

## Original Article

## High-risk human papillomavirus, other than type 16/18, in predominantly older Taiwanese women with high-grade cervical preinvasive lesions

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**Abstract**

**Objective:** To investigate the various genotypes of human papillomavirus (HPV) in Taiwanese women patients with abnormal cervical cytology and analyze the associations between HPV types, cervical preinvasive lesions, and the medical characteristics of these patients.

**Materials and Methods:** We performed HPV genotyping GeneChip procedures and colposcopies for 784 women with abnormal Papanicolaou smears. The characteristics of the patients and the status of the HPV infection were correlated.

**Results:** A total of 706 (90.1%) of the 784 women were positive for HPV infection, including 641 patients with high-risk HPV (HR-HPV). Among the patients with high-grade squamous intraepithelial lesions (HSILs), the average age of the 273 patients with other HR-HPV types ( $48.6 \pm 13.8$  years) was significantly older than the 222 patients infected with HPV 16/18 ( $39.8 \pm 11.8$  years) ( $p < 0.001$ ). The proportion of patients with HSILs who were older than 40 years and infected with other HR-HPV types (76.6%) was also significantly higher than those with HPV 16/18 (20.3%) ( $p < 0.001$ ).

**Conclusion:** Women older than 40 years and having abnormal Pap smears and HR-HPV infections other than type 16/18 should be managed carefully because of the risk for HSILs.

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**Keywords:** high-grade squamous intraepithelial lesion; human papillomavirus; Taiwanese women

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**Introduction**

Cervical cancer is the most prevalent gynecological malignancy and results in considerable morbidity and mortality. The prevalence of cervical cancer has been significantly reduced globally because Pap smear screening programs are conducted in most countries; however, it continues to be a major health concern among women in less developed

countries [1]. zur Hausen first proposed the association between the human papillomavirus (HPV) and cervical cancer, and the role of HPV in the carcinogenesis of cervical cancer has now been confirmed [2]. Patients with persistent infections of the high-risk HPV (HR-HPV) types have an increased risk of developing cervical carcinoma. HPV 16 and 18 are the two leading genotypes that are detected in women with cervical neoplasm worldwide [3,4]. The addition of the HPV test to Pap smear testing has provided a more sensitive and cost-effective way for the early detection of cervical lesions, especially in patients with atypical squamous cells of undetermined significance (ASCUS) [5,6]. At present, it has been suggested that a combination of the HPV test and Pap smear

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for women aged 30 years or older is an effective way to minimize unnecessary management of these lesions [7,8]. This study was conducted to investigate the HPV genotypes in Taiwanese women patients with abnormal cervical cytology results and analyze the associations between HPV types, cervical preinvasive lesions, and the characteristics of these patients.

## Materials and Methods

### Patient recruitment

From January 1, 2000 to December 31, 2007, consecutive patients with cytological abnormalities presenting to our institution were recruited for this investigation. The research protocol was approved by the Ethics Committee of the National Taiwan University Hospital, and written informed consent forms were obtained from each patient. The medical characteristics of these patients were retrieved from the medical records. Results of the Pap smear tests were reviewed according to the terminologies specified in the 2001 Bethesda System [9]. The study included patients with cytological abnormalities, including ASCUS, atypical squamous cells not excluding high-grade squamous intraepithelial lesions (HSILs), atypical glandular cells not otherwise specified, atypical glandular cells favoring neoplasm, low-grade squamous intraepithelial lesions (LSILs), HSILs, and malignant diseases. The intervals between performing the Pap smears, HPV DNA testing, and colposcopic examinations were less than 3 months. Cervical biopsies and/or endocervical curettages were performed if indicated at the colposcopic clinics of our hospital. The indications for a loop electrosurgical excision procedure (LEEP) or conization consisted of a colposcope-directed cervical biopsy or a endocervical curettage showing cervical intraepithelial neoplasia (CIN) 2/3 or adenocarcinoma *in situ*, cytological HSIL with an unsatisfactory colposcopy, and discrepancies between the results of cytological analysis and cervical biopsy. The intervals between colposcope-directed biopsies and LEEP or conization were less than 1 month. We enrolled those patients who underwent hysterectomies for the primary treatment of CIN 2/3, adenocarcinoma *in situ*, or other gynecologic benign lesions such as uterine fibroids or ovarian tumors, or patients who had had a hysterectomy within 12 weeks after conization for further analysis. Patients who had a cone diagnosis of cervical cancer, refused to receive HPV DNA testing, underwent a hysterectomy more than 12 weeks after primary diagnosis or conization, or refused to participate in the study were excluded. Patients who had ever received an HPV vaccine and patients with a past history of cervical cancer or precancerous lesions were also excluded.

