

## RESEARCH COMMUNICATION

# Type-specific Human Papillomavirus Distribution in Invasive Cervical Cancer in Korea, 1958-2004

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### Abstract

**Objective:** To describe the HPV genotype distribution and to investigate the underlying secular trend in the relative contribution of HPV types 16-18 in invasive cervical cancer (ICC) over a period of 47 years (1958-2004) in South Korea. **Methods:** Paraffin embedded ICC samples were obtained from historical archives of two hospitals in Korea. HPV detection and genotyping was performed by SPF<sub>10</sub> PCR, DEIA and LiPA<sub>25</sub> assays (version 1). **Results:** Of 874 ICC cases, 742 were considered suitable for HPV DNA testing after histological evaluation. Squamous cell carcinoma was the major histological type (93.0%). HPV was detected in 674 of the 742 specimens (90.8%). The five most common types identified as single types among HPV-positive cases were HPV16 (63.1%), HPV18 (8.5%), HPV33 (4.5%), HPV58 (3.9%) and HPV31 (3.0%). Multiple infections were detected in 5%. HPV16-18 together accounted for 72% of all HPV-positive cervical cancers with no statistically significant differences by time at diagnosis (adjusted model- $p>0.05$ ). **Conclusion:** This present study confirmed the role of HPV infection as the main factor in cervical cancer in Korea. HPV16-18 accounted for more than 70% in cervical cancer and there was no statistically significant secular trend for the past 50 years.

**Keywords:** Human Papillomavirus - cervical cancer - trends - Korea

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### Introduction

Even though a small reduction of the cervical cancer burden in Korea has been observed, the burden of disease remains high (Shin et al., 2008). In 2005, approximately 4,000 invasive cervical cancers (ICC) and 3,000 carcinomas in situ (CIS) were newly diagnosed and 1,000 women died from this cancer (Won et al., 2009). The age-standardized incidence rates of cervical cancer have been reduced from 19.0 per 100,000 women in 1993-1995 to 16.7 in 2003-2005. In relation to early detection of cervical cancer, a decrease in mortality was observed among women 30-69 years old (Shin et al., 2008) however elderly women over 70 years old showed increased incidence of cervical squamous cell carcinoma (SCC) (Jo et al., 2007).

Clinical and molecular epidemiological studies have clearly established that infections by certain human papillomaviruses (HPVs) types are causally linked to cervical cancer development (Bosch et al., 1995; zur Hausen, 1996; Munoz et al., 2004). 14 HPV types were significantly associated with development to invasive cervical cancer and called oncogenic or high-risk types (Munoz et al., 2003). A large multicentric case-control

study (Munoz et al., 2004) and meta-analyses of 130 studies in 6 continents (Clifford et al., 2003; Smith et al., 2007) showed that the most common HPV types in cervical cancer were HPV16 and 18, both accounting for 70% of all cervical cancer cases worldwide.

In Korea, although there are several reports on the HPV type distribution in cervical cancers, they have several limitations: many of them, are based on a small number of cases, only a few HPV types had been tested for, most of the assays used for the detection of HPV DNA had not been fully validated, and were mostly studied with samples collected in the last decade.

Two prophylactic HPV vaccines (Gardasil® (Merck & Co., Whitehouse Station, NJ USA) and Cervarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium)) have been introduced in Korea and administered in private sectors since 2007 (Konno et al., 2008). Both vaccines aim at preventing infection with HPV16 and 18, responsible for about 70% of cervical cancers. The future impact of both vaccines will in part depend on the past and future distribution of HPV types in cervical cancer. Assessing the distribution of HPV types in cervical cancer over the last few decades might be helpful to predict changes in

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the HPV type distribution.

To describe the HPV genotype distribution and to investigate the underlying secular trend of HPV type distribution in ICC in Korea, we conducted a study based on paraffin blocks from ICC cases diagnosed over the past 50 years, using a validated HPV DNA test. The present study is part of an ongoing project coordinated by the Institut Català d'Oncologia (ICO, Barcelona) (RIS HPV TT).

## Materials and Methods

### Study materials

Paraffin embedded tissue blocks of ICC were consecutively collected from 2 hospitals. 1,001 blocks from 785 cases diagnosed between 1958 and 1998 were collected from National Medical Center in Seoul, and from 89 cases diagnosed between 1999 and 2004 were collected from Dong-A University Hospital in Busan. Basic information (i.e., age, year of diagnosis, and histopathological diagnosis) was also collected from medical records.

