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Prevalence of Human Papillomavirus Genotypes in Northern Taiwanese Women

Kuo-Chien Tsao,[1,2,3] Chung-Guei Huang,[1,2,3]** Yung-Bin Kuo,[4]* Ting-Chang Chang,[5,6] Chien-Feng Sun,[1,7] C. Allen Chang,[4,8] Su-Li Yang,[1] and Err-Cheng Chan[2]***

[1]*Department of Laboratory Medicine, Lin-kou Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[2]*Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan*
[3]*Research Center for Emerging Viral Infections, Chang Gung University, Taoyuan, Taiwan*
[4]*College of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan*
[5]*Department of Obstetrics and Gynecology, Lin-Kou Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[6]*College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[7]*Department of Pathology, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[8]*Department of Biomedical Imaging and Radiological Sciences, National Yang Ming University, Taipei, Taiwan*

The prevalence of *Human Papillomavirus* (HPV) in the general population of northern Taiwan is described. A total of 343 consecutive cervical swabs from women visiting the medical center for routine gynecologic care were included. Cervical cell cytology was examined by the Papanicolaou (Pap) test, and a PCR-based hybridization gene chip analysis was used to identify HPV genotypes. The HPV prevalence in the overall population was 32.4%. When divided into two groups according to cytology, 20.9% of women with normal cytology were HPV positive while 75.3% of women with abnormal cytology were HPV positive. Among positive samples, 68.5% were single type infections while 31.5% harbored multiple HPV types. A total of 32 types of HPV were identified; the leading five were HPV16 (5.8%), HPV58 (5.3%), HPV53 (4.1%), HPV52 (3.8%), and HPV18 (2.3%). Our results constitute baseline data and may provide important implications for future prophylactic programs. The relatively high prevalence of HPV 58, 53, and 52 among northern Taiwanese women has important implications for vaccine development. ***J. Med. Virol. 82:1739–1745, 2010.***

KEY WORDS: human papillomavirus; genotype; distribution

INTRODUCTION

Cervical cancer continues to be one of the leading female genital cancers worldwide [Parkin et al., 2001]. The cervical cancer burden in Taiwan remains high and is accompanied by an incidence of invasive cancer of 18.6 out of every 100,000 cases [Tay et al., 2008]. Genital *Human Papillomavirus* (HPV) infection appears to be the most common sexually transmitted virus, and many studies have demonstrated a link between HPV and cervical lesions [Kiviat et al., 1992; Bosch et al., 1995; zur Hausen, 2000; Burd, 2003; Munoz et al., 2003]. To date, 118 HPV genotypes have been identified, of which at least 15 are strictly related to cervical cancer [Munoz et al., 2003; Bernard, 2005]. Almost all of cervical cancers are attributable to persistent HPV infection. However, the prevalence of genital tract HPV infection has been reported to range from 1.4% to 25.6% in the population exhibiting normal cytology [Clifford et al., 2005].

Tumorigenicity of HPV differs markedly among HPV genotypes [Kjaer et al., 2002; Clifford et al., 2003], and geographic differences in the frequency of HPV genotypes have also been reported to exist [Clifford et al., 2003, 2005; Munoz et al., 2004]. For example, some genotypes such as type 52 and 58 are rare in Western countries; however, they are relatively prevalent in Asian populations [Huang et al., 1997; Lai et al., 1999]. Hence, an accurate assessment of the regional, community-based distribution of HPV genotypes is extremely important for prevention of cervical cancer and for public hygiene management, yet such data are limited in Taiwan.

Kuo-Chien Tsao, Chung-Guei Huang and Yung-Bin Kuo contributed equally to this work and should be considered co-first authors.

