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Cytomorphologic Features According to HPV DNA Type in Histologically Proven Cases of the Uterine Cervix

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Background: This study investigated whether human papillomavirus (HPV) genotype is related to koilocytic changes in cervical cytology and histology, and what factors cause discrepancies among cytology, HPV DNA chip tests, and biopsies. **Methods:** We examined 174 of 949 cases histologically confirmed by both cytology and HPV DNA chip testing. We analyzed koilocytic changes in cytology and biopsies according to HPV genotype. **Results:** HPV-16 significantly coincided with nuclear size variation and hyperchromasia, although the cytomorphologic features correlated with other HPV genotypes were not statistically significant. By analyzing 68 cases in which there were discrepancies between the HPV DNA chip test and histological results, we confirmed that artifacts or glycogen acanthosis resulted in the over-diagnoses of four HPV-negative cases with normal cytology. Four diagnostic errors and four sampling errors were present in eight HPV-positive cases. The degree of nuclear size variation significantly influenced the cytologically under-diagnosed cases ($p=0.006$). **Conclusions:** Other than HPV-16, HPV genotype exhibited no cytological or histological differences. The discrepancy between the results of HPV DNA chip test and histology was created by glycogen acanthosis, immature squamous metaplasia, artifacts, and sampling errors.

Key Words: HPV; DNA chip test; Cervical cytology; Biopsy

It is now well established that human papillomavirus (HPV) infection, particularly in high-risk (HR) groups, is related to the development of cervical squamous cell carcinoma (SqCC). Most HPV infections have a benign clinical course and resolve spontaneously. An estimated 3-10% of HPV carriers are infected with HR HPV and are consequently at risk for developing cervical cancer. HR and low-risk (LR) groups are as follows: HR: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -60, -66, -68, and -70; LR: HPV-6, -11, -32, -34, -40, -43, -44, -53, -54, -55, -61, and -62. A new HPV detection technique capable of providing useful data regarding HPV positivity and type has been introduced. The HPV DNA chip harbors multiple HPV probes and can detect different HPV types simultaneously. The distribution of HPV genotypes varies geographically, and HPV-16, -58, and -52 seem to be the most prevalent types in northeast Asia.[1-5] The five most common HPV types in Korean women with normal cytology are HPV-18, -53, -70, -16, and -52 or -58, in descending order of frequency, whereas HPV-16, -58, -18, -52, and -56 or -53 are the most prevalent in women with abnormal cytology.[2]

The majority of HPVs belong to the genus alpha-papilloma virus, which can be further subdivided into species and strains. It is equivocal whether natural HPV infection infers cross-protection against other related strains from the same species. However, HPV vaccines offer cross-protection against related HPV strains, and alpha-7 (HPV-18, -39, -45, -59, -68) and alpha-9 (HPV-16, -31, -33, -35, -52, -58, -67) are related to high oncogenic risk.[6,7] Although a recent study[8] showed that cooperative functions of both the E5 and E6 proteins induce koilocyte formation in human cervical cells, no data are available regarding cytomorphologic correlations according to HPV genotype or risk group.

We investigated whether HPV genotype is related with the koilocytic changes in cervical cytology or histology, and what factors influence the discrepancies among cytology, biopsy, and HPV DNA chip test results.

MATERIALS AND METHODS

Patient selection

We examined 949 cases of cervical cytology between April 2007 and April 2011. All cytology specimens were obtained

from the gynecology outpatient clinic, and the HPV DNA chip test was performed at either that time or within 6 months. A tissue biopsy such as punch biopsy, loop electrosurgical excision procedure conization, or hysterectomy was performed in 174 cases. We analyzed the cervical cytology findings and HPV genotype results in the histologically proven cases.