### Pathology and laboratory procedures

**Paraffin block processing-** Blocks were re-embedded whenever necessary. At least four paraffin sections were obtained for each block (sandwich method). First and last sections were used for histopathological evaluation after Hematoxylin and Eosin (H&E) staining. The in-between sections of the blocks were kept in Eppendorf tubes for HPV DNA testing. Paraffin embedded blocks were processed under strict conditions to avoid potential contamination. A tissue-free paraffin block was cut after processing each study block to detect any HPV carry-over from block to block. For each block a new blade was used and the microtome was cleaned with HistoClear II and 70% alcohol.

To further control for possible sources of contamination

paraffin blocks containing non HPV related lesions processed at the same time as the cervical cancer specimens in the local pathology laboratory were blindly included in the process at a ratio of 5% of the total ICC cases. These specimens were labelled as controls.

### Histopathological evaluation

The paraffin blocks processing and the pathology reassessment of the histopathological diagnosis was done by the reference pathology laboratory for the study at ICO, and was performed following the consensus criteria established by an expert panel of pathologists. The pathology evaluation included: diagnosis of histological type (ICC: squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, other types; non-invasive cervical cancer; and control tissue specimen); presence of mucose or pre-neoplastic lesions adjacent to ICC (Cervical Intraepithelial Neoplasia-CIN1/2/3; Adenocarcinoma In Situ-AIS); degree of necrosis; amount of tumour infiltration; and adequacy of the sample for further HPV testing. A block was determined to be adequate for HPV testing if invasive cancer was observed in the two H&E stained sections of the specimen. In case of discrepancies between the local and the reference pathology laboratories, the results obtained at the reference lab prevailed.

### HPV DNA detection and typing

250µl of freshly Proteinase K solution was used to extract DNA. SPF<sub>10</sub> PCR was performed using 10µl of the DNA extract in a final reaction volume of 50µl. All samples were run with a 1:10 dilution. The amplified PCR products were tested using a probe hybridization with a cocktail of conservative probes recognizing, at least, 54 mucosal HPV genotypes in a microtiter plate format for the detection of HPV DNA. Optical densities (OD<sub>450</sub>) were read on a microtiter plate reader. HPV DNA positive samples were subsequently analysed by LiPA<sub>25</sub> (version 1, Labo Biomedical Products, Rijswijk, The Netherlands), a

**Table 1. Demographic and Histological Characteristics of Invasive Cervical Cancer Cases Tested for HPV DNA in South Korea**

	Number of ICC cases HPV analysed	% of cases	Number of HPV positive cases	Crude HPV prevalence % (95% CI)
Age (years)				
≤39	163	22.0	152	93.3 (88.2-96.6)
40-49	269	36.2	249	92.6 (88.8-95.4)
50-59	174	23.5	157	90.2 (84.8-94.2)
≥60	136	18.3	116	85.3 (78.2-90.8)
Histological type				
Squamous cell carcinoma	690	93.0	639	92.6 (90.4-94.4)
Adenocarcinoma	45	6.1	28	62.2 (46.5-76.2)
Adenosquamous	5	0.6	5	100.0 (47.8-100.0)
Other <sup>a</sup>	2	0.3	2	100.0 (15.8-100.0)
Date of diagnosis <sup>b</sup>				
1958-1969	212	28.6	194	91.5 (86.9-94.9)
1970-1979	156	21.0	144	92.3 (86.9-96.0)
1980-1989	156	21.0	142	91.0 (85.4-95.0)
1990-1998	130	17.5	114	87.7 (80.8-92.8)
1999-2004	88	11.9	80	90.9 (82.9-96.0)
Total	742	100.0	674	90.8 (88.5-92.8)

"ICC": Invasive Cervical Cancer; "95% CI": 95% Confidence Interval; a) Other histological diagnosis: 2 neuroendocrine carcinomas (small cell carcinomas); b) For the period 1958-1998 samples were from Seoul and for the period 1999-2004 from Busan.