*Correspondence to: Err-Cheng Chan, (concerning methodology) Department of Medical Biotechnology and Laboratory Science, Chang Gung University, 259 Wen-Hua 1st Road, Kweishan, Taoyuan, Taiwan. E-mail: chanec@mail.cgu.edu.tw; K. C. Tsao (concerning for clinical virology) Department of Laboratory Medicine, Chang Gung Memorial Hospital, Lin-Kou Medical Center, 5, Taoyuan 333, Taiwan.
E-mail: kctsao@adm.cgmh.org.tw

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Probing for HPV DNA has proven to be a valuable complementary test to conventional Papanicolaou (Pap) staining for improving the efficacy of cervical cancer screening [Herrington et al., 1996; Kulasingam et al., 2002; Kim et al., 2005; Molijn et al., 2005]. Furthermore, its clinical utility has accorded HPV genotyping an essential role in determining appropriate clinical management strategies for cervical cancer screening and follow-up subsequent to HPV vaccination [Meijer et al., 2006]. The FDA-approved Hybrid Capture II HPV DNA test (HC II) (Digene Corporation, Gaithersburg, MD), which can detect 13 carcinogenic HPV types, is the HPV DNA detection method used most commonly [Solomon et al., 2001]. Unfortunately, this cocktail detection method does not identify specific HPV genotypes.

The polymerase chain reaction (PCR)-based microarray genechip (Easychip® HPV Blot; King Car, Yi-Lan, Taiwan), which utilizes PCR to amplify the HPV L1 gene followed by reverse hybridization with immobilized probes, offers an all-in-one method to detect 39 types of HPV in a single hybridization reaction. This kit is manufactured under class III Good Manufacturing Practices in Taiwan and has manifested sensitivity and reliability comparable to HC II [Huang et al., 2004, 2006; Lai et al., 2007].

Identification of HPV genotypes is necessary not only for screening and diagnostic purposes but also for monitoring possible changes in the distribution of HPV genotypes after introduction of an HPV vaccine. However, because genital HPV infection is not a reportable disease and because only a few studies have focused on the general population, data regarding prevalence and HPV genotype-specific distribution patterns among distinct cytological and/or histological grades of cervical abnormality in Taiwanese women are limited [Jeng et al., 2005]. The current study was designed to investigate HPV prevalence, genotype distribution and extent of multiple infections in women of counties in northern Taiwan.

MATERIALS AND METHODS

Study Subject Recruitment and Sample Collection

A total of 343 female residents in cities of northern Taiwan who visited clinics for cervical cancer screening or for follow-up of cervical intraepithelial neoplasia (CIN) were enrolled consecutively between January 2008 and April 2008 by the Department of Gynecology, Chang Gung Memorial Hospital (CGMH) in Taoyung, Taiwan. The median age of participating women was 42 years old (ranging from 15 to 78 years old), and their demographic characteristics are shown in Table I. One cervical swab was collected using a cytobrush for Papanicolaou test and another swab was taken and transported in a storage medium (King Car, Yi-Lan, Taiwan) for HPV detection. Cytology was classified according to the Bethesda system and categorized as negative, atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC), a low-grade squamous intraepithelial lesion (LSIL) or a high-grade squamous intraepithelial lesion (HSIL) [Solomon, 1991; Solomon et al., 2002]. All the samples were analyzed independently by two pathologists. Institutional Review Boards of CGMH approved this study.

TABLE I. Demographic Characteristics of Study Population

Characteristics	Number (n = 343)	%
Age (15–78 years old)		
≤25 years	5	1.5
26–34 years	70	20.4
≥35 years	268	78.1
Education[a]		
Elementary school	33	17.1
Junior high school	28	14.5
Senior high school	57	29.5
College	75	38.9
Contraceptives		
Ever use intrauterine device		
Yes	4	1.2
No	339	98.8
Ever used oral contraceptive		
Yes	3	0.9
No	340	99.1
Tubal ligation		
Yes	17	5.0
No	326	95.0

[a]Among them, 150 educational degree data were not available.

DNA Extraction and PCR Conditions

QIAamp® DNA Blood Mini Kits (Qiagen, Valencia, CA) were used to extract cervical cell DNA according to the manufacturer's protocol. HPV type-specific primer sequences, conditions for PCR amplification and general precautions were detailed previously [Huang et al., 2006; Lin et al., 2008]. Briefly, 100 ng of purified DNA was used as template for each 50 μl PCR reaction run in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus, Emeryville, CA). The quality of the sample DNA was evaluated by amplification of a 136-bp fragment of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene. Consensus primers for SPF1/GP6(+) were used to amplify a fragment of approximately 184-bp within the L1 open reading frame of HPV. Each round of PCR was performed with positive and negative controls to confirm the test's accuracy. All participating investigators were double-blinded to the tested cervical specimens.