Cervical cytology and histology

Based on the 2001 Bethesda System, diagnosis of the cervical cytology was classified as negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), SqCC, and atypical glandular cells (AGC). After comparing the cytological results to the histological and HPV DNA chip test results, only NILM was considered a negative cytological result and HSIL or SqCC were considered high-grade lesions. The histological diagnosis of cervical lesions was classified as normal or an inflammatory lesion, condyloma, cervical intraepithelial neoplasia 1 to 3 (CIN 1 to 3) or SqCC, and the lesions from CIN 2 to SqCC were considered high-grade lesions. All cytological and histological slides were reviewed and quantitatively evaluated for the following cytomorphological parameters: abortive koilocytosis, dyskeratosis, parakeratosis, nuclear hyperchromasia, nuclear size variation, bi-/multinucleation, keratohyalin-like granule/measle cells, inflammation, and atrophy. The degrees of abortive koilocytosis, bi-/multinucleation, and keratohyalin-like granule/measle cells were divided into three grades; -, absence of the above findings; +, present, but less than 1 atypical cell per 5 high power fields (HPFs); ++, present, more than 1 atypical cell per 5 HPFs. The degrees of dyskeratosis and parakeratosis were also divided into three grades according to the severity: -, absent; +, present, but mild; ++, evident. The degrees of nuclear hyperchromasia and nuclear size variations were divided into four grades: -, absence of nuclear hyperchromasia or nuclear size variation; +, mild nuclear enlargement with increased stainability without nuclear membrane irregularity/minor variation in nuclear size or presence of longitudinal nuclear grooves, and wrinkled or spindle-shaped nuclei; ++, presence of definitive nuclear hyperchromasia or nuclear size variation, but quantitatively few; +++, marked presence of definitive nuclear hyperchromasia or size variation, or presence of bizarre cells. Only severe cases were considered positive for inflammation and atrophy. The histological diagnosis and degree of koilocytic changes apparent in the slides were examined. The cytological and histological results were compared with the HPV DNA chip test results according to HPV genotype and species.

HPV genotyping

A commercially available HPV DNA chip (Biomedlab Co., Seoul, Korea) was used. The HPV DNA chip uses a polymerase chain reaction-based DNA microarray system as a genotyping method and harbors 29 HPV probes (HR: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -60, -66, -68, and -70; LR: HPV-6, -11, -32, -34, -40, -43, -44, -53, -54, -55, -61, and -62; and "other" type). The detected HPV genotypes were assessed according to species for cross-protection evaluation within the same species: alpha-1 (HPV-32); alpha-3 (HPV-61, -62); alpha-5 (HPV-51); alpha-6 (HPV-53, -56, -66); alpha-7 (HPV-18, -39, -45, -59, -68, -70); alpha-8 (HPV-40, -43); alpha-9 (HPV-16-, 31, -33, -35, -52, -58); alpha-10 (HPV-6, -11, -44, -55); alpha-11 (HPV-34); alpha-13 (HPV-54); G4 (HPV-60).[6]

Statistical analyses

Data analyses were carried out using SPSS ver. 18 (SPSS Inc., Chicago, IL, USA). The chi-square test was used to assess the statistical significance of HPV genotypes by cytological or histological diagnoses and to compare sensitivity, specificity, and the positive and negative predictive values (PPV and NPV, respectively) among the HPV DNA chip test, cervical cytology and histological diagnoses. p-values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics

The mean age of the patients was 42.3 years (range, 18 to 92 years; standard deviation, 10.68 years). The cytological results showed the presence of 42% NILM, 41.6% ASCUS, 2.3% ASC-H, 11.4% LSIL, 2.0% HSIL, 0.2% SqCC, and 0.4% AGC. The percentage of LSIL was higher in the third (14.3%, 17/119) and fourth (14.3%, 42/293) decades, and HSIL increased from the fourth decade onward (Table 1).

The HPV DNA chip test was performed in patients mainly

Table 1. Cytological results according to age

Age (yr)	NILM	ASCUS	ASC-H	LSIL	HSIL	SqCC	AGC	Total
10-19	1	0	0	0	0	0	0	1
20-29	41	52	8	17	1	0	0	119
30-39	117	126	2	42	6	0	0	293
40-49	117	139	6	29	7	0	3	301
50-59	95	51	4	17	3	0	1	171
60-69	22	24	2	3	2	1	0	54
70-79	5	2	0	0	0	1	0	8
80-89	1	0	0	0	0	0	0	1
90-99	0	1	0	0	0	0	0	1
Total (%)	399 (42.0)	395 (41.6)	22 (2.3)	108 (11.4)	19 (2.0)	2 (0.2)	4 (0.4)	949 (100.0)

NILM, negative for intraepithelial lesion or malignancy; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SqCC, squamous cell carcinoma; AGC, atypical glandular cells.

Table 2. Results of the human papillomavirus DNA chip test according to age

Age (yr)	HR	LR	Other	Positive cases/Total No. of cases (%)
10-19	1	0	0	1/1 (100.0)
20-29	41	12	0	53/119 (44.5)
30-39	64	19	0	83/293 (28.3)
40-49	49	16	2	67/301 (22.3)
50-59	22	20	2	44/171 (25.7)
60-69	11	6	0	17/54 (31.5)
70-79	3	1	0	4/8 (50.0)
80-89	0	0	0	0/1 (0.0)
90-99	1	0	0	1/1 (100.0)
Total	192	74	4	270/949 (28.5)

HR, high-risk; LR, low-risk.

in their 40s and 50s (30.9% and 31.7%, respectively), and 270 cases showed positive results. The number of positive results on the HPV DNA chip test was the highest in the third decade (44.5%), followed by the eighth (50%), seventh (31.5%), fourth (28.3%), and sixth (25.7%) decades. The highest percentage in the HR group was found in the third decade (33.3%). The ratio of HR to LR groups decreased with age and was higher in the group aged < 50 years than in older patients (3.30 vs 1.37) (Table 2).