reverse hybridization technique that detects 25 high-risk and low-risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74). The sequence variation within the SPF<sub>10</sub> primers allows the recognition of these different HPV genotypes, except for types 68 and 73, as their interprimer regions are identical and cannot be distinguished by this test. After PCR, 10µl of the amplimers were used to perform reverse hybridization for HPV genotype identification. Positive hybridization on the strips is visualized as a purple band by means of a precipitating colour substrate on the probe site. Specimens that were HPV DNA positive but did not hybridize with any of the 28 probes were coded as HPV type X (uncharacterized type). SPF<sub>10</sub> PCR DEIA and LiPA<sub>25</sub> assay (version 1) were performed at ICO and DDL Diagnosis Laboratory (The Netherlands).

*Statistical analysis*

Data analysis was performed with the Statistical Package STATA 10. Overall HPV positive and HPV type-specific proportions were determined. 95% Confidence Intervals (95% CIs) for proportions were estimated. To estimate the HPV type distribution, single and multiple HPV infections were considered either separately or combined. A stratified analysis of the overall HPV and type-specific relative contribution by age at diagnosis, year of diagnosis and histological characteristics was performed. Age and time at diagnosis were recoded as categorical variables (age: ≤39, 40-49, 50-59, and ≥60 years; and time: 1958-69, 1970-79, 1980-89, 1990-98, 1999-2004). Sources of variation of the most frequent HPV types (HPV16, HPV18 and other HPV types-non HPV16-18) were evaluated by unconditional logistic regression, including the following variables: age at diagnosis, time at diagnosis and histological type. Statistical significance for all analysis was set at the 2-sided 0.05 level.

*Ethical issues*

Specimens were received anonymously (without name and/or original medical record number) at the reference laboratory in Barcelona (ICO). All protocols were approved by international and ICO ethics committees and all the study progress was overseen by an international steering committee specifically formed for the supervision and advising in critical issues of the project.

**Results**

A total of 1,001 paraffin blocks from 874 women with cervical cancer were processed. In the blocks from 132 women the diagnosis of ICC could not be confirmed, leaving 742 cases that were tested for HPV DNA. HPV DNA was detected in 674 of 742 specimens (90.8%). Table 1 shows number of cases and the prevalence of HPV in cervical cancer by patient's age, histological type and time at diagnosis. 58.2% of the cases were diagnosed in women under 49 years of age. Squamous cell carcinoma was the major histological type (93.0%), and the others were 45 adenocarcinomas (ADC), 5 adenosquamous cell carcinomas and 2 small cell carcinomas. Over hundred cases were collected in each time period, except for 1999-

2004 with 88 cases.

Table 2 shows the type-specific HPV distribution of the invasive cervical cancers. Most common types (single types among HPV-positive cases) were HPV16 (63.1%), 18 (8.5%), 33 (4.5%), 58 (3.9%) and 31 (3.0%). 5% of cases (34/674) had multiple HPV infections and there were 19 type-unknown cases. Figure 1 shows the type-

