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Original Article

Human Papillomavirus Prevalence and Genotype Distribution in Normal and ASCUS Specimens: Comparison of a Reverse Blot Hybridization Assay with a DNA Chip Test

Sunghyun Kim[1], In-soo Lee[2] and Dongsup Lee[2,†]

[1]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan 609-757, Korea
[2]Department of Clinical Laboratory Science, Hyejeon College, Hongsung, Chungnam 350-702, Korea

High-risk (HR) human papillomavirus (HPV) genotypes are strongly associated with cervical cancer, whereas other HPV genotypes are not. To identify the various HPV genotypes in clinical samples, we conducted HPV genotyping using a DNA chip test and reverse blot hybridization assay (REBA) in normal cytology samples and atypical squamous cells of undetermined significance (ASCUS) cytology samples. We also investigated the HPV infection rate and HPV genotype prevalence in women with normal cytology and ASCUS cytology. Liquid-based cytology preparations were used for the initial screening of 205 subjects with normal cytology and ASCUS cytology. The HPV infection rate was 49.8% when using the DNA chip assay and 61.0% when using the REBA test. In patients with normal cytology, the HR-HPV positive rate was 21.9% with the DNA chip assay and 43.9% with the REBA test. In contrast, 8.3% of patients with ASCUS were HR-HPV positive when using the DNA chip assay, and 13.6% were positive when tested with the REBA test. The infection rate of HR-HPV in the 40~50-year age group was significantly higher than that of the other age groups. Based on the cytological analysis of the normal and ASCUS samples, the five most prominent HPV genotypes were HPV 16, 18, 68, 33, and 58 using the DNA chip test, and they were HPV 16, 18, 53, 33, and 66 when using the REBA test. In conclusion, the findings show that the results of the REBA test are comparable to those of the DNA chip test. Most strikingly, the REBA test detected the HR-HPV genotype associated with cervical carcinoma similar to that detected with the DNA chip method. Therefore, the REBA test is a useful method to detect clinically important HR-HPV genotypes.

Key Words: Human papillomavirus, Uterine cervix cancer, DNA chip, Reverse blot hybridization assay

INTRODUCTION

Human uterine cervical cancer is the third most common cancer among women worldwide (Torre et al., 2015; de Sanjosé et al., 2007; Bosch et al., 2005). Every year, approximately, 500,000 new patients are diagnosed, and 270,000 women die from cervical cancer worldwide each year. Cervical cancer-related mortality is the third highest after breast cancer and lung cancer (Pisani et al., 1990; Kim et al., 2001). According to the Korean National Cancer Center, in 2003-2005, there was an average of 132,941 cancer patients per year. Of these, with the exception of carcinoma in situ, the crude incidence rate of cervical cancer was 4% (4,035/100,000 cases), making it the sixth most common cancer after breast cancer, thyroid cancer, stomach cancer, colon cancer, and lung cancer.

Uterine cervical cancer is only weakly associated with

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†Corresponding author: Dongsup Lee. Department of Clinical Laboratory Science, Hyejeon College, Hongsung, Chungnam 350-702, Korea.
Tel: +82-41-630-5342, Fax: +82-41-630-5364
e-mail: dslee@hj.ac.kr
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genetic disposition, and acquired factors are considered the main contributing factors (Vizcaino et al., 1998). Uterine cervical cancer begins with a precancerous stage and progresses to invasive carcinoma, with human papillomavirus (HPV) infection being the main etiologic cause (zur Hausen, 2000). In particular, high-risk (HR) HPV genotypes are closely associated with the development of cervical cancer (Gaarenstroom et al., 1994). Certain HPV genotypes cause invasive cervical carcinoma or squamous intraepithelial lesions upon infection of cervical epithelial cells (Cattani et al., 2009). Much is known about the mechanisms by which HPV causes cervical cancer. The HPV E6/E7 oncoprotein induces degradation and inactivation of the tumor suppressor protein p53 and retinoblastoma protein (pRb) resulting in deregulation of the cell cycle checkpoints (Irene et al., 2006; Yugawa et al., 2009). This results in immortalization of the uterine cervix squamous epithelium and inhibition of apoptosis (Dyson et al., 1989; Scheffner et al., 1990).

