

ASCUS 환자에서 고위험 사람유두종바이러스 검사와 자궁경검사의 유용성

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= Abstract =

The Usefulness of Concomitant High-Risk Human Papillomavirus Test and Colposcopy in Combination with the Papanicolaou Test in ASCUS Patients

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The objective of this study was to ascertain whether or not the high-risk human papillomavirus (HPV) test, when coupled with Papanicolaou (Pap) smears, would prove useful in the screening and management of patients in whom abnormal Pap smear results had been obtained. Concomitant high-risk HPV detection using the hybrid capture II test and colposcopy with a Pap smear were performed with 176 patients, all of whom had been screened for both cervical carcinoma and precancerous lesions. We concomitantly performed colposcopies on these patients. Upon the follow-ups, the histologic diagnoses of these patients were confirmed via either biopsy or hysterectomy. The rate of high-risk HPV detection was correlated with cytologic diagnoses and colposcopic findings. The group composed of the high-risk HPV-positive ASCUS patients exhibited a 55.7% rate of cervical intraepithelial neoplasia (CIN), a significantly higher rate than the 7.5% result obtained in the high-risk HPV-negative ASCUS group. HPV test showed high sensitivity (87%) and low specificity (62.6%) in detection of CIN and colposcopy also showed high sensitivity (88%) and low specificity (22%). Any combination of these tests improve sensitivity, but not specificity. High-risk HPV tests, when coupled with Pap smears, constituted a useful triage approach with regard to colposcopy-directed biopsies in patients in whom a cytologic diagnosis of ASCUS had been rendered.

Key words: Human papillomavirus, ASCUS, Colposcopy

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INTRODUCTION

The diagnostic criteria for atypical squamous cells of undetermined significance (ASCUS) are, to some degree, subjective, and reported rates of ASCUS have ranged from 1.6% to 9.2%.¹ Moreover, reports of the rates of cervical intraepithelial neoplasia (CIN) upon follow-up biopsies obtained subsequent to an ASCUS Papanicolaou (Pap) result range from 10% to 50%.²⁻⁵ Therefore, the management of women with ASCUS Pap smears remains controversial, whereas patient guidelines have been established for cases in which Pap smear results are negative, or indicate low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), or carcinoma. The administration of human papillomavirus (HPV) testing, coupled with Pap tests for the triage of CIN lesions, enables the selection of patients for colposcopic examination and biopsy. However, the advantages of HPV tests or colposcopy used as a screening test remain to be adequately determined.

Some gynecologists proposed multiple combined screening tests are justifiable due to low sensitivity of Pap test. In order to determine the usefulness of the combination of a high-risk HPV test and colposcopy as a screening method, we conducted a comparison of sensitivity, specificity, positive predictive value, and negative predictive value for the Pap test, the HPV test using the hybrid capture tube (HCT) II method, colposcopy, and of combinations of these tests, with regard to the detection of CIN in patients in whom a biopsy had already confirmed the conditions.

MATERIALS and METHODS

The subject group in this study consisted of 176 patients, all of whom had been subjected to concomitant high-risk HPV tests using the hybrid capture II tube method (HCT II; Digene Diagnostics, Inc, Beltsville, MD) and colposcopy with a Pap test as an initial screening. All of these patients had been diagnosed recently via biopsy, between January 1999 and December 2000, at the Korea University Guro Hospital. All the

patients included in this study were initially selected by the gynecologist. The cervicovaginal smears were diagnosed by two pathologists, according to the guidelines provided by the Bethesda System. Cytological diagnoses of atypical glandular lesions of undetermined significance, and also diagnoses of adenocarcinoma, were excluded due to the small number of these cases represented in the sample group.

For the HCT II test, specimens were collected from the uterine cervix with a Digene specimen collection kit, then stored at -20°C until analysis. In the initial step, the specimens were denatured with alkali. Then, 150 μl of the denatured samples were hybridized to probe B (high-risk cocktail: probe for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Specimen and reagent storage, as well as all tests, were conducted in accordance with the relevant manufacturer's instructions. HPV status was expressed as the ratio of relative light units (RLU) of HPV DNA in the sample to those in the positive control, and this was set at 1 pg/ml HPV DNA. In order to be considered positive for HPV, the ratio of the RLU of the HPV DNA in the sample to that of the positive control was required to be greater than 1.

Surgical specimens for histological confirmation were obtained via colposcopy-directed biopsies, loop electro-surgical excisions, or total hysterectomies. Two pathologists confirmed all of these histological diagnoses, in the absence of any knowledge regarding patient HPV status.

For the statistical analysis of the results of the cytologic diagnoses, HPV tests, and colposcopic findings, we employed Mann-Whitney U tests and Jonckheere-Terpstra tests, depending on the grouping variables.