**Table 2. HPV Type-specific Distribution in Invasive Cervical Cancer Cases from South Korea**

	HPV type-specific positive ICC cases n	HPV type-specific relative contribution % (95% CI)
Single types	621	92.1 (89.8-94.1)
HPV16	425	63.1 (59.3-66.7)
HPV18	57	8.5 (6.5-10.8)
HPV33	30	4.5 (3.0-6.3)
HPV58	26	3.9 (2.5-5.6)
HPV31	20	3.0 (1.8-4.5)
HPV45	19	2.8 (1.7-4.4)
HPV59	10	1.5 (0.7-2.7)
HPV35	7	1.0 (0.4-2.1)
HPV39	6	0.9 (0.3-1.9)
HPV52	6	0.9 (0.3-1.9)
HPV68or73	6	0.9 (0.3-1.9)
HPV56	4	0.6 (0.2-1.5)
HPV51	3	0.4 (0.1-1.3)
HPV6	1	0.1 (0.0-0.8)
HPV11	1	0.1 (0.0-0.8)
Multiple types	34	5.0 (3.5-7.0)
HPV31&33	4	0.6 (0.2-1.5)
HPV16&74	3	0.4 (0.1-1.3)
HPV16&18	2	0.3 (0.0-1.1)
HPV16&31	2	0.3 (0.0-1.1)
HPV16&33	2	0.3 (0.0-1.1)
HPV31&33&44	2	0.3 (0.0-1.1)
HPV11&16	1	0.1 (0.0-0.8)
HPV11&31	1	0.1 (0.0-0.8)
HPV11&45	1	0.1 (0.0-0.8)
HPV16&18&58	1	0.1 (0.0-0.8)
HPV16&52	1	0.1 (0.0-0.8)
HPV16&56	1	0.1 (0.0-0.8)
HPV16&68or73	1	0.1 (0.0-0.8)
HPV18&33&56	1	0.1 (0.0-0.8)
HPV18&52	1	0.1 (0.0-0.8)
HPV31&52	1	0.1 (0.0-0.8)
HPV31&58	1	0.1 (0.0-0.8)
HPV33&52	1	0.1 (0.0-0.8)
HPV45&52&70	1	0.1 (0.0-0.8)
HPV45&53&66&70	1	0.1 (0.0-0.8)
HPV52&56	1	0.1 (0.0-0.8)
HPV52&58	1	0.1 (0.0-0.8)
HPV52&66	1	0.1 (0.0-0.8)
HPV52&68or73	1	0.1 (0.0-0.8)
HPV53&56	1	0.1 (0.0-0.8)
Type X (Unknown)	19	2.8 (1.7-4.4)

Potential impact of HPV vaccines (HPV16/18)

HPV16, 18, 16&18	484	71.8 (68.2-75.2)
HPV16, 18, 16&18, 16&OT, 18&OT, 16&18&OT	498	73.9 (70.4-77.2)
Total HPV-positive ICC cases	674	100.0

ICC, Invasive Cervical Cancer; OT, other HPV types neither HPV16 nor HPV18; 95% CI, 95% Confidence Interval

**Table 3. Factors Associated to Detection of Hpv16, Hpv18 and Other Hpv Types (Non Hpv16-18) in Invasive Cervical Cancer Cases from South Korea**

	Total HPV positive cases	HPV16		HPV18		Other HPV types (non HPV16-18)	
		HPV16	Adjusted OR	HPV18	Adjusted OR	Other HPV	Adjusted OR
		% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
<b>Age (years)</b>							
≤39	145	73.8 (65.8-80.7)	2.9 (1.6-5.3)	7.6 (3.8-13.2)	0.7 (0.2-2.2)	11.0 (6.4-17.3)	0.4 (0.2-0.8)
40-49	232	61.6 (55.0-67.9)	1.7 (1.0-2.9)	12.5 (8.5-17.5)	1.2 (0.4-3.1)	18.1 (13.4-23.7)	0.7 (0.4-1.3)
50-59	133	63.9 (55.1-72.1)	1.7 (1.0-3.1)	5.3 (2.1-10.5)	0.6 (0.2-1.8)	25.6 (18.4-33.8)	1.0 (0.5-2.0)
≥60	84	47.6 (36.6-58.8)	1	9.5 (4.2-17.9)	1	27.4 (18.2-38.2)	1
p-value for linear trend			p<0.05		p>0.05		p<0.05
<b>Histological type</b>							
Squamous cell carcinoma	560	65.4 (61.3-69.3)	1	6.8 (4.8-9.2)	1	19.5 (16.3-23.0)	1
Adenocarcinoma	27	18.5 (6.3-38.1)	0.1 (0.1-0.3)	55.6 (35.3-74.5)	15.4 (6.7-35.8)	18.5 (6.3-38.1)	1.0 (0.4-2.7)
Other <sup>a</sup>	7	57.1 (18.4-90.1)	0.7 (0.2-3.4)	28.6 (3.7-71.0)	5.1 (0.9-28.6)	14.3 (0.4-57.9)	0.6 (0.1-5.2)
<b>Date of diagnosis</b>							
1958-1969	194	64.4 (57.3-71.2)	1.2 (0.7-2.1)	11.9 (7.7-17.3)	1.2 (0.5-3.1)	14.9 (10.2-20.8)	0.6 (0.3-1.2)
1970-1979	144	66.0 (57.6-73.7)	1.4 (0.8-2.4)	9.0 (4.9-14.9)	1.0 (0.4-2.7)	18.8 (12.7-26.1)	0.7 (0.4-1.3)
1980-1989	142	65.5 (57.1-73.3)	1.4 (0.8-2.3)	6.3 (2.9-11.7)	0.7 (0.3-2.0)	20.4 (14.1-28.0)	0.8 (0.4-1.5)
1990-1998	114	54.4 (44.8-63.7)	1	8.8 (4.3-15.5)	1	26.3 (18.5-35.4)	1
p-value for linear trend			p>0.05		p>0.05		p>0.05
Total	594	63.1 (59.1-67.0)		9.3 (7.1-11.9)		19.4 (16.3-22.8)	