HPV is classified according to DNA sequence similarity. Each species is further subdivided into HPV genotypes based on genetic homology: <90% genetic homology, subtypes with 90~98% genetic homology, and variants that share >98% of their genomes (Yugawa et al., 2009). Most HPV infections are transient, with 70% resolving within 1 year and 90% resolving within 2 years (Ylitalo et al., 2000; Zur Hausen et al., 2002). Fourteen HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) cause persistent infections, which subsequently lead to cervical cancer (Agarossi et al., 2009).

The incidence of uterine cervical cancer has begun to decline since the implementation of periodic cancer screening programs, which detect cervix dysplasia and in situ carcinoma. The cervix Papanicolaou smear (Pap. smear) test has been important in decreasing the cervical cancer incidence rate and accompanying mortality (Sung et al., 2009). The Pap smear is a widely cervical screening test due to its low cost and simple procedure. However, its main drawback is its false-negative error rate of 20~50%. In addition, previous reports have questioned its sensitivity for detecting uterine cervix high-grade squamous intraepithelial lesions and its predictive value (Vizcaino et al., 1998). The progression of a precancerous lesion to invasive carcinoma in uterine cervical cancer occurs slowly. Therefore, early detection is vital. The HPV DNA test can be used with the Pap smear to detect HR-HPV groups. Several PCR-based multiplex detection methodologies have been shown to be effective in detecting HPV infections. Clinicians and researchers have used these assays.

In this retrospective study, we analyzed the HPV infection rate and the prevalence of normal cervical exfoliative cells (i.e., within normal limits, negative for intraepithelial lesions or malignancy, and negative for reactive cellular changes associated with inflammation) and ASCUS (atypical squamous cells of undetermined significance)-positive cervical exfoliative cells collected from 205 women. These samples were previously assessed by Pap smears. We compared the utility of the DNA chip assay and reverse blot hybridization assay (REBA) as a molecular diagnostic tool for assessing malignant tumors. We also assessed the HPV infection rate and genotypes in the infected samples.

MATERIALS AND METHODS

Clinical samples

Liquid-based cytology samples were obtained with a ThinPrep Pap test (Hologic Inc., Acton, MA, USA) from 205 women between the ages of 22 and 85. The clinical samples were archived in the Department of Pathology, Yonsei University Wonju Severance Christian Hospital between September 2010 and July 2011. All the subjects included in this study were normal, inflammation, and ASCUS, except dysplasia, carcinoma in situ (CIS), and squamous cell carcinoma (SCC). This study was approved by the Institutional Ethics Committee at Yonsei University Wonju College of Medicine (approval no. YWMR-12-4-010). All subjects provided written informed consent.

ThinPrep preparation

Thin-layer slides were prepared using the ThinPrep 5,000 Processor (Product Insight Inc., Acton, MA, USA) according to the manufacturer's instructions. The prepared slides were stained by the Papanicolaou method and evaluated according to the 2001 Bethesda System by cytopathologists and pathologists.

DNA extraction

Clinical specimens were collected and vortexed for 1 min. The volume was adjusted to 40 μL with PBS (pH 7.2) and centrifuged at 8,000×g for 5 min. The supernatant was discarded, and 300~500 μL of washing buffer were added to the pellet. DNA extraction was performed using a QIAamp DNA Mini Kit (Qiagen, Amsterdam, Netherlands) according to the manufacturer's instructions.

HPV DNA chip test

HPV genotyping was carried out with the GG HPV DNA Genotyping Chip Kit (Goodgene, Seoul, Republic of Korea) according to the manufacturer's recommendations. This assay is able to detect the amplicons of 15 HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 69) and seven LR-HPV genotypes (HPV 6, 11, 34, 40, 42, 43, and 44). The HPV genotyping method requires nested-PCR to amplify the target region using MY11/MY9 primers, followed by a P5/GP6 primer pair. The nested-PCR conditions consisted of an initial denaturation step for 5 min at 94℃, followed by 15 cycles consisting of denaturation for 30 sec at 94℃ and extension for 30 sec at 65℃. These cycles were followed by another 45 cycles consisting of 30 sec at 94℃ and 30 sec at 54℃. The final extension step occurred at 72℃ for 7 min. After PCR amplification of the target region, subsequent steps were performed according to the manufacturer's recommendation. The PCR products were loaded onto a probe-labeled glass Goodgene HPV Chip, and the resulting signal was read using a scanner (Axon Instruments, Foster City, CA, USA).