Correlations among the histological diagnosis, cytological diagnosis, and HPV DNA chip test

We diagnosed 174 patients who underwent cervical biopsy as follows: 20 NILM, 83 ASCUS, 13 ASC-H, 37 LSIL, 17 HSIL, two SqCC, and two AGC. Excluding the two patients with AGC, the cytological results coincided with the histological results in 110 cases. However, 38 cases exhibited lower grade lesions via cytological diagnoses compared to those on the histological diagnoses (17 NILM with condyloma or CIN 3, 10 ASCUS with CIN 2 or CIN 3, five ASC-H with CIN 2 or CIN 3, six LSIL with CIN 2 or CIN 3), and 24 cases exhibited higher grade lesions via cytological diagnoses compared to those on the histological diagnoses (21 ASCUS-LSIL with benign histological findings and 3 HSIL with condyloma or CIN 1). Two cases with NILM and CIN 3 were reviewed and revealed material inadequacy, suggesting a sampling error or hemorrhage (Table 3).

Table 3. Results of cervical cytology compared to the histological results

Histology	Cytology							
	NILM	ASCUS	ASC-H	LSIL	HSIL	SqCC	AGC	Total
WNL	3	18	2	1	0	0	0	24
Condyloma	15	53	5	26	2	0	1	102
CIN 1	0	2	1	4	1	0	0	8
CIN 2	0	5	3	5	3	0	1	17
CIN 3/CIS	2	5	2	1	10	1	0	21
SqCC	0	0	0	0	1	1	0	2
Total	20	83	13	37	17	2	2	174

NILM, negative for intraepithelial lesion or malignancy; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SqCC, squamous cell carcinoma; AGC, atypical glandular cells; WNL, within normal limits; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*.

In the 270 HPV-positive cases, the percentage of low-grade lesions determined by cytology was significantly higher than that in high-grade lesions ($p < 0.001$): ASCUS (94 cases, 34.8%), ASC-H (17 cases, 6.3%), LSIL (68 cases, 25.2%), HSIL (17 cases, 6.3%), and SqCC (two cases, 0.7%). Infections in the HR group occurred more frequently according to the severity of the cytology (percentage of the HR and LR groups in their respective cytological diagnoses: 12.5% vs 4.5% in NILM, 14.2% vs 9.6% in ASCUS, 46.3% vs 14.8% in LSIL, and 84.2% vs 5.3%

in HSIL, 100% vs 0% in SqCC) (Table 4).

Eight of the 24 cases with normal histology (33.3%) had HPV-positive results, whereas 49 of 102 cases with condyloma (48.0%), seven of eight cases with CIN 1 (87.5%), 13 of 17 cases with CIN 2 (76.5%), 19 of 21 cases with CIN 3/carcinoma *in situ* (90.5%), and two patients with SqCC (100%) had HPV-positive results. HPV positivity increased according to the severity of the histological results ($p < 0.001$). The HR HPV group had more frequent cases with CIN ($p < 0.001$) (Table 5).

Accuracy of cervical cytology and the HPV DNA chip test

The accuracy of cervical cytology and the HPV DNA chip test according to the histological results is summarized in Table 6. Cervical cytology had higher sensitivity (88.6%), whereas the HPV DNA chip test had higher specificity (66.7%) and PPV (91.8%) for detecting HPV infections. The combination of both tests provided reasonable sensitivity and specificity (95.3% and 12.5%, respectively), and the NPV was higher than each test separately (30.0% vs 15.0%, 21.1%). Additionally, the HPV DNA chip test showed sensitivity of 85.0%, specificity of 47.7%, and NPV of 92.1% for detecting CIN 2 or worse.

Results of the HPV DNA chip test

HPV-16 was the most common genotype (13.6%), followed by HPV-58 (11.3%), HPV-70 (7.7%), HPV-66 (7.4%), HPV-53 (7.4%), HPV-52 (6.5%), and HPV-18 (4.7%) (Table 7). The relationship between HPV genotype and cytology was not statistically significant. However, of the five major HPV genotypes, only HPV-16 was significantly correlated with high-grade CIN ($p < 0.001$).