Cases included in the analysis: HPV specific single types among HPV positive invasive cervical cancer cases and cases from Seoul (1958-1998); a) Other histological diagnosis: Adenosquamous; cell carcinoma and neuroendocrine tumours; “OR”: Odds Ratio; “95% CI”: 95% Confidence Interval

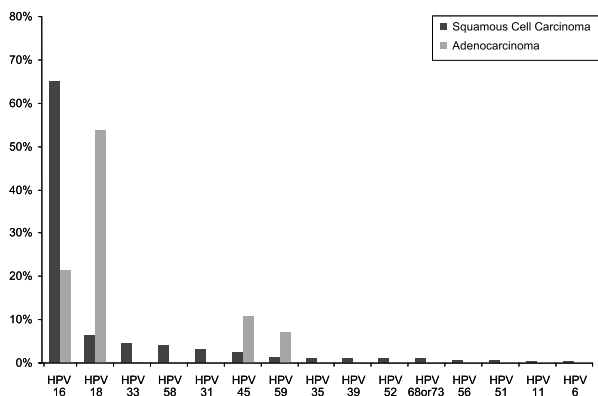
specific HPV distribution of the cervical cancer cases by histological diagnosis. HPV18 was the predominant type among ADCs (53.6%), whereas HPV16 was predominant among SCCs (64.9%).

Table 3 shows the analysis of the factors related to the HPV type detection. In order to exclude any possibility of regional variation, the analysis was run for the samples from Seoul and the period from 1958 to 1998. HPV16 and 18, as single types, accounted for 72% of all HPV-

positive cervical cancers. HPV18 was more frequent than HPV16 among ADCs (adjusted OR=15.4; 95% CI=6.7-35.8), conversely HPV16 was more frequently identified in SCCs (adjusted models: p<0.05). Regarding age at diagnosis, the prevalence of HPV16 significantly decreased with age, from 73.8% among women under 40 years of age to 47.6% among women 60 years or older and non HPV16-18 types increased from 11.0% among women under 40 years of age to 27.4% among women 60 years or older (adjusted models: p for trend<0.05). Finally, not statistically significant trends were observed in the HPV type detection over the studied period (p for linear trend >0.05).

**Discussion**

In the era of HPV vaccination, it is very important to know about the HPV type distribution and the underlying secular trend of it in cervical cancers. The reason is that one of the determinants of the impact of HPV vaccination in the prevention of cervical cancer is the attributable fraction related to HPV16 and 18, and it is unknown whether the current fraction of about 70% has been stable and will be maintained in the decades following