HPV Genotyping

The presence and genotype of HPV in all cervical swab samples were tested using the EasyChip HPV Blot kit (King Car, Yi-Lan, Taiwan). The details of the HPV blotting format and HPV typing procedures were described previously [Huang et al., 2004, 2006]. Briefly, oligonucleotide probes for 39 genotypes 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, 83 [MM7], 84 [MM8], LIAE5,

and MM4) were immobilized on a nylon membrane, which was used for reverse-blot hybridization and to detect HPV DNA in a single reaction. Fifteen microliters of the resultant amplicons were hybridized to the membrane and detected with a streptavidin-alkaline phosphatase conjugate and NBT/BCIP (5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium) substrate. After the blot was dried, HPV genotypes were determined using a standard visual assessment protocol as described previously [Huang et al., 2004, 2006].

Statistical Analysis

Samples deemed positive for high risk HPV (HR-HPV) were categorized as single or multiple infections. The data were analyzed by an SPSS 12.0 statistical package for Windows. Pearson's χ^2 test was used to evaluate the significance of differences between designated groups. All tests were two-sided, and a P-value <0.05 was considered statistically significant.

RESULTS

HPV Prevalence

Overall, HPV was detected in 32.4% (111/343) of the samples with the use of an EasyChip HPV Blot kit, and a total of 32 different HPV types were identified in this study population. The distribution of HPV genotypes among HPV positive samples is listed in Table II. The five most prevalent HPV genotypes were HPV 16 (5.8%), HPV 58 (5.3%), HPV 53 (4.1%), HPV 52 (3.8%), and HPV 18 (2.3%). Among women who tested positive for HPV, 68.5% (n = 76) were infected with a single type, and 31.5% (n = 35) were infected with multiple types of the virus. The distribution of genotypes in multiple-type HPV infections is listed in Table III.

HPV Genotype Distribution According to Cytology

Among the 343 participants, 284 samples were tested for HPV Blot and Pap smear simultaneously. The results of the valid cervical histological diagnoses and HPV status are presented in Table IV. Among them, 215 (75.7%) presented normal cytology while 69 (24.3%) revealed histologically confirmed cervical abnormalities. Positive HPV rates in normal and abnormal Pap smears were 20.9% (45/215) and 75.3% (52/69), respectively. Among the 111 HPV positive samples, 97 cases gave valid cytology data. Fifty-two cases (53.6%) presented with abnormal cytology, and their histological diagnoses were distributed as follows: 15 (28.8%) ASC-

TABLE II. Overall HPV Genotype-Specific Distribution in Descending Order of Prevalence

	No. of patients (%)		
	Total (N = 343)	Multiple type	Single type
HPV(−)	232 (67.6%)		
HPV(+)	111 (32.4%)		
HPV 16	20 (5.8%)	9 (45.0%)	11 (55.0%)
HPV 58	18 (5.3%)	6 (33.3%)	12 (66.7%)
HPV 53	14 (4.1%)	5 (35.7%)	9 (64.3%)
HPV 52	13 (3.8%)	8 (61.5%)	5 (38.5%)
HPV 18	8 (2.3%)	4 (50.0%)	4 (50.0%)
HPV 39	8 (2.3%)	7 (87.5%)	1 (12.5%)
HPV 31	7 (2.0%)	1 (14.3%)	6 (85.7%)
HPV 84 (MM8)	6 (1.7%)	3 (50.0%)	3 (50.0%)
HPV 6	5 (1.5%)	2 (40.0%)	3 (60.0%)
HPV 81 (CP8304)	5 (1.5%)	2 (40.0%)	3 (60.0%)
HPV 33	4 (1.2%)	4 (100%)	0 (0%)
HPV 54	4 (1.2%)	3 (75.0%)	1 (25.0%)
HPV 62	4 (1.2%)	2 (50.0%)	2 (50.0%)
HPV 68	4 (1.2%)	1 (25.0%)	3 (75.0%)
HPV 70	4 (1.2%)	3 (75.0%)	1 (25.0%)
HPV 35	3 (0.9%)	3 (100%)	0 (0%)
HPV 51	3 (0.9%)	2 (66.7%)	1 (33.3%)
HPV 56	3 (0.9%)	1 (33.3%)	2 (66.7%)
HPV 66	3 (0.9%)	1 (33.3%)	2 (66.7%)
HPV 11	2 (0.6%)	2 (100%)	0 (0%)
HPV 32	2 (0.6%)	1 (50.0%)	1 (50.0%)
HPV 45	2 (0.6%)	0 (0%)	2 (100%)
HPV 69	2 (0.6%)	1 (50.0%)	1 (50.0%)
HPV 71 (CP8061)	2 (0.6%)	1 (50.0%)	1 (50.0%)
HPV 82	2 (0.6%)	2 (100%)	0 (0%)
HPV 42	1 (0.3%)	0 (0%)	1 (100%)
HPV 43	1 (0.3%)	1 (100%)	0 (0%)
HPV 44	1 (0.3%)	0 (0%)	1 (100%)
HPV 55	1 (0.3%)	1 (100%)	0 (0%)
HPV 61	1 (0.3%)	1 (100%)	0 (0%)
HPV 67	1 (0.3%)	1 (100%)	0 (0%)
HPV 72	1 (0.3%)	1 (100%)	0 (0%)