Reverse blot hybridization assay (REBA)

The REBA HPV-ID® (YD Diagnostics, Yongin, Republic of Korea) was carried out according to the manufacturer's recommendations. This assay is able to detect the amplicons of 15 HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 66, and [59/68]) and 10 LR-HPV genotypes (HPV 6, 11, 42, 43, 44, 70, 72, 84, and [81/87]). This method requires nested-PCR to amplify the target region using MY11/MY9 primers, followed by a GP5/GP6 primer pair. The nested-PCR conditions consisted of an initial denaturation step for 5 min at 94℃, followed by 15 cycles comprising denaturation for 30 sec at 94℃ and extension for 30 sec at 65℃. The subsequent 45 cycles were 30 sec at 94℃, and 30 sec at 54℃. The final extension step was performed at 72℃ for 7 min. After PCR amplification of the target region, the following steps were performed according to the manufacturer's recommendations. For the REBA HPV-ID®, PCR products were added to the genotype-specific probe labeled membrane strip, leftover PCR products were washed, and then the colorimetric signals from the hybridized PCR products were generated and observed without using a scanner (Axon Instruments, Foster City, CA, USA).

RESULTS

Total HPV infection rate

A total of 154 (75.1%) normal and 51 (24.9%) ASCUS samples were used in this study. The samples were previously evaluated by liquid-based cytology. The total HPV infection rate with the DNA chip assay was 49.8% and 61.0% with the REBA test, irrespective of the liquid-based cytology diagnosis. The positivity of the REBA test was 11.2% higher than that of the DNA chip assay. Of the HPV-positive samples, the HR-HPV infection rate was 30.2% with the DNA chip assay and 57.6% with the REBA test, irrespective of multiple infections (Table 1). The LR-HPV infection rate was 7.3% and 13.2% with the DNA chip assay and REBA test, respectively.

HPV infection rate according to the subject's age

We assessed the infection rate of five age groups (Table 2). Of the samples diagnosed as normal or ASCUS, 10 (4.9%) were within the 20~29-year group, 42 (20.5%) were within the 30~39 year group, 73 (35.6%) were within the 40~49 year group, 59 (28.8%) were within the 50~59 year group, and 21 (10.2%) were within the >60-year group. We evaluated the infection rate in each group using the DNA chip assay and the REBA test. The HPV infection rate within each age group using the DNA chip assay and the REBA test was as follows: 2.0% versus 2.4% (20~29 years), 9.8%

Table 1. Total HPV infection rate and HR-HPV infection rate according to the DNA chip test and REBA

	DNA chip, *n* (%)		REBA, *n* (%)	
Total HPV infection	Negative	Positive	Negative	Positive
	103 (50.2%)	102 (49.8%)	80 (39.0%)	125 (61.0%)
HR-HPV infection*	62 (30.2%)		118 (57.6%)	

*HR-HPV; high-risk HPV

Table 2. HPV prevalence according to age

Age (*n*, %)	DNA chip		REBA	
	HPV negative	HPV positive	HPV negative	HPV positive
Total (205, 100%)	103 (50.2%)	102 (49.8%)	80 (39.0%)	125 (61.0%)
20~29 (10, 4.9%)	6 (2.9%)	4 (2.0%)	5 (2.4%)	5 (2.4%)
30~39 (42, 20.5%)	22 (10.7%)	20 (9.8%)	19 (9.3%)	23 (11.2%)
40~49 (73, 35.6%)	38 (18.5%)	35 (17.1%)	29 (14.1%)	44 (21.5%)
50~59 (59, 28.8%)	28 (13.7%)	31 (15.1%)	21 (10.2%)	38 (18.5%)
<60 (21, 10.2%)	9 (4.4%)	12 (5.9%)	7 (3.4%)	14 (6.8%)

Table 3. HR-HPV infectivity according to age

Age group	DNA chip	REBA
	HR-HPV infection (*n*=62) *n* (%)	HR-HPV infection (*n*=118) *n* (%)
20~29	4 (6.5%)	5 (4.2%)
30~39	11 (17.7%)	21 (17.8%)
40~49	19 (30.6%)	40 (33.9%)
50~59	22 (35.5%)	38 (32.2%)
<60	6 (9.1%)	14 (11.9%)

versus 11.2% (30~39 years), 17.1% versus 21.5% (40~49 years), 15.1% versus 18.5% (50~59 years), and 5.9% versus 6.8% (>60 years). In all the age groups, the analytical sensitivity of the REBA test was higher than that of the DNA chip assay. In all the age groups, 62 samples were diagnosed as HR-HPV with the DNA chip test and 118 samples were diagnosed as HR-HPV with the REBA test (Table 3). The total HR-HPV positive rate was higher with the REBA test than with the DNA chip assay, but the positivity rate of the two assays was similar within each age group.

