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CLINICAL ARTICLE

# Prevalence and genotype distribution of human papillomavirus in women with cervical cancer or high-grade precancerous lesions in Chengdu, western China

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ABSTRACT

*Objective:* To study the prevalence and genotype distribution of human papillomavirus (HPV) among women with cervical cancer or high-grade squamous intraepithelial lesions (HSIL) in western China. *Methods:* Cervical cast-off cells from 144 women with cervical cancer and 63 women with HSIL were tested for HPV genotypes using an oligonucleotide microarray. *Results:* The overall HPV prevalence was 80.6% in cases of carcinoma and 61.9% in cases of HSIL. The most common genotypes were HPV-16 (carcinoma, 68.1%; HSIL, 34.9%) and HPV-58 (carcinoma, 8.3%; HSIL, 17.5%). Other high-risk types included HPV-18, -31, -33, -35, -45, and -52, with HPV-18 more common in adenocarcinomas than in squamous cell carcinomas (21.4% vs 3.1%; $P<0.02$). The HPV prevalence was lower among patients older than 49 years ($P<0.02$). *Conclusion:* The prevalence of HPV-16 and HPV-58 was high. This finding may help to improve HPV vaccination and cervical cancer prevention programs in western China.


## 1. Introduction

Cervical cancer is one of the most common cancers among women worldwide, particularly in low-income countries [1]. In China, cervical carcinoma is the most common malignancy in women, with over 100 000 new cases and nearly 50 000 deaths every year [2]. It is believed that human papillomavirus (HPV) infection, which is detected in nearly 90% of all invasive carcinoma tissues, is the most common risk factor for cervical cancer [3]. More than 60 different HPV genotypes have been found in the female genital tract, with some categorized as high-risk genotypes owing to their strong oncogenic potential [4]. High-risk HPV genotypes are believed to increase the risk of cervical precancerous lesions and trigger the progression of these lesions to carcinoma. At least 18 high-risk HPV genotypes have been identified in human genital ducts; the most prevalent ones worldwide are HPV-16 and HPV-18 [4,5].

An important approach to preventing cervical cancer is the prevention of HPV infection by prophylactic vaccination. The availability of HPV vaccines will help to not only curb the incidence and mortality of cervical cancer, but also reduce the cost burden of cervical cancer-screening programs. Because HPV-16 and HPV-18 are the most common genotypes, vaccines for HPV prevention have been designed mainly to act against these 2 subtypes and could effectively protect 70% of women worldwide [6]. However, the distribution of HPV genotypes in cervical cancers varies in different countries and regions. In China, the prevalence and distribution of HPV genotypes are still unclear because China covers 9.6 million $km^2$ and has a population of over 1.3 billion. In northern China, HPV-16 and HPV-18 are the major risk factors for cervical cancer and precancerous lesions, whereas HPV-58 and HPV-52 are detected more frequently in cancer patients in some areas of southern China [7,8]. Epidemiologic data regarding HPV infection rates in western China are lacking. However, the determination of HPV prevalence and genotype distribution would have a crucial role in evaluating the cost-effectiveness of prophylactic cervical cancer vaccines in this area.

Chengdu is the capital of Sichuan province, located in western China, and is one of the largest cities in the country—covering 12 000 $km^2$ and with a population of approximately 10.6 million (as estimated in 2004 by the Population and Family Planning Committee of Chengdu Government). In 2002, the cervical cancer prevalence rate in Chengdu was estimated by the Local Committee of Public Health to be 16.1 cases per 100 000 reproductive women. However, to the best of our knowledge, there have been no reports of HPV prevalence and genotype distribution among women with cervical cancer or precancerous disease in this area. The aim of the present study was, therefore, to determine the prevalence and distribution of HPV genotypes among women with cervical cancer or high-grade precancerous lesions in the Chengdu area.

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## 2. Materials and methods

In total, 207 patients, 144 with cervical cancer and 63 with high-grade precancerous lesions (high-grade squamous intraepithelial lesions [HSIL]), were included in the present study. The mean ages of the patients were 43.4 ± 8.3 years for women with carcinoma and 29.7 ± 5.7 years for women with HSIL. All patients were from the Chengdu area and visited West China Second Hospital, Sichuan University, Chengdu, China, between January 1, 2007, and September 31, 2009. Patients with low-grade squamous intraepithelial lesions or metastatic cervical carcinoma were excluded, as were those who were unwilling to undergo an HPV test. Cervical biopsy or loop electrosurgical excision procedure (LEEP) was used to determine the pathologic diagnosis, which was made by 2 pathologists (JL and YZ). Before biopsy or LEEP, cervical cast-off cells were collected for DNA extraction and HPV genotyping. The study was approved by the hospital Ethics Committee and informed consent was obtained from all participants.

The cervical cancers were staged as IB (n = 68) or IIA (n = 76), according to the staging system of the International Federation of Gynecology and Obstetrics (FIGO). In total, 130 cancers were diagnosed as squamous cell carcinoma and 14 were diagnosed as adenocarcinoma. All patients received radical hysterectomy and full lymphectomy. Clinicopathologic features, including differentiation grade, lymph node metastasis, vascular invasion, and interstitial invasion, were also recorded. The tumors were pathologically graded as well differentiated, moderately differentiated, or poorly differentiated. Lymph node metastasis was defined by the pathologic detection of carcinoma cells in 1 or more lymph nodes. Vascular invasion was defined by the presence of a carcinoma embolus in a blood or lymphatic capillary on microscopy.