**Figure 1. HPV Type-specific Distribution in Invasive Cervical Cancer Cases by Histological Diagnosis**

**Table 4. Hpv Type-Specific Distribution in Invasive Cervical Cancers from 30 Korean Studies: A Pooled Analysis**

Reference	No. cases	SCC/ADC	Any HPV	16	18	31	33	35	39	45	51	52	56	58	59	66	68	Time at diagnosis	Method
Park et al., 2008	54	NA	54	11	4	1	3	2	2	0	1	2	0	8	0	1	1	2004-2006	HPV DNA Chip (MyGene)
Lee et al., 2007	160	133/27	133	83	30	1	5	4	2	6	1	2	1	9	1	1	0	Not described	HPV DNA Chip (MyGene)
Wui et al., 2006	26	24/2	26	14	5	1	2	0	0	0	0	0	0	6	0	0	0	2004-2005	HPV DNA Chip (MyGene)
Lee et al., 2006	38	34/4	32	20	5	1	6	0	0	1	0	0	1	2	0	0	0	1999-2000	HPV DNA Chip (MyGene)
Park et al., 2005	85	66/19	74	47	6	3	1	3	0	1	0	4	4	8	2	0	0	2002	HPV DNA Chip (Biomedlab)
Lin et al., 2005	67	47/20	38	26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Not described	HPV DNA Chip (Biomedlab)
Lee et al., 2005	53	NA	47	27	15	1	1	0	1	2	0	1	0	4	1	1	0	Not described	HPV DNA Chip (Biomedlab)
An et al., 2005	135	0/135	121	51	35	0	6	0	0	1	0	0	0	0	0	0	1	1997-2001	HPV DNA Chip (Biomedlab) or type-specific PCR
Suh et al., 2004	38	0/38	28	12	15	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1996-2001	Type-specific PCR
Park et al., 2004	62	55/7	59	47	9	1	2	2	2	0	0	2	2	6	0	0	1	2000-2002	HPV DNA Chip (Biomedlab)
Park et al., 2003	145	121/24	119	70	12	3	3	10	1	3	0	6	3	12	4	1	2	2001-2003	HPV DNA Chip (Biomedlab)
Lee et al., 2003	68	68/0	52	29	6	2	2	4	0	2	1	2	0	3	1	0	0	1992-1995	HPV DNA Chip (Biomedlab)
Kim et al., 2003	84	58/26	71	57	8	0	4	1	0	1	0	0	0	1	1	0	0	1998-2001	HPV DNA Chip (Biomedlab)
Hwang et al., 2003	72	NA	65	38	3	0	9	3	0	1	1	1	0	6	0	0	0	Not described	HPV DNA Chip (Biomedlab)
Cho et al., 2003	49	45/4	43	30	2	0	1	0	2	0	1	1	0	4	0	0	1	2000	HPV DNA Chip (Biomedlab)
An et al., 2003	50	44/6	48	32	8	0	0	0	NA	NA	NA	NA	NA	5	NA	NA	1	2001-2002	HPV DNA Chip (Biomedlab)
Kim et al., 2002	24	NA	20	4	0	NA	2	2	NA	NA	NA	4	NA	2	NA	NA	NA	2000-2001	HPV DNA Chip (Biomedlab)
Kwon et al., 2001	41	NA	37	17	18	0	1	NA	NA	NA	NA	1	NA	0	NA	NA	NA	Not described	Type-specific PCR
Kim et al., 2001	40	37/3	27	15	5	0	2	1	NA	NA	NA	2	NA	5	NA	NA	NA	Not described	Type-specific PCR
Na et al., 2000	70	70/0	53	25	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1995-1997	Hybrid Capture and type-specific PCR
Hwang et al., 1999	41	28/3	38	15	4	3	4	1	NA	NA	NA	2	NA	6	NA	NA	NA	Not described	Type-specific PCR
Ahn et al., 1999	35	35/0	29	27	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1995-1996	Type-specific PCR
Kim et al., 1997	51	NNA	33	29	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1994-1995	Type-specific PCR
Hong et al., 1997	26	26/0	19	10	3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1995	Not described
Park et al., 1996	30	29/1	23	23	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Not described	Southern blotting
Kim et al., 1995	30	NA	21	16	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Not described	Southern blot hybridization
Kang et al., 1995	52	46/6	40	37	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1985	Type-specific PCR
Sohn et al., 1994	57	49/8	41	32	9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1993-1994	Type-specific PCR
Park et al., 1994	45	39/6	42	34	5	2	2	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	1991-1992	Type-specific PCR
Kim et al., 1994	39	35/4	17	12	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Not described	Southern blot hybridization
SUMMARY			Any HPV	16	18	31	33	35	39	45	51	52	56	58	59	66	68		
Total (N)	1,767		1,450	889	250	19	57	33	10	19	5	30	11	87	10	4	7		
Prevalence (%) <sup>a</sup>	100.0		82.1	50.3	14.1	1.1	3.2	1.9	0.6	1.1	0.3	1.7	0.6	4.9	0.6	0.2	0.4		
Relative contribution (%) <sup>b</sup>			100.0	61.3	17.2	1.3	3.9	2.3	0.7	1.3	0.3	2.1	0.8	6.0	0.7	0.3	0.5		

“SCC”: Squamous cell carcinoma; “ADC”: Adenocarcinoma/adenosquamous cell carcinoma/others; “NA”: Not Available; a) Prevalence (among analysed cases; N=1,767); b) Relative contribution (among HPV-positive cases; N=1,450); Multiple infections are included in the HPV detection and type-specific calculations

the introduction of HPV vaccination. Some hints in the degree of stability of this fraction can be obtained by the assessment of time trends of HPV types in cervical cancer during the past decades. In the present study, HPV16 and 18 consistently accounted for 72% of HPV-positive cervical cancers, and no statistically significant changes were observed for the past 50 years in Korea.