HR-HPV infection rate in single infections and multiple infections

To compare the infection rate between single and multiple infections, normal and ASCUS liquid-based cytology samples diagnosed according to the Bethesda System were used (Table 4). The infection rate of HR-HPV with the DNA chip and REBA test was 30.3% and 57.6%, respectively. The HPV single infection rate was 21.5% with the DNA chip assay and 29.3% with the REBA test, and the HPV multiple infection rate was 8.8% and 28.3% with the DNA chip assay and REBA test, respectively. The positivity of the REBA test was higher than that of the DNA chip assay in both HPV single and multiple infection cases. The positivity of the REBA test was 19.5% points higher in HPV multiple infection cases than the DNA chip assay. The HPV single infection rate of normal cytology samples was higher with both the DNA chip and REBA tests, and the HPV single and multiple infection rates were the same in the ASCUS cytology samples.

HPV infection rate in normal and ASCUS samples

We evaluated the total HPV infection rate in the normal/

Table 4. HR-HPV single and multiple infection rates according to the DNA chip test and REBA (n=205)

HR-HPV infection*	DNA chip		REBA	
	Single infection n (%)	Multiple infection** n (%)	Single infection n (%)	Multiple infection** n (%)
Total	44 (21.5%)	18 (8.8%)	60 (29.3%)	58 (28.3%)
Normal	31 (15.1%)	14 (6.8%)	46 (22.4%)	44 (21.5%)
ASCUS	13 (6.3%)	4 (2.0%)	14 (6.8%)	14 (6.8%)

*HR-HPV infection: high-risk HPV infection (only high-risk samples)
**multiple infection; infected with above two HPV genotypes (only high-risk samples)

Table 5. Infection rates of HPV tested with the DNA chip and REBA in samples diagnosed by cytology

DNA chip (n=205)								
Cytological diagnosis	n (%)	HPV positive (%)	HPV infection patterns (%)					
			H-S	H-M	H and L-M	L-S	L-M	Others
Normal	154 (75.1%)	70 (34.1%)	26 (12.7%)	13 (6.3%)	6 (2.9%)	5 (2.4%)	0 (0%)	20 (9.8%)
ASCUS	51 (24.9%)	32 (15.6%)	11 (5.4%)	4 (2.0%)	2 (1.0%)	2 (1.0%)	0 (0%)	13 (6.3%)
Total	205 (100%)	102 (49.8%)	37 (18.0%)	17 (8.3%)	8 (3.9%)	7 (3.4%)	0 (0%)	33 (16.1%)

REBA (n=205)								
Cytological diagnosis	n (%)	HPV positive (%)	HPV infection patterns (%)					
			H-S	H-M	H and L-M	L-S	L-M	Others
Normal	154 (75.1%)	96 (46.8%)	38 (18.5%)	35 (17.1%)	17 (8.3%)	4 (2.0%)	0 (0%)	2 (1.0%)
ASCUS	51 (24.9%)	29 (14.1%)	13 (6.3%)	10 (4.9%)	5 (2.4%)	1 (0.5%)	0 (0%)	0 (0%)
Total	205 (100%)	125 (61.0%)	51 (24.9%)	45 (22.0%)	22 (10.7%)	5 (2.4%)	0 (0%)	2 (1.0%)

H-S; high-risk HPV single infection, H-M; high-risk HPV multiple infection, H&L-L; high- and low-risk HPV multiple infection, L-S; low-risk HPV single infection, L-M; low-risk HPV multiple infection, other; other HPV genotype infection.

inflammation and ASCUS group according to HR- and LR- and other HPV genotypes associated with infection (Table 5). The total HPV infection rate was 49.8% and 61.0% with the DNA chip and REBA tests, respectively. In the normal group, the HPV infection rate with the DNA chip and REBA tests was 34.1% and 46.8%, respectively. In the ASCUS group, the HPV infection rate was 15.6% with the DNA chip assay and 14.1% with the REBA test. According to the DNA chip assay, excluding the other HPV genotype infections, the cytological test in the normal and ASCUS group revealed HR-HPV single infection rates of 12.7% and 5.4%, respectively. The rate of HR-HPV multiple infections was high in the HR-HPV infection group. According to the REBA test, excluding the other HPV genotype infections, the rate of HR-HPV single infections was 18.5% and 14.4% in the normal and ASCUS groups, respectively. HR-HPV infections were predominant in the normal and ASCUS groups in cases of multiple infections.