Multiple HPV infections were identified in 56 of 949 cases, consisting of 47 double, seven triple, and two quadruple infections. Multiple HPV infections were common in LSIL (21/56

Table 4. Results of cervical cytology compared to the human papillomavirus (HPV) DNA chip test

	NILM	ASCUS	ASC-H	LSIL	HSIL	SqCC	AGC	Total
HPV DNA chip test								
Negative	329	301	5	40	2	0	2	679
Positive								
HR	50	56	16	50	16	2	2	192
LR	18	38	1	16	1	0	0	74
Other	2	0	0	2	0	0	0	4
Total	399	395	22	108	19	2	4	949

NILM, negative for intraepithelial lesion or malignancy; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SqCC, squamous cell carcinoma; AGC, atypical glandular cells; HR, high-risk; LR, low-risk.

Table 5. The results of the human papillomavirus (HPV) DNA chip test compared to the histological results

Histology	Cytology (-)			Cytology (+)			Total
	HPV (-)	HPV (+)		HPV (-)	HPV (+)		
		HR	LR		HR	LR	
WNL	3	0	0	13	4	4	24
Condyloma	7	6	2	46	33	8	102
CIN 1	0	0	0	1	5	2	8
CIN 2	0	0	0	4	13	0	17
CIN 3/CIS	0	2	0	2	17	0	21
SqCC	0	0	0	0	2	0	2
Total	10	8	2	66	74	14	174

Cytology (-), negative for intraepithelial lesion or malignancy; cytology (+), other cytologic lesions; HR, high-risk; LR, low-risk; WNL, within normal limits; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; SqCC, squamous cell carcinoma.

Table 6. The accuracy of cervical cytology, the human papillomavirus (HPV) DNA chip test, and the combination of both tests according to the histological results

		Histology						Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		WNL	Condyloma	CIN 1	CIN 2+3	SqCC	Total				
Cytology	(-)[a]	3	15	0	2	0	20	88.6	12.5	86.3	15.0
	(+)	21	87	8	36	2	154				
HPV DNA chip test	(-)	16	53	1	6	0	76	60.0	66.7	91.8	21.1
	(+)	8	49	7	32	2	98				
Combination of cytology and HPV DNA chip (cytology [+]/ DNA chip [+])	Both (-)	3	7	0	0	0	10	-	-	-	-
	Either (+)	13 (0+13)	54 (8+46)	1 (0+1)	8 (2+6)	0	76 (10+66)	85.0	47.7	34.7	92.1
	Both (+)	8	41	7	30	2	88	95.3	12.5	87.2	30.0
	Total	24	102	8	38	2	174	-	-	-	-

[a]The negative cytologic result means only negative for intraepithelial lesion or malignancy.
WNL, within normal limits; CIN, cervical intraepithelial neoplasia; SqCC, squamous cell carcinoma; PPV, positive predictive value; NPV, negative predictive values.

cases). The four most common multiple infection genotypes were HPV-66 (15/56 cases), HPV-16 (13/56 cases), HPV-58 (12/56 cases), and HPV 53 (11/56 cases). However, no difference was observed between any HPV genotype of multiple infections and cytological severity. Of 174 cases that underwent tissue biopsy, multiple infections were detected in 31 cases, frequently occurring in cases of condyloma and CIN 1 ($p<0.001$).

In total, 340 detected HPVs were classified according to species. Alpha-9 (HPV-16, -31, -33, -35, -52, and -58) and alpha-7 (HPV-18, -39, -45, -59, -68, and -70) constituted 41.2% and 22.1% of the cases, respectively. Alpha-9 was significantly higher in HSIL ($p<0.001$) and alpha-6 was higher in ASCUS-LSIL ($p=0.013$). Alpha-7 was mainly present in NILM-LSIL ($p=0.055$). Twenty-one of 56 cases (37.5%) with multiple infections occurred within the same species. Fourteen double infections occurred within the same species and consisted of seven alpha-9, four alpha-7, two alpha-6, and one alpha-10. Six triple infections occurred within the same species and consisted of two alpha-6, three alpha-9, and one alpha-7, and a quadruple infection occurred in one alpha-6 and two alpha-9 cases. Although several reports have suggested that one HPV infection reduces the risk of contracting HPV infection from the same species by sharing group-specific immune protection or general protection, our results did not corroborate these findings. Alpha-9 was significantly related to CIN in 209 detected HPVs with histological results ($p<0.001$).

Table 7. Distribution of human papillomavirus (HPV) genotypes

HPV genotype	HR			HPV genotype	LR		
	Single	Multiple	Total (%)		Single	Multiple	Total (%)
16	33	13	46 (13.6)	70	19	7	26 (7.7)
58	26	12	38 (11.3)	40	9	6	15 (4.5)
66	10	15	25 (7.4)	6	8	6	14 (4.2)
53	14	11	25 (7.4)	54	4	2	6 (1.8)
52	15	7	22 (6.5)	61	5	0	5 (1.5)
18	11	5	16 (4.7)	11	4	0	4 (1.2)
33	7	7	14 (4.2)	55	3	0	3 (0.9)
35	7	7	14 (4.2)	43	1	1	2 (0.6)
56	9	2	11 (3.3)	44	0	2	2 (0.6)
68	5	6	11 (3.3)	32	1	0	1 (0.3)
39	5	5	10 (3.0)	34	0	1	1 (0.3)
31	5	2	7 (2.1)	62	1	0	1 (0.3)
45	3	3	6 (1.8)	Other	4	0	4 (1.2)
59	2	2	4 (1.2)				
51	2	1	3 (0.9)				
60	1	0	1 (0.3)	Total	214	123	337 (100)

HR, high-risk; LR, low-risk.