### HPV detection and genotyping by GeneChip

We performed HPV tests using the cervical swabs of the recruited patients before colposcopies, cervical biopsies, or conizations were considered. DNA extraction and polymerase

chain reaction (PCR) were then performed from the cells acquired from the cervical swab as described previously [10]. In brief, short PCR fragment 1/general primer 6 + PCR was performed for 40 cycles. The resultant PCR products were hybridized with a commercial HPV genotyping array membrane (King Car Food Industrial Co., Ltd., I-lan, Taiwan) in a single reaction. HPV Blotting contains 38 types of HPV (6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71 [CP8061], 72, 74, 81 [CP8304], 82 [MM4], 83 [MM7], 84 [MM8], and L1AE5) oligonucleotide probes (20–30 mer) on a nylon membrane. The hybridization and detection procedures were described previously [11].

Direct sequence analysis was performed on the HPV-positive products periodically. The HPV genotypes MM4, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82 were regarded as HR-HPV types; 26 and 53 were regarded as probable HR-HPV types; and the other types were regarded as low-risk HPV (LR-HPV) types [12]. When HPV-negative results were noted in the first round, a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) PCR was performed to validate the HPV negativity. If the GAPDH PCR was also negative, another PCR for GAPDH and HPV infection was performed. In cases of GAPDH positivity and a negative repeat of the HPV Blot, the final result was designated as HPV negative.

After primary treatment with LEEP, conization, or hysterectomy, the patients underwent regular Pap smear tests and/or colposcopic examinations at 3- to 6-month intervals in the first 3 years, and then annually for follow-up.

### Statistical analysis

The data were computerized and analyzed using SPSS for Windows, version 10.0.7C, SPSS Inc., (Chicago, IL, USA). Comparisons between unpaired groups were made using Student *t* test, Mann–Whitney *U* test, one-way analysis of variance (ANOVA),  $\chi^2$  test, and Kruskal–Wallis test.

## Results

A total of 784 women matched the study criteria and were recruited for this study (Fig. 1). The age of these patients ranged from 20 to 87 years, with a mean age of 45.2 years, and 199 (25.4%) were menopausal women. Of the 784, 594 (75.8%) women received LEEP or conization, and 52 (6.6%) underwent a hysterectomy as the primary treatment. A total of 646 women had a histological diagnosis, and the other 138 women did not have a cervical biopsy because of negative colposcopic findings. Among the patients with a histological diagnosis of preinvasive cervical lesions, we noted positive section margins on the LEEP/conization specimens in 59 patients. A total of 21 patients subsequently underwent hysterectomies, of which 17 (80.9%) had residual CIN lesions in their hysterectomy specimens. Among the other patients with negative section margins on the LEEP/conization specimens, 34 underwent a hysterectomy for other indications and out of