HPV prevalence and genotype distribution in the cytological test

We next evaluated the HPV prevalence and genotype distribution in the normal limit and inflammation group as assessed by the liquid-based cytology test (Table 6). In multiple infections, several HPV genotypes were observed in a single individual, and therefore the total infection was

Table 6. Genotype-specific HPV infection rate in women with normal and ASCUS cytology according to the DNA chip test and REBA

	Genotypes	DNA chip			REBA		
		Normal	ASCUS	Total genotype infection	Normal	ASCUS	Total genotype infection
High-risk	16	20 (15.3%)	6 (4.6%)	26 (19.8%)	54 (22.9%)	14 (5.9%)	68 (28.6%)
	18	16 (12.2%)	3 (2.3%)	19 (14.5%)	53 (22.3%)	13 (5.5%)	66 (27.7%)
	31	0 (0%)	0 (0%)	0 (0%)	3 (1.3%)	0 (0%)	3 (1.3%)
	33	6 (4.6%)	1 (0.8%)	7 (5.3%)	12 (5.0%)	7 (2.9%)	19 (8.0%)
	35	2 (1.5%)	1 (0.8%)	3 (2.3%)	2 (0.8%)	0 (0%)	2 (0.8%)
	39	1 (0.8%)	2 (1.5%)	3 (2.3%)	0 (0%)	2 (0.8%)	2 (0.8%)
	45	1 (0.8%)	1 (0.8%)	2 (1.5%)	1 (0.4%)	3 (1.3%)	4 (1.7%)
	51	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	0 (0%)	1 (0.4%)
	52	0 (0%)	1 (0.8%)	1 (0.8%)	3 (1.3%)	3 (1.3%)	6 (2.5%)
	53	−	−	−	16 (6.7%)	4 (1.7%)	20 (8.4%)
	56	2 (1.5%)	2 (1.5%)	4 (3.1%)	1 (0.4%)	0 (0%)	1 (0.4%)
	58	6 (4.6%)	1 (0.8%)	7 (5.3%)	4 (1.7%)	0 (0%)	4 (1.7%)
	66	1 (0.8%)	2 (1.5%)	3 (2.3%)	8 (3.4%)	1 (0.4%)	9 (3.8%)
	68	7 (5.3%)	1 (0.8%)	8 (6.1%)	1 (0.4%)	0 (0%)	1 (0.4%)
Low-risk	6	2 (1.5%)	0 (0%)	2 (1.5%)	0 (0%)	0 (0%)	0 (0%)
	11	2 (1.5%)	0 (0%)	2 (1.5%)	1 (0.4%)	0 (0%)	1 (0.4%)
	32	−	−	−	1 (0.4%)	0 (0%)	1 (0.4%)
	40	4 (3.1%)	3 (2.3%)	7 (5.3%)	2 (0.8%)	0 (0%)	2 (0.8%)
	42	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	0 (0%)	1 (0.4%)
	43	2 (1.5%)	0 (0%)	2 (1.5%)	1 (0.4%)	1 (0.4%)	2 (0.8%)
	44	1 (0.8%)	1 (0.8%)	2 (1.5%)	0 (0%)	1 (0.4%)	1 (0.4%)
	54	−	−	−	4 (1.7%)	1 (0.4%)	5 (2.1%)
	70	−	−	−	1 (0.4%)	0 (0%)	1 (0.4%)
	72	−	−	−	1 (0.4%)	0 (0%)	1 (0.4%)
	81/87	−	−	−	4 (1.7%)	0 (0%)	4 (1.7%)
	84	−	−	−	7 (2.9%)	4 (1.7%)	11 (4.6%)
Other HPV genotype		20 (15.3%)	13 (9.9%)	33 (25.2%)	2 (0.8%)	0 (0%)	2 (0.8%)
Total		93 (71.0%)	38 (29.0%)	131 (100%)	184 (77.3%)	54 (22.7%)	238 (100%)