Cytomorphological parameters of HPV infection and HPV genotype

The degree of nuclear hyperchromasia ($p<0.001$) or nuclear size variation ($p<0.001$), and bi-/multinucleation ($p<0.001$) were significantly related with histological diagnosis in cases that underwent a tissue biopsy and had cytological signs of an HPV infection (Table 8). The severity and extent of koilocytic changes in tissue biopsies were significantly correlated with the histological results ($p<0.001$). According to HPV risk group, only the severity of nuclear hyperchromasia and nuclear size variation were significantly correlated with the HR group ($p=0.002$, for both). HPV-16 was significantly related to the degrees of dyskeratosis, nuclear hyperchromasia, and nuclear size variation (Fig. 1), and HPV-58 demonstrated significant abortive koilocytic changes and bi-/multinucleation (Table 9). However, the cytomorphological features correlating all HPV genotypes were not statistically significant.

Table 8. Cytomorphological parameters of koilocytic change according to histological diagnosis

Cytomorphologic parameters		WNL (n=24)	Condyloma-CIN 1 (n=100)	CIN 2-SqCC (n=40)	Total (n=174)	p-value
Abortive koilocytes	-	6	12	8	26	
	+	16	93	32	141	
	++	2	5	0	7	0.132
Dyskeratosis	-	5	24	9	38	
	+	19	81	31	131	
	++	0	5	0	5	0.551
Parakeratosis	-	19	97	29	145	
	+	5	12	9	26	
	++	0	1	2	3	0.110
Nuclear hyperchromasia	-	6	39	2	47	
	+	15	52	15	82	
	++	3	19	19	41	
	+++	0	0	4	4	<0.001
Nuclear size variation	-	1	3	2	6	
	+	16	76	8	100	
	++	7	26	21	54	
	+++	0	5	9	14	<0.001
Bi-/multinucleation	-	15	67	12	94	
	+	9	40	17	66	
	++	0	3	11	14	<0.001
Keratohyalin-like granule/measle cells	-	7	39	12	58	
	+	15	47	15	77	
	++	2	24	13	39	0.156
Inflammation	-	12	56	17	85	
	+	12	54	23	89	0.655
Atrophy	-	22	105	38	165	
	+	2	5	2	9	0.748

WNL, within normal limits; CIN, cervical intraepithelial neoplasia; SqCC, squamous cell carcinoma.

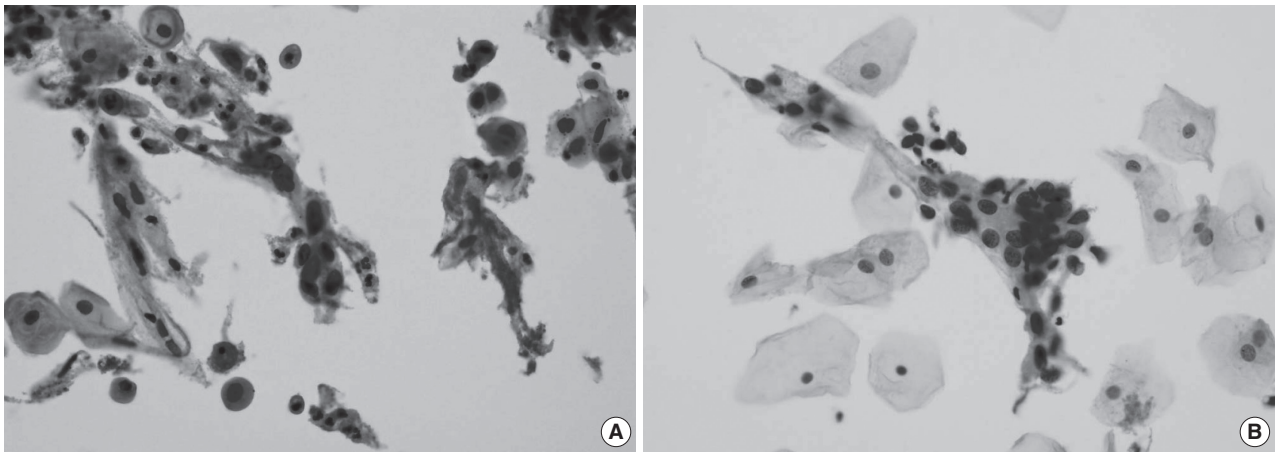

Fig. 1. Human papillomavirus (HPV) infected nuclei from a high-grade squamous intraepithelial lesion are more hyperchromatic and variable in size in HPV-16 (A) than those in HPV-33 (B) (Papanicolaou stain).