TABLE III. Distribution of HPV Multiple Infections

Multiple HPV types (n = 35)	No. of patients
Double types	28
HPV 16/54, 16/53, 16/39, 16/18, 16/6, 16/33	2, 1, 1, 1, 1, 1
HPV 58/18, 58/39, 58/61	2, 2, 1
HPV 53/39	1
HPV 52/62, 52/70, 52/35, 52/51, 52/69, 52/55	1, 1, 1, 1, 1, 1
HPV 39/62, 39/43	1, 1
HPV 31/33	1
HPV 84(MM8)/81(CP8304)	1
HPV 6/11	1
HPV 33/35, 33/82	1, 1
HPV 54/35	1
HPV 70/71(CP8061)	1
Triple types	5
HPV 16/53/39	1
HPV 58/18/66	1
HPV 52/68/81(CP8304)	1
HPV 84(MM8/51/11)	1
HPV 70/82/72	1
Quadruple types	2
HPV 16/53/56/67	1
HPV 53/52/84(MM8)/32	1

US, 30 (57.7%) LSIL, 6 (11.5%) HSIL, and 1 (1.9%) was classified as AGC. The prevalence of high-risk (including probable high risk) and low-risk HPV genotypes with varying degrees of abnormal cytology is depicted in Table V. HPV positivity in cytological cervical samples was stratified according to the subsequent histological diagnosis. A strong association exists between an increasing severity of histological diagnosis and the presence of higher risk HPV genotypes. Furthermore, HR-HPV genotypes were substantially more frequent (79/97; 81.4%) than LR-HPV genotypes (18/97; 18.6%) in present test specimens ($P = 0.001$). Association of cervical cytology with the pattern of HPV infection is shown in Table VI. Cells given an abnormal histological designation (21/52; 40.4%) appeared to have a greater incidence of multiple infections than those of normal cytology samples (10/45; 22.2%); however, this association was not significant ($P = 0.056$). Single type HR-HPV infection was found in 53.8% of women with abnormal cytology, and LR-HPV infection was found in 5.7% in the same group.

DISCUSSION

Information about the distribution of HPV types among the general public is necessary for all societies. The present study provides information on the distribution of HPV genotypes in female residents of north Taiwanese who visited the clinic for routine HPV screening. It is important to consider the study population when interpreting HPV prevalence because infection type and frequency of HPV are different in distinct geographic areas [Clifford et al., 2005]. Aside from geographical differences, variation may also be the result of detection methods employed and varied specimen type and amount. Therefore, precise HPV identification heavily depends on the selection of a proper HPV genotyping method. Many investigators have shown that a PCR-based blot assay is much more reliable than other methods [van Doorn et al., 2002; van den Brule et al., 2002; Huang et al., 2004; Molijn et al., 2005]. While the FDA-approved Hybrid Capture II (HC II) HPV DNA test is the most widely used HPV DNA detection method, it does not have the capability to identify a specific HPV genotype. Furthermore, previous studies have shown that the HC II HPV test cross-reacts with at least 15 HPV genotypes not included in its current high-risk probe cocktail set [Poljak et al., 2002; Huang et al., 2006]. In this article, HPV genotypes were identified by using the EasyChip HPV Blot kit, which is not only comparable to HC II with regard to detection of HPV infection but is also able to identify 39 HPV genotypes in a single assay [van Doorn et al., 2002].