shown. According to the DNA chip test, the total number of infections was 131, whereas there were 238 total infections according to the REBA test. These results indicate that the REBA test was able to detect more HPV genotypes. In the normal samples, HPV 16 was detected in 20 cases (15.3%) by the DNA chip test and in 54 (22.9%) cases by the REBA test. In the ASCUS samples, HPV 16 was detected in 6 (4.6%) cases by the DNA chip assay and 14 (5.9%) cases by the REBA test. These results suggest that the sensitivity of the assays is different. With the DNA chip test, the HPV infection rate was 71.0% and 29.0% in the normal and ASCUS groups, respectively. With the REBA test, the HPV infection rate was 77.3% and 22.7% in the normal and ASCUS groups, respectively. In the most frequently infected HR-HPV 16 group, the infection rate as determined by the DNA chip assay and the REBA test was 19.8% and 28.6%, respectively. Of the most common HR-HPV genotypes, the DNA chip test detected HPV 16, 18, 68, 33, and 58, whereas the REBA detected HPV 16, 18, 53, 33, and 66.

DISCUSSION

Uterine cervical cancer progresses from a precancerous lesion for 5~20 years and is limited to abnormal cancer cells of the cervix. The mechanism underlying uterine cervical cancer is not known. Therapy is effective when the disease is detected during the precancerous lesion or early invasive cancer stage. However, once the disease has progressed to the metastatic cervical cancer stage, therapeutic options are often limited. Since the introduction of cervical liquid-based cytology as a screening test in the 1940s, the incidence of cervical cancer and consequent mortality has dropped significantly. However, high false-negative results have been problematic (Gaarenstroom et al., 1994). Thus, a novel diagnostic method with higher sensitivity and specificity is needed. Currently, the Hybrid-Capture II test is most commonly used, but this method can detect only HR- and LR-HPV genotypes. Peyton et al. (1998) pointed out that cross-reactivity among probes resulted in nonspecific reactions. In the Republic of Korea, HPV genotype tests are still considered as a special test and only used to supplement the traditional Pap smears (Clifford et al., 2003).

According to a report by the Korea National Cancer Center (2008), the HPV infection rate was 29.2% in the 40~49-year group, 20.0% in the 50~59-year group, and 18% in the >60-year group. The most common therapy for cervical cancer is the removal of HPV-infected tissues, but it may not be possible to remove all the tissue. HPV infections are recurrent and have a long incubation period. In the current study, we used a DNA chip and a PCR-REBA in normal and ASCUS samples and evaluated the HPV infection rate, prevalence, and genotype distribution. In addition, we examined the correlation between the infection rate of HR-HPV and cervical cancer in a normal and ASCUS group previously evaluated with the liquid-based cytology test. Both methods identified the HPV genotypes in both single and multiple HPV infection samples. In previous studies, multiple infections, as well as age and HR-HPV genotypes, contributed to persistent infection. Persistent HPV infection is a contributing factor to cervical dysplasia and progression to precancerous lesions (Ho et al., 1995). The results of our study suggest that both the DNA chip and REBA tests can discriminate multiple HPV infections in both normal and ASCUS samples and that they are useful diagnostic tools to screen potential cervical cancer patients.

The HPV infection rate was 49.8% using the DNA chip assay and 61.0% using the REBA test, showing that the latter has higher sensitivity. The HR-HPV infection rate of single and multiple HPV infections was 30.2% and 57.6% with the DNA chip assay and REBA test, respectively. This indicates that the REBA test is much more sensitive than the DNA chip assay in detecting HR-HPVs (Table 1, 5). The HPV infection detection rate was higher using the REBA test than the DNA chip test in all age groups (Table 3). We propose that HR-HPV infection and age are correlated with persistent HPV infection. In this situation, a follow-up test would be immediately performed for the early detection of dysplasia and prevention of the progression to uterine cervical cancer.

The prevalence and genotype distribution of HR-HPV genotypes was HPV 16, 18, 68, 33, and 58 according to the DNA chip test and HPV 16, 18, 53, 33, and 66 according to the REBA test (Table 6). These results are different from those of a recent study by Sung et al. (Sung et al., 2009). In the present study, the five most common HPV genotypes were HPV 16, 58, 18, 52, and 53, and the global HPV prevalence was HPV 16, 18, 45, 31, and 33 (Agarossi et al., 2009).