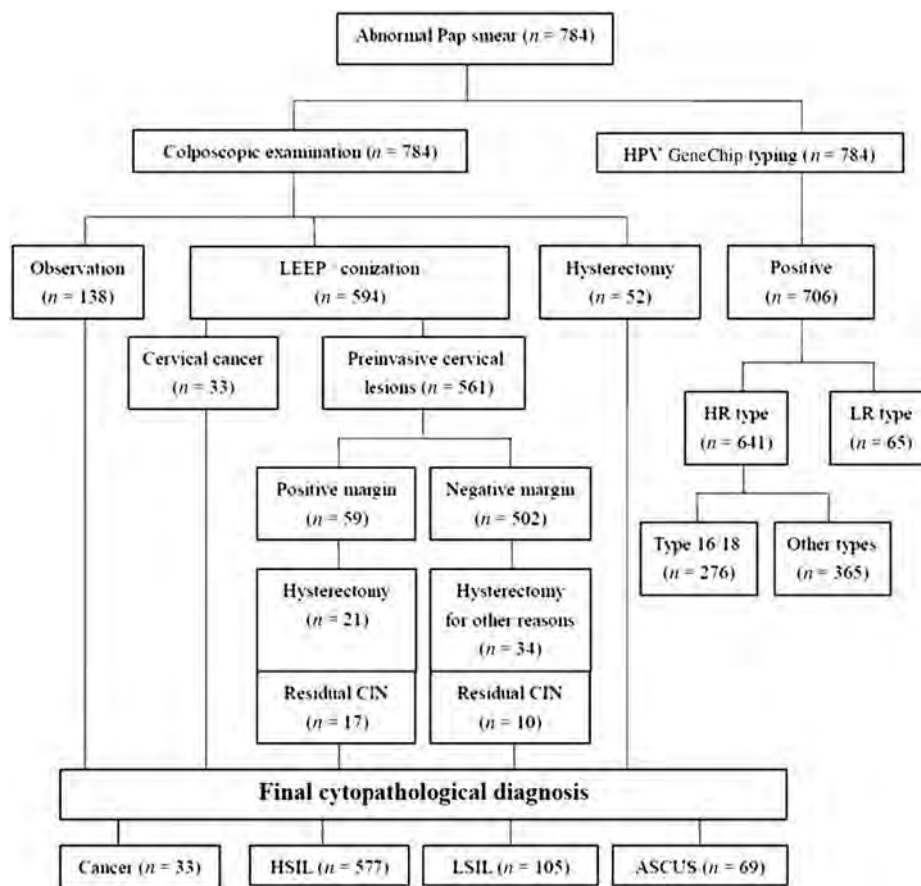


Fig. 1. Flowchart of the current investigation. The final cytopathological diagnoses, treatments, outcomes, and HPV genotyping are summarized. ASCUS = atypical squamous cells of undetermined significance; CIN = cervical intraepithelial neoplasia; HPV = human papillomavirus; HR = high risk; HSIL = high-grade squamous intraepithelial lesion; LEEP = loop electrosurgical excision procedure; LR = low risk; LSIL = low-grade squamous intraepithelial lesion.

these, 10 patients (29.4%) still had residual CIN lesions. The final diagnosis was based on the worst of both the cytological and histological findings. Cervical cancer was diagnosed in 33 patients (4.2%), HSIL in 577 patients (73.6%), LSIL in 105 patients (13.4%), and ASCUS in 69 patients (8.8%).

The HPV infection was detected by a genotyping chip in 706 (90.1%) of the 784 women with abnormal Pap smears. Of them, 641 (90.8%) were infected with HR-HPV and 65 (9.2%) were infected with LR-HPV. There were 159 women who were infected with more than one HPV type. Of the 641 women infected with HR-HPV, 276 (43.1%) of them were infected with HPV 16 and/or 18, and 365 (56.9%) of them were infected with the other HR-HPV types. The distribution of HPV types for all the 784 women is summarized in Table 1. The percentages of HR-HPV infection increased from ASCUS to invasive cancer (Fig. 2,  $p < 0.001$ , Mann–Whitney  $U$  test). When focusing on individual HPV genotypes, the percentages of HPV 16 ( $p < 0.001$ ) and HPV 18 ( $p = 0.039$ ) infection significantly increased from ASCUS to invasive cancer; HPV 31 ( $p = 0.071$ ) and HPV 58 ( $p = 0.067$ ) infections showed marginal increase, but HPV 33 ( $p = 0.311$ ), HPV 35 ( $p = 0.650$ ), and HPV 52 ( $p = 0.159$ ) were not significantly different (Mann–Whitney  $U$  test).