Various studies of HPV infection in Taiwanese women have reported overall positive rate ranging from 10.8% to 21% [Jeng et al., 2005; Sun et al., 2005; Lin et al., 2006; Chao et al., 2008], yet our results show that the overall prevalence, using the HPV Blot kit, is substantially higher (34.4%). Jeng et al. demonstrated that the overall HPV prevalence of Taiwanese women (age 21–65 years old) in metropolitan Taipei by using an HPVDNAChip (Biomedlab Co., Seoul, Korea) was 19.9%, and Lin et al. reported a hospital-based study in southern Taiwan (age 16–78 years old) that the HPV positive ratio, using semi-nested PCR test, was 19.3% (also see Supplementary Fig. 1). The reasons for a higher rate of HPV positivity in this study are unclear, but it may be attributable to socio-cultural factors or changes in the sexual habits of participants. Another possible explanation may be rooted in the particular assay method used. Previous studies have shown that HPV prevalence is higher in studies using a hybridization-based method compared with those using a PCR-based assay [de Sanjose et al., 2007].

Distribution of individual HPV genotypes varies across geographic areas and ethnic groups [Bosch et al., 1995; Huang et al., 1997; Walboomers et al., 1999; van Muyden et al., 1999; Sebbelov et al., 2000; Clifford et al., 2003; Munoz et al., 2003]. A meta-analysis

TABLE IV. HPV Positivity Rate in Cytological Results According to Pap Smears

Cytology	Number samples tested (n = 284)	HPV(+) (n = 97)	HPV(−) (n = 187)	*P*
Normal	215	45 (20.9%)	170 (79%)	<0.001
Abnormal	69	52 (75.3%)	17 (24.7%)	

TABLE V. Prevalence of HR-HPV and LR-HPV According to Histological Diagnosis

	Cytology (n = 97)						
		Abnormal (n = 52)					
HPV type	Normal (n = 45)	ASC-US (n = 15)	LSIL (n = 30)	HSIL (n = 6)	AGC (n = 1)	Total	P^a
HR-HPV 79 (81.4%)	30 (66.7%)	13 (86.7%)	29 (96.7%)	6 (100%)	1 (100%)	49 (94.2%)	0.001
LR-HPV 18 (18.6%)	15 (33.3%)	2 (13.3%)	1 (3.3%)	0 (0%)	0 (0%)	3 (5.8%)	

HR-HPV, high-risk HPV; LR-HPV, low-risk HPV; ASC-US, atypical squamous cells of uncertain significance; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions; AGC, atypical glandular cells.
[a]By Pearson's χ^2 test.

encompassing 85 HPV studies demonstrated that the most common strains worldwide were HPV Types 18 and 16. However, in Asia, HPV Types 58 and 52 were identified more frequently [Clifford et al., 2003]. Therefore, detailed information on the distribution of HPV genotypes within a region, particularly in Asia, is important for both primary cervical cancer screening and prophylactic vaccination policy decision making [van Muyden et al., 1999; Harper et al., 2006].

Data on the distribution of HPV genotypes in northern Taiwanese women remain controversial. Chao et al. [2008] showed that the three most frequently found HPV types were HPV 52, 18, and 58. Jeng et al. [2005] showed that the most common HPV types in Taipei women were 16, 18, and 58. It was found that the five most prevalent types were HPV 16, 58, 53, 52, and 18, which comprised about 65.8% of all HPV infections. In contrast, HPV 16/18 only comprised about 25.2% of all HPV infections. A possible explanation could be differences between studied populations. Interestingly, it was found that HPV 18 was a minor contributor (2.3%) to HPV infection. This is in agreement with previous reports which found that the prevalence of HPV 18 in the general population is low and variable depending on the population studied [Bernard et al., 2006; Trottier and Franco, 2006]. Another interesting finding was the relatively high prevalence of HPV 53, which was isolated in 14 patients, five of whom had multiple type infections. Collectively, these findings further emphasize the importance of HPV 58, 53 and 52 in the Taiwanese population and may have relevance for decision making on cervical cancer prevention programs in Taiwan.