Table 9. Cytomorphological parameters of koilocytic change according to the human papillomavirus (HPV) risk group and the two most prevalent HPV genotypes (HPV-16 and HPV-58)

Cytomorphologic parameters		HR (n=82)	LR (n=16)	Total (n=98)	p-value	HPV-16		p-value	HPV-58		p-value
						+ (n=26)	-[a] (n=183)		+ (n=28)	-[a] (n=181)	
Abortive koilocytes	-	16	2	18	0.468	4	30	0.498	9	25	0.050
	+	62	14	76		22	144		18	148	
	++	4	0	4		0	9		1	8	
Dyskeratosis	-	19	2	21	0.140	8	37	0.031	7	38	0.891
	+	58	14	72		15	141		20	136	
	++	5	0	5		3	5		1	7	
Parakeratosis	-	65	15	80	0.628	19	153	0.390	20	152	0.109
	+	15	1	16		6	27		8	25	
	++	2	0	2		1	3		0	4	
Nuclear hyperchromasia	-	15	6	21	0.002	6	53	0.050	3	56	0.075
	+	34	10	44		8	87		13	82	
	++	29	0	29		10	40		11	39	
	+++	4	0	4		2	3		1	4	
Nuclear size variation	-	5	0	5	0.002	4	6	0.002	0	10	0.210
	+	33	11	44		8	109		14	103	
	++	34	5	39		13	50		9	54	
	+++	10	0	10		1	18		5	14	
Bi-/multinucleation	-	38	10	48	0.037	11	101	0.447	8	104	0.003
	+	32	6	38		12	68		14	66	
	++	12	0	12		1	14		6	11	
Keratohyalin-like granule/ Measle cells	-	29	5	34	0.986	10	61	0.711	7	64	0.118
	+	35	7	42		9	79		10	78	
	++	18	4	22		7	43		11	39	
Inflammation	-	38	7	45	0.668	10	88	0.357	16	82	0.243
	+	44	9	53		16	95		12	99	
Atrophy	-	79	15	94	0.695	26	174	0.248	28	172	0.228
	+	3	1	4		0	9		0	9	

[a]The negative result includes negative cases for HPV and positive cases for non-HPV-16 or non-HPV-58.
HR, high-risk; LR, low-risk.

Discrepancy among the histological diagnosis, cytological diagnosis, and HPV DNA chip test

Thirty-eight cases had higher degree lesions on the histological examination compared to those on the cytological diagnosis, and 24 cases had lower degree lesions on the histological diagnosis. The nuclear features of the cases with discrepancies were reviewed, as only the nuclear changes in the koilocytes were re-

Table 10. The degree of koilocytic change according to the discrepancy between cytology and biopsy results

	Coincidence			
	Cytology = Biopsy	Cytology < Biopsy	Cytology > Biopsy	Total
Degree of nuclear hyperchromasia				
Absence	29	13	4	46
Mild	53	13	15	81
Moderate	25	11	5	41
Marked	3	1	0	4
Total	110	38	24	172
p-value		0.513	0.525	0.481
Degree of nuclear size variation				
Absence	1	5	0	6
Mild	66	16	16	98
Moderate	33	13	7	53
Marked	10	4	1	15
Total	110	38	24	172
p-value		0.006	0.814	0.013
Severity of koilocytic change in tissue biopsy				
Absence	6	1	18	25
Minimal	29	9	3	41
Moderate	59	20	3	82
Bizarre	16	8	0	24
Total	110	38	24	172
p-value		0.730	<0.001	<0.001
Extent of koilocytic change in tissue biopsy				
Absence	6	1	18	25
Lower	34	14	2	50
Mid	69	23	4	96
Upper	1	0	0	1
Total	110	38	24	172
p-value		0.765	<0.001	<0.001

Cytology = biopsy, cases that the cytologic diagnosis coincides with the histologic diagnosis; Cytology < biopsy, cases that the cytologic diagnosis is lower grade lesion than the histologic diagnosis; Cytology > biopsy, cases that the cytologic diagnosis is higher grade lesion than the histologic diagnosis.

lated to the cytological or the histological results. The degree of nuclear size variation was significantly related with the cytological diagnosis (p = 0.013). In particular, nuclear size variation was greater in cases with a higher degree of histological diagnosis rather than those on the cytological diagnosis (p = 0.006). Cases with a lower histological diagnosis compared to those on the cytological diagnosis exhibited milder degrees and lower locations of koilocytic change (p < 0.001, for both). All eight cases with bizarre koilocytes were found in the cytologically under-diagnosed cases, but the difference was not significant (Table 10).