We also analyzed the relationships among age, cervical preinvasive lesions, and HPV infection. In 105 patients with

LSIL, the mean ages did not differ among the women with HPV 16/18 infections ( $45.6 \pm 15.6$  years old), the other HR-HPV types of infection ( $42.2 \pm 10.6$ ), the LR-HPV type infections, and those without HPV infection ( $41.7 \pm 13.4$ ) ( $p = 0.565$ , one-way ANOVA test). In 577 patients with HSIL, 222 patients were infected with HPV 16/18, 273 patients with other HR-HPV types, and 82 patients with either LR types or were without HPV infection (Table 2). In patients with HSIL, the average age of the 273 patients with other HR-HPV types ( $48.6 \pm 13.8$  years) was significantly older than the 222 patients infected with HPV 16/18 ( $39.8 \pm 11.8$  years) ( $p < 0.001$ , one-way ANOVA test). The proportion of the HSIL patients older than 40 years and infected with other HR-HPV types (76.6%) was also significantly higher than that of the HPV 16/18 patients (20.3%) ( $p < 0.001$ , Kruskal–Wallis test). In addition, a higher proportion of the HSIL patients infected with other types of HR-HPV were in menopause as compared to those infected with HPV 16/18 ( $p < 0.001$ , Mann–Whitney  $U$  test).

## Discussion

The HR-HPV infection is involved in the carcinogenesis of the uterine cervix. In our study, HR-HPV was detected in all of

Table 1  
Distribution of HPV genotypes in all patients.

HPV types	Total (n)	%	ASCUS (n)	%	LSIL (n)	%	HSIL (n)	%	CC (n)	%
	784		69		105		577		33	
MM4	3	0.4	0	0.0	1	1.0	2	0.4	0	0
16	217	27.7	11	15.9	16	15.2	174	30.2	16	48.5
18	64	8.2	3	4.4	3	2.9	52	9.0	6	18.2
26	3	0.4	1	1.5	2	1.9	2	0.4	0	0
31	32	4.1	0	0.0	2	1.9	30	5.2	0	0
33	48	6.1	4	5.8	3	2.9	36	6.2	5	15.2
35	20	2.6	2	2.9	4	3.8	15	2.6	1	3.0
39	23	2.9	4	5.8	4	3.8	15	2.6	0	0
45	11	1.4	0	0.0	2	1.9	9	1.6	0	0
51	23	2.9	2	2.9	6	2.9	36	6.2	0	0
52	103	13.1	3	4.4	15	14.3	81	14.0	4	12.1
53	34	4.3	0	0.0	15	14.3	19	3.3	1	3.0
56	19	2.4	1	1.5	8	7.6	10	1.7	0	0
58	94	12.0	2	2.9	8	7.6	78	13.5	4	12.1
59	17	2.2	2	2.9	3	2.9	10	1.7	0	0
68	16	2.0	0	0.0	1	1.0	8	1.4	0	0
82	10	1.3	0	0.0	0	0.0	3	0.5	0	0
LR types	65	8.3	7	10.1	18	17.1	40	6.9	0	0
Negative	78	9.9	27	39.1	10	9.5	41	7.1	0	0

Percentages add up to more than 100 due to some patients being infected with multiple HPV types. ASCUS = atypical squamous cells of undetermined significance; CC = cervical cancer; HPV = human papillomavirus; HSIL = high-grade squamous intraepithelial lesion; LR = low risk; LSIL = low-grade squamous intraepithelial lesion; n = patient number.

the patients with invasive cancer, 86% of the patients with HSIL, 72% of the patients with LSIL, and 51% of the patients with ASCUS. The proportions of HR-HPV infection increased from ASCUS to more aggressive lesions, especially in women infected with HPV 16, 18, 52, and 58 genotypes. The increased proportion of the HR type in the HSILs and invasive cancers may imply that these genotypes had the ability to evade the immune protection of the host and cause oncogenic changes in the cervix. The prevalent HPV genotypes in our study were compatible with the previous studies in which HPV 52 and 58 were the most prevalent types of cervical pre-invasive and invasive lesions in addition to HPV 16 and 18 [13,14] in Asia.