In this study, 15 HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) were classified as high risk (HR), 3 HPV genotypes (26, 53, and 66) were categorized as probable high risk and 12 (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 91) were grouped as low-risk (LR) genotypes [Munoz et al., 2003; Clifford et al., 2005]. Many studies have been conducted that have focused on the distribution of HPV genotype in cervical cancer; however, few epidemiological studies have investigated the distribution of HR-HPV genotype in the general female Taiwanese population [Lai et al., 2007]. This study reported that the detection rate of HR-HPV (53.8%) was significantly higher than LR-HPV (5.7%) in women with abnormal cytology and that the overall prevalence of HPV infection (HR and LR) increased across all histological grades of disease (Tables V and VI). Of note, the overall prevalence of HR-HPV infection (single and multiple) was significantly greater in the histologically abnormal group (49/52; 94.2%) versus the normal group (30/45; 66.7%), reaching a level of 100% among subjects with histological diagnoses of HSIL. These results support the claim that the infection pattern of HPV genotypes is strongly related to the severity of cervical abnormalities, and the data confirm the clinical relevance of identifying specific HR-HPV genotypes.

Incorporating diagnoses of multiple HPV infections into the clinical management of cervical lesions and the prediction outcomes of HPV infection is an important issue that should be examined extensively. Several studies reported that the presence of multiple HR-HPV genotypes tends to increase with the severity of cervical

TABLE VI. Cervical Histological Diagnosis Versus Different HPV Infection Patterns

	HPV(+) (n = 97)							
	Single type			Multiple type				
Cytology	HR-HPV	LR-HPV	Total	HR-HPV (1)	LR-HPV (2)	Mix type (1) + (2)	Total	P^a
Normal (N = 45)	23 (51.1%)	12 (26.6%)	35 (77.8%)	4 (8.8%)	3 (6.6%)	3 (6.6%)	10 (22.2%)	0.056
Abnormal (N = 52)	28 (53.8%)	3 (5.7%)	31 (59.6%)	10 (19.2%)	1 (1.9%)	10 (19.2%)	21 (40.4%)	

HR-HPV, high-risk HPV; LR-HPV, low-risk HPV.
[a]By Pearson's χ^2 test.

disease [Morrison et al., 1991; Becker et al., 1994; Fife et al., 2001; Rousseau et al., 2003; Bello et al., 2009] and seems to act synergistically in cervical carcinogenesis [Trottier et al., 2006]. Globally, the prevalence of multiple HPV infections in HPV positive cases is variable, ranging from about 9% to 50% in European countries [Forslund et al., 2002; Matos et al., 2003]. Results from PCR-based detection methods suggest that mixed infections with multiple HPV types occur in 20–30% of infected women [Hildesheim et al., 1993; Wheeler et al., 1993]. In this study, multiple HPV infections, comprising between two and four HPV types, were detected in 10.2% of all subjects and 31.5% in HPV positive samples. Our results paralleled those of a previous study in which HPV multiple-type infections accounted for 35.8% of the infected group and 7.9% of the whole study population of Taiwanese women [Jeng et al., 2005].

From a prevention point of view, it is valuable to identify women at high risk for cervical cancer from the general population. Our study shows that 94.2% of abnormal cytology smears were HR-HPV positive. More importantly, 37.9% of specimens infected with HR-HPV were cytologically normal. The HPV Blot test can detect HPV infection and concomitantly provide genotype information. Therefore, results from the Blot test, in combination with analysis of Pap smear cytology, could be used in screening for early stage cervical lesions and help to identify high risk sub-clinical women who may require more frequent follow-up examinations.

In conclusion, this study adds valuable insight into the prevalence and distribution of specific HPV genotypes within northern Taiwanese women. Moreover, our results may provide essential information for determining the appropriate clinical management strategies for cervical cancer screening and cost-effective multivalent HPV vaccine policy in our country.

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