We reviewed the 68 cases with discrepancies between the HPV DNA chip test and histological diagnosis. Sixty cases were negative based on the HPV DNA chip test, but 39 cases revealed definitive HPV infection on tissue biopsy. Seventeen cases with negative HPV DNA chip results had koilocytic changes via cytology (14 ASCUS, two LSIL, and one AGC). Four cases with negative cytological results and negative HPV DNA chip test results were over-diagnosed as condyloma due to the perinuclear halo created by artifacts or glycogen acanthosis. In contrast, eight cases had positive HPV DNA chip test results, and no evidence of HPV infection was present on the tissue biopsy. Four cases were due to targeting misses of the biopsy (absence of overlying mucosa or transformation zone), and the other four cases were diagnostic errors: two cases of immature squamous metaplasia were diagnosed as condyloma, one case of condyloma was missed in a hysterectomized specimen of uterine leiomyoma, and we missed one case of focal minimal lesion on a punch biopsy. The HPV genotypes of the under-diagnosed cases were HPV-6, -53, and -62 (LR group) and HPV-66 (HR group).

DISCUSSION

Prevalence of HPV genotype

It is now well established that HPV infection is related to squamous cell lesions of the uterine cervix. HPV-16 is the most common type of HPV infection worldwide. The second most common type is HPV-18 in Western regions and HPV-58 in Asian regions.[2,9,10] We identified two major HPV genotypes in this study: HPV-16 (13.6%) and HPV-58 (11.3%). Other prevalent types were HPV-70 (7.7%), -66 (7.4%), -53 (7.4%), -52 (6.5%), and HPV-18 (4.7%). A recent multi-center study on the prevalence and genotypic distribution of HPV in Korea[2] revealed five major HPV genotypes (HPV-16, -18, -58, -53, and -52, in order of frequency). The five most common HPV types in women with abnormal cervical cytology were HPV-16, -58, -18, -52, and -56 or -53. HPV-70 comprised a large percentage of cases with normal cytology (8.3%), but was a minor group in cases with abnormal cytology (2.3%). However, a more recent study by Jeong *et al*.[11] showed that HPV-70 is one of the most common genotypes in the Korean patient group (HPV-16, -58, -18, -52, -53, -70, -6, and -11) and predominant in those >40 years of age. In our study, we found 26 HPV-70 positive cases. Of these 26 cases, 25 had relatively low-grade cervical cytology (four NILM, 14 ASCUS, one ASC-H, six LSIL), and one HSIL case had a multiple infection in the HR group. Therefore, we inferred that there was an increasing trend for HPV-70 infection or that HPV-70 was simply not detected due to the skip of HPV DNA test in cases with benign cytology, al-

though the prevalence of HPV-70 is actually high in Korea.

Jeong *et al*.[11] analyzed the prevalence of HPV and HPV genotypes in the uterine cervix using the HPV DNA chip, cytology, and biopsy in 2,086 Korean women. They reported that the HR HPV infections were composed of 20.5% ASCUS, 17.9% benign lesion, and 17.4% HSIL. LR HPV infections were composed of 31.2% ASCUS, 22.6% benign lesion, and 19.8% LSIL. In our study, LSIL comprised the third largest percentage of both HR and LR HPV infections (HR: 29.2% ASCUS, 26.0% NILM, and 26.0% LSIL; LR: 51.4% ASCUS, 24.3% NILM, and 21.6% LSIL).

Accuracy of cervical cytology and the HPV DNA chip test

Lee *et al*.[12] reported that cervical cytology (ThinPrep) demonstrated 88.4% sensitivity, 54.5% specificity, 79.8% PPV, and 69.9% NPV for HPV detection, whereas the HPV DNA chip test demonstrated 86.2% sensitivity, 46.2% sensitivity, 76.5% PPV, and 62.2% NPV. Although these authors found higher accuracy for the HPV DNA chip test compared to that in our study, their study was designed for patients with an abnormal cervical cytology smear and/or abnormal cervicography. Therefore, they surmised that the accuracy might be lower in the general population. In our study, the sensitivity (88.6%) of cervical cytology was higher than that of the HPV DNA chip test, whereas the HPV DNA chip test had a higher PPV (91.8%). An *et al*.[13] reported that the HPV DNA chip has 96.0% sensitivity, 51.9% specificity, 45.7% PPV, and 96.9% NPV for detecting HSIL or worse. We found similar results for the HPV DNA chip test for high-grade CIN-SqCC (sensitivity, 85.0%; specificity, 47.7%; PPV, 34.7%; and NPV, 92.1%). The combination of cervical cytology and the HPV DNA chip test reasonably increased sensitivity (95.3%) and NPV (30.0%), suggesting their combined usefulness for detecting HPV infection compared to either test individually ($p = 0.002$).

