

Previous studies have shown that education level [22], type of vaginitis, history of hyperlipidemia, family history of cancer, number of pregnancies [23], number of sexual partners [21], age, race [24], age difference of heterosexual partners [25], smoking, immunosuppression, and personal hygiene and care [19] were risk factors for HPV infection and development of CC lesions in women. Our present study found that HPV infection was strongly associated with the method of contraception, the number of pregnancies, and the number of births.

Conclusion

In this study, we for the first time performed a large-scale cross-sectional study to investigate the HPV infection rate in Xichang, Sichuan, China. Among 3522 subjects,

a total of 13.77% cases showed cervical abnormalities. Further colposcopy showed that there were 58 cases of CIN1-3 and cancerous lesions. With HPV test, 112 subjects were detected as HPV positive, which was positively correlated with the method of contraception, number of pregnancies and number of births. The HPV infection, especially the high-risk HPVs, is positively associated with cervical lesion progression.

Data availability

The datasets generated during this study are not publicly available since they will contain patient data and the informed consent agreement does not include sharing data publicly. However, the data are available from the corresponding author on reasonable request.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dan Li, Xiangyu Deng, Xu Wu, Rong Huang, Fukuan Du, Mingxing Li, Yueshui Zhao, Jing Shen, Yu Chen, Pingxiu Zhang, Congcui Hu. The first draft of the manuscript was written by Xu Wu, Xiangyu Deng,Dan Li, Rong Huang, Zhangang Xiao and Qinglian Wen, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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