The patients avoided vaginal douching or medication for 3 days before sampling. The cast-off cells were collected from cervical lesions via plastic cervical swabs (HybriBio, Hong Kong, China). Each plastic swab full of cells was mixed with 1 mL of physiologic saline and stored immediately at 4 °C until further use. After thawing, the supernatants were removed by centrifugation at 9660 *g* for 5 minutes. The pellets were used for DNA extraction (HybriBio, Hong Kong, China).

The HPV genotyping was performed with an HPV GenoArray test kit (HybriBio, Hong Kong, China), as described previously [9]. To amplify the HPV DNA, 1 μL of the supernatant of the sample containing the extracted DNA was added to 24 μL of a polymerase chain reaction (PCR) mixture containing 4-mmol/L $MgCl_2$, 50-mmol/L KCl, 7.5-U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 200-μmol/L dATP, dCTP, and dGTP, 600-μmol/L dUTP, 1-U uracil *N*-glycosylase, 100 pmol each of the biotinylated PGMY primers PGMY09 and PGMY11, and 2.5 pmol each of the 5′-biotinylated β-globin primers GH20 and CP04. The PCR was performed with the following parameters: initial denaturation at 95 °C for 9 minutes, followed by 40 cycles at 95 °C for 20 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes. In some experiments, the PCR products were separated by electrophoresis in 2.0% agarose gels. The genotyping was performed via hybridization (using a flow-through hybridization technique) of the PCR products to a gene chip containing genotype-specific oligonucleotides that were immobilized on a nylon membrane. The hybridized DNA fragments were detected via direct visualization of colorimetric changes on the chip, which covered 21 HPV genotypes—5 low-risk types (6, 11, 42, 43, and 44); 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68); and 2 intermediate-risk types (CP8304 and 53)—that are common among Chinese women.

Data were analyzed with the $\chi^2$ test or the Cochran–Armitage trend test, as appropriate, via SAS version 9.0 (SAS Institute, Cary, NC, USA). $P<0.05$ was considered statistically significant.

**Table 2**
Prevalence of multiple HPV infections in women with cervical carcinoma or high-grade squamous intraepithelial lesions.[a]

| HPV genotypes involved | Number of women affected |
|---|---|
| Carcinoma (n = 144) | |
| 16, 33, and 58 | 1 (0.7) |
| 16 and 52 | 3 (2.1) |
| 16 and 58 | 1 (0.7) |
| 16 and 45 | 1 (0.7) |
| 16 and 18 | 1 (0.7) |
| 16 and 6 [b] | 2 (1.4) |
| High-grade squamous intraepithelial lesions (n = 63) | |
| 16 and 33 | 1 (1.6) |
| 18 and 58 | 1 (1.6) |
| 52 and 58 | 1 (1.6) |

Abbreviation: HPV, human papillomavirus.
[a] Values are given as number (percentage).
[b] Low-risk type.

## 3. Results

The prevalence of HPV infection and the distribution of the different HPV genotypes are shown in Tables 1 and 2. Human papillomavirus DNA was detected in 116 of 144 patients (80.6%) with carcinoma and 39 of 63 patients (61.9%) with HSIL. The following HPV genotypes were detected: HPV-6, -16, -18, -31, -33, -35, -58, -45, and -52.

As expected, HPV-16 was the most common genotype; it was detected in 98 (68.1%) patients with carcinoma. The second most common genotype was HPV-58, which was detected in 12 (8.3%) carcinoma patients. HPV-18 was the third most common genotype and was detected in 7 (4.9%) carcinoma patients. In addition, HPV-33, -45, -52, and the low-risk HPV-6 were detected in carcinoma patients. The most common genotypes among patients with HSIL were HPV-16 and HPV-58, which were detected in 22 (34.9%) and 11 (17.5%) cases, respectively. HPV-18, -31, -33, -35, -45, and -52 were also detected in patients with HSIL, but they were rare. Infection with multiple HPV genotypes occurred in 9 (6.3%) women with carcinoma and 3 (4.8%) women with HSIL; the difference was not statistically significant.

We also studied the correlation of HPV prevalence and clinicopathologic features among carcinoma patients. Human papillomavirus infection was present in 42 (89.4%), 56 (80.0%), and 18 (66.7%) patients younger than 40 years, 40–49 years, and older than 49 years, respectively ($P<0.02$; Table 3). There was no association between carcinoma histology and overall HPV prevalence (HPV DNA was present in 106 [81.5%] cases of squamous cell carcinoma and 10 [71.4%] cases of adenocarcinoma; $P>0.05$). However, HPV-18 infections were

**Table 1**
HPV genotype distribution in cervical cancer and HSIL.[a]

| | HPV positive | HPV-16 | HPV-58 | HPV-18 | HPV-33 | HPV-45 | HPV-52 | HPV-31 | HPV-6 [b] | HPV-35 | Multiple types |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HSIL (n = 63) | 39 (61.9) | 22 (34.9) | 11 (17.5) | 1 (1.6) | 2 (3.2) | 1 (1.6) | 2 (3.2) | 2 (3.2) | 0 (0.0) | 1 (1.6) | 3 (4.8) |
| Cancer (n = 144) | 116 (80.6) | 98 (68.1) | 12 (8.3) | 7 (4.9) | 1 (0.7) | 1 (0.7) | 4 (2.8) | 0 (0.0) | 2 (1.4) | 0 (0.0) | 9 (6.3) |

Abbreviations: HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.
[a] Values are given as number (percentage).
[b] Low-risk type.