Cytomorphological parameters and HPV genotype

It is well established that the interaction of the E6, E7 and, to a lesser extent, E5 genes control the cell cycle and are related to the malignant progression of HPV-transformed cells.[14] Krawczyk *et al*.[8] recently demonstrated cooperative functions for both the E5 and E6 proteins for inducing koilocyte formation in human cervical cells *in vitro*, and that the E6 protein from both LR and HR HPVs is capable of potentiating koilocytosis with the E5 protein. However, previous HPV genotype studies were mainly concerned with the prevalence of HPV infection or genotypic distribution, and there are no available data for the cytopathic effect of HPV infection according to genotype or HPV risk group. We considered the possibility that cytomorphological features might be related to the genotype or the HPV HR group. Therefore, we applied cytomorphological parameters of HPV infection to our analysis. Cramer *et al*.[15] found that partial koilocytosis and multinucleation are associated with detecting HR HPV DNA, whereas only partial koilocytosis was associated with the presence of LR HPV DNA. Bollmann *et al*.[16] investigated non-classic cytomorphological signs of HPV infection and concluded that minimal nuclear change improves the sensitivity of cytology for detecting HPV, including abortive koilocytosis, mild dyskeratosis, mild nuclear hyperchromasia, mild nuclear variations, bi-/multinucleation, measle cells, parakeratosis, macrocytes, cytoplasmic folding, and keratohyalin-like granules. They indicated that it was not possible to cytomorphologically discriminate between the LR and HR HPV-positive cases. In our study, a significant difference was observed with respect to the degree of nuclear hyperchromasia or nuclear size variation and bi-/multinucleation according to the histological diagnosis, and the severity of nuclear hyperchromasia or nuclear size variation was significantly correlated with the HR HPV group. This result suggests that only nuclear changes were meaningful for diagnosing HPV infection. Furthermore, HPV-16 was significantly related to the degrees of dyskeratosis, nuclear hyperchromasia, and nuclear size variation, and HPV-58 demonstrated relatively less significant nuclear change but significant abortive koilocytic change and bi-/multinucleation. However, the cytomorphological features correlating to all other HPV genotypes were not statistically significant. Additionally, HPV-58 infection in the histological review exhibited koilocytic changes in the lower portion of the epithelial layer compared to those in non-HPV-58 infections (data not shown). Therefore, it is possible that HPV-58 expresses milder koilocytic changes in cytology or histology than those in other HPV genotypes.

Review of discrepant cases among the histological and cytological diagnosis and HPV DNA chip test

We examined the discrepant cases to determine the factors that caused the discrepancy among the cytology, HPV DNA chip, and biopsy results. The degree of nuclear size variation via cytology increased significantly in cases with higher grade lesions determined by histological diagnosis rather than cytologi-

cal diagnosis. Considering that the degree of nuclear hyperchromasia was not significant in the same cases, we believe that nuclear size variation might be underestimated when making cytological diagnoses. In the histological review, a milder degree and lower location of the koilocytic changes were noted in the cytologically over-diagnosed cases, and we considered the possibility that cervical biopsy specimens might have been obtained from less severe lesions.

Our study had a small number of patients with high-grade CIN or SqCC, because patients with HSIL or grossly evident lesions skipped the HPV DNA chip test and directly underwent the final procedure. This limited the analysis of the relationship between HPV genotype and the development of high-grade CIN or SqCC.

In conclusion, cervical cytology was a more sensitive and less specific test than that of the HPV DNA chip test, but the HPV DNA test was particularly useful for high-grade squamous lesions. The combination of cervical cytology and the HPV DNA chip test improved the detection rate of HPV infections compared to an individual evaluation by either cervical cytology or the HPV DNA chip test. Nuclear changes among the cytomorphological parameters of HPV infections were the most important factor for diagnosis, and HPV-16 significantly coincided with the degree of nuclear hyperchromasia and nuclear size variation. However, the cytomorphological features did not correlate with all HPV genotypes. In cases in which there were discrepancies between cytological and histological results, the increased nuclear size variation significantly influenced a false cytological diagnosis, particularly for higher grade lesions on the histological diagnosis compared to the cytological diagnosis. The discrepancies between the HPV DNA chip test and histological results were caused by over-diagnosis as a result of glycogen acanthosis, immature squamous metaplasia, artifacts, and sampling errors.

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