The worldwide HPV type distribution in cervical cancer slightly varies among countries, but the dominant types are HPV16 and 18 accounting for 70% of all cervical cancers in almost every country (Clifford et al., 2003; Smith et al., 2007). In Korea, studies on the HPV type distribution in cervical cancers were reported since 1994. We reviewed 30 published studies up to December 2008 and summarized a pooled estimation of the HPV type distribution. Table 4 shows the list of studies, detected HPV types, times at diagnosis and typing methods (Ahn et al., 1999; An et al., 2003; 2005; Hong et al., 1997; Hwang, 1999; Hwang et al., 2003; Kang et al., 1995; Kim et al., 1997; 2001; 2002; 2003; Kim and Kim, 1995; Kim and Park, 1994; Kwon et al., 2001; Lee et al., 2003; 2005; 2006; 2007; Lin et al., 2005; Na and Choi, 2000; Park et al., 1994; 1996; 2003; 2004; 2005; Park and Koh, 2008; Sohn et al., 1994; Suh et al., 2004; Wui et al., 2006). Based on the pooled analysis, HPVs were detected in 82.1% of 1,767 cervical cancer cases. The most common HPV types among all invasive cervical cancers analyzed were HPV16 (50.3%) and HPV18 (14.1%). HPV16 and 18 were responsible for approximately 64.5% of cervical cancers, similar to the results from global meta-analyses (Clifford et al., 2003; Smith et al., 2007) and a Korean meta-analysis (Bae et al., 2008).

Very consistent findings can therefore be seen when putting these various reports together along with the present study. However, it is not possible to assess secular trends of type specific HPV prevalence in previous studies because most of the cases were collected in recent years.

We found no statistically significant secular trend in the prevalence of HPV16 and 18 in invasive cervical cancers in the present study. Regarding age at diagnosis, the prevalence of HPV 16 was higher in younger age group and a significant decreasing trend with age for HPV16-related cases was observed. Similar findings have been reported from the US (Wheeler et al., 2009). This may suggest that young women could be more severely affected by HPV16 and 18 (i.e., more prevalent and strongly persisting) than by other oncogenic types (Porrás et al., 2009), whereas cervical cancers in older women may develop from repeated infections with weaker oncogenic HPV types, and cumulative effects of cofactors (Moscicki et al., 2006).

HPV type-specific distribution was different according to histological type of cervical cancer. In the present study, HPV18 was significantly more prevalent in ADC. This result is consistent with other previous studies (Clifford et al., 2003; Smith et al., 2007). In SCC, the most predominant types HPV16 and 18, were followed by HPV33, 58, 31 and this distribution is different from that reported in other regions (HPV16, 18, 45, 31 and 33 in order) (Smith et al., 2007).

Our study has strengths and limitations. The samples

were collected from only two hospitals located in the southern and northern region of the Republic of Korea. Therefore it was hard to examine any regional variations. However, it is worthy to note that this is the largest study that investigates secular trends of HPV type specific prevalence in cervical cancer during the last 50 years.

In conclusion, our results confirmed the role of HPV infection as the main factor in cervical cancer in Korea. HPV16 and 18 accounted for 72% in cervical cancer and there was no statistically significant secular trend of HPV16 and HPV18 relative contributions for the past 50 years. The above allows to forecast that HPV vaccination has the potential to reduce 72% of cervical cancer in Korea provided that a high coverage of the susceptible population is achieved and that the protection conferred by the vaccine be long lasting. This is the largest study of its nature in Korea to date. The study run in a very high sensitivity PCR technique to test for HPV showing a very high HPV detection even when using paraffin blocks diagnosed many years before (this is particular the case for squamous cervical cancer). This study allows for a robust estimate of the stable contribution of the major 2 types HPV16 and 18 over time. The study shows that HPV52 and 56 were not common in the region as postulated by other authors.

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