The aim of this study was to evaluate the HPV infection rate of normal (within normal limits, negative for intraepithelial lesions or malignancy, and negative for reactive cellular changes associated with inflammation) and ASCUS samples. We suggest that the results from this study will shorten the follow-up tests to prevent oncogenic progression. This study shows that the REBA test can be used to determine HPV genotypes and that the sensitivity of this assay is higher than that of the DNA chip test. However, the increased sensitivity of HPV DNA detection assays, such as the DNA chip and REBA tests, can also be limited by specificity and accuracy. Methods that can detect HPV E6/E7 oncogenes and cancer cell biomarkers need to be developed.

REFERENCES

Agarossi A, Ferrazzi E, Parazzini F, Perno CF, Ghisoni L. Prevalence and type distribution of high-risk human papillomavirus infection in women undergoing voluntary cervical cancer screening in Italy. J Med Virol. 2009. 81: 529-535.

Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) study group. J Natl Cancer Inst. 1995. 87: 796-802.

Cattani P, Zannoni GF, Ricci C, D'Onghia S, Trivellizzi IN, Di Franco A, Vellone VG, Durante M, Fadda G, Scambia G, Capelli G, De Vincenzo R. Clinical performance of human papillomavirus E6 and E7 mRNA testing for high-grade lesions of the cervix. J Clin Microbiol. 2009. 47: 3895-3901.

Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer. 2003. 88: 63-73.

de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch FX. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007. 7: 453-459.

Dyson N, Howley PM, Münger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science. 1989. 243: 934-937.

Gaarenstroom KN, Melkert P, Walboomers JM, Van Den Brule AJ, Van Bommel PF, Meyer CJ, Voorhorst FJ, Kenemans P, Helmerhorst TJ. Human papillomavirus DNA and genotypes: prognostic factors for progression of cervical intraepithelial neoplasia. Int J Gynecol Cancer. 1994. 4: 73-78.

Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, Basu J, Tachezy R, Lewis R, Romney S. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst. 1995. 87: 1365-1371.

Hong SR, Kim IS, Kim DW, Kim MJ, Kim AR, Kim YO, Kim HS, Rha SH, Park GS, Park YK, Park YS, Park HS, Suh KS, Sohn JH, Shin MK, Oh HK, Yun KJ, Yoon HK, Lee SN, Lee AW, Lee HJ, Cho HY, Choi C, Jung WW. Prevalence and genotype distribution of cervical human papillomavirus DNA in Korean women: a multicenter study. Korean J Pathol. 2009. 43: 342-350.

Kraus I, Molden T, Holm R, Lie AK, Karlsen F, Kristensen GB, Skomedal H. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. J Clin Microbiol. 2006. 44: 1310-1317.

Kim YS, Lee HJ, Lee GG, Park SY, Lee GE, Suh JT, Huh JY. Detection of human papillomavirus type 16 and 18 in cervical specimens by nested polymerase chain reaction. Korean J Clin Pathol. 2001. 21: 210-214.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003. 348: 518-527.

Peyton CL, Schiffman M, Lörincz AT, Hunt WC, Mielzynska I, Bratti C, Eaton S, Hildesheim A, Morera LA, Rodriguez AC, Herrero R, Sherman ME, Wheeler CM. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. J Clin Microbiol. 1998. 36: 3248-3254.

Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer. 1999. 83: 870-873.

Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell. 1990. 63: 1129-1236.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015. E-pub.

Vizcaino AP, Moreno V, Bosch FX, Muñoz N, Barros-Dios XM, Parkin DM. International trends in the incidence of cervical cancer: I. Adenocarcinoma and adenosquamous cell carcinomas. Int J Cancer. 1998. 75: 536-545.

Ylitalo N, Sørensen P, Josefsson AM, Magnusson PK, Andersen PK, Pontén J, Adami HO, Gyllensten UB, Melbye M. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. Lancet. 2000. 355: 2194-2198.

Yugawa T, Kiyono T. Molecular mechanisms of cervical carcinogenesis by high-risk human papillomaviruses: novel functions of E6 and E7 oncoproteins. Rev Med Virol. 2009. 19: 97-113.

zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst. 2000. 92: 690-698.