The HSIL in relation to HR-HPV, other than type 16/18, predominantly occurred in woman older than 40 years. These women may have recovered from previous HPV 16/18-

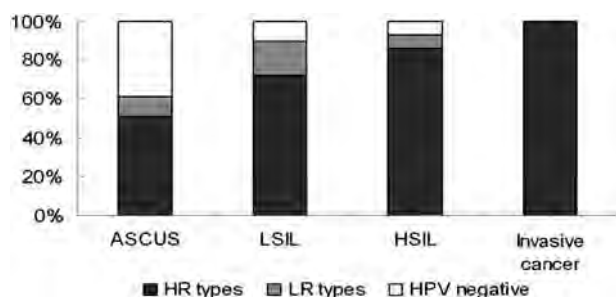


Fig. 2. Proportion of HPV types in all patients. The proportion of HR-HPV infection increased as the cervical lesions progressed from ASCUS to aggressive lesions ( $p < 0.001$ , Mann–Whitney  $U$  test). ASCUS = atypical squamous cells of undetermined significance; HPV = human papillomavirus; HR = high risk; HSIL = high-grade squamous intraepithelial lesion; LR = low risk; LSIL = low-grade squamous intraepithelial lesion.

associated cervical lesions at a younger age, before they received the screening in our study. Consequently, we excluded women with a previous history of cervical pre-invasive lesions or cervical cancer from enrolling in this study to reduce the interference from this factor. In the study of HPV genotypes in 1086 Taiwanese women with CIN 2/3 conducted by Chao et al, HPV 16 and 18 comprised 21.3%, whereas HPV 52, 58, and 33 represented 55.5% in women older than 50 years. In women under the age of 50, HPV 16 and 18 comprised 32.1%, whereas HPV 52, 58, and 33 represented 47.9%. These results were consistent with our findings [15]. The current HPV vaccine for children/young women aged between 9 and 26 years has been proven to prevent HPV 16- and 18-related HSIL effectively [16,17]. Women with HSIL derived from other HR-HPV types will be the more important problem in the era of HPV vaccination worldwide. The addition of the HPV test to the Pap smear is currently suggested in the screening of cervical cancer [7], and the HPV test is recommended as a triage test for women with ASCUS [5,18]. However, controversy exists over the utility of the HPV test for general cervical cancer screening [19]. Type-specific HPV screening should be the next step for HPV testing instead of the nontyping Hybrid Capture assays [20]. Our study could assist in the clinical utilization of HPV genotyping testing in patients with abnormal cervical cytological findings. Women older than 40 years, who have abnormal Pap smear results and an HR-HPV infection other than type 16/18, should be carefully managed to avoid the risk of developing cervical HSIL.

In conclusion, this investigation provides useful information about the association of HPV genotypes and cervical preinvasive lesions. In the era of an HPV vaccination against



Table 2  
Relationship between age and HPV genotypes in the 577 patients with HSIL.

Patient numbers	HPV 16/18	Other HR-HPV types	LR-HPV or HPV negative	p
	222	273	82	
Age (y/o)	39.8 ± 11.8	48.6 ± 13.8	47.8 ± 13.4	<0.001
Age ≥ 40 (y/o)				
Yes	45 (20.3%)	209 (76.6%)	59 (72.0%)	<0.001
No	177 (79.7%)	64 (23.4%)	23 (28.0%)	
Menopause				
Yes	33 (14.9%)	91 (33.3%)	23 (28.0%)	<0.001
No	189 (85.1%)	182 (66.7%)	59 (72.0%)	

HPV = human papillomavirus; HR = high risk; HSIL = high-grade squamous intraepithelial lesion; LR = low risk.

type 16/18, the other HR-HPV-related lesions will play a more important role in clinical practice. A combination of Pap smear testing and HPV genotyping will be the trend of screening in the future.

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