RESULTS

The subject group members' ages ranged from 18 to 78 years, with a mean age of 43 years. Of 176 patients, 36 (20.5%) were diagnosed as being within normal limits (WNL), 114 (64.8%) were diagnosed with atypical squamous cells of undetermined significance (ASCUS), 6 (3.4%) were diagnosed with low-grade squamous

Table 1. Correlation between cytologic diagnosis and detection of high-risk HPV

| HPV | WNL (%) (n=36) | ASCUS (%) (n=114) | LSIL (%) (n=6) | HSIL (%) (n=18) | SCC (%) (n=2) | Total (%) (n=176) |
|------|-------------------|----------------------|-------------------|--------------------|------------------|----------------------|
| HPV- | 19 (52.8) | 53 (46.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 72 (40.9) |
| HPV+ | 17 (47.2) | 61 (53.5) | 6 (100) | 18 (100) | 2 (100) | 104 (59.1) |

HPV, human papillomavirus; WNL, within normal limits; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

* Mann-Whitney U test, significance < 0.001.

Table 2. Relationship between colposcopic finding and detection of high-risk HPV

| HPV | Normal (%) (n=31) | SPI (%) (n=53) | HPV/LSIL (%) (n=34) | HSIL (%) (n=45) | Invasive Ca (%) (n=13) | Total (%) (n=176) |
|------|----------------------|-------------------|------------------------|--------------------|---------------------------|----------------------|
| HPV- | 21 (67.7) | 26 (49.1) | 13 (38.2) | 9 (20.0) | 3 (23.1) | 72 (10.9) |
| HPV+ | 10 (32.3) | 27 (50.9) | 21 (61.8) | 36 (80.0) | 10 (76.9) | 104 (59.1) |

HPV, human papillomavirus; SPI, suspicious for human papillomavirus infection; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; Ca, carcinoma.

* Mann-Whitney U test, significance < 0.001.

intraepithelial lesions (LSIL), 18 (10.2%) were diagnosed with high-grade squamous intraepithelial lesions (HSIL), and 2 (1.1%) were diagnosed with squamous cell carcinoma (SCC). The elevated prevalence of ASCUS/SIL categories might be attributable primarily to our patient selection strategy, which was to include patients who had undergone concomitant high-risk HPV tests and colposcopies, coupled with Pap tests. As is indicated in Table 1, the detection rate of high-risk HPV was correlated positively with cytologic diagnoses ($p < 0.001$). The WNL category was shown to include the formerly-separated 'benign cellular change' group, which may, at least in part, explain the high detection rate of high-risk HPV in our study.

The colposcopic findings included the following: 31 (17.6%) cases were normal, 53 (30.1%) were suspicious for HPV infection, 34 (19.3%) were diagnosed as HPV infection and LSIL, 45 (25.6%) as HSIL, and 13 (7.4%) as invasive carcinoma. The colposcopic findings and the rate at which high-risk HPV was detected were correlated significantly ($p < 0.001$), as is shown in Table 2.

The biopsy results, used as a gold standard, were as follows: 61 (34.7%) cases were determined to be normal

or were associated with reactive cervicitis, 38 (21.6%) cases involved koilocytosis or flat condyloma, 36 (20.5%) were associated with CIN1, 35 (19.9%) with CIN2 and CIN3, and 6 (3.4%) cases received diagnoses of invasive carcinoma. The relationship between histologic diagnoses and high-risk HPV status within each of the cytologic result categories are indicated in Table 3. In the ASCUS category, the percentages of CIN and carcinoma cases found by histologic diagnoses were remarkably higher in the high-risk HPV-positive group (55.7%) than in the high-risk HPV-negative group (7.5%; Mann-Whitney U test, $p < 0.001$). Four cases in the ASCUS category without high-risk HPV were confirmed to have CIN and invasive carcinoma. These cases were reviewed, and confirmed as ASCUS. 34 cases with positive high-risk HPV in the ASCUS category were confirmed as 10 CIN 1, 7 CIN 2, 15 CIN 3, and 2 invasive carcinoma. One case of invasive carcinoma was confirmed as endocervical adenocarcinoma, and the other proved to be a case of endometrial adenocarcinoma. Review of the Pap smears of these patients revealed that one of the CIN1 cases and 2 of the CIN2 cases also exhibited LSIL. 5 of the CIN3 cases also exhibited HSIL, and the other 26

Table 3. Relationship between histologic diagnosis and detection of high-risk HPV in each cytologic category

| Cytologic category | WNL (%) (n=61) | Flat condlyoma (%) (n=38) | CIN1 (%) (n=36) | CIN2/3 (%) (n=35) | Ca (%) (n=6) |
|--------------------|-------------------|------------------------------|--------------------|----------------------|-----------------|
| WNL(n=36) | 14 (38.9) | 5 (13.9) | 16 (44.4) | 1 (2.8) | 0 (0.0) |
| HPV-(n=19) | 10 (52.6) | 3 (15.8) | 6 (31.6) | 0 (0.0) | 0 (0.0) |
| HPV+(n=17) | 4 (23.5) | 2 (11.8) | 10 (58.8) | 1 (5.9) | 0 (0.0) |
| ASCUS(n=114) | 46 (40.4) | 30 (26.3) | 12 (10.5) | 23 (20.2) | 3 (2.6) |
| HPV-(n=53) | 33 (62.3) | 16 (30.2) | 2 (3.8) | 1 (1.9) | 1 (1.9) |
| HPV+(n=61) | 13 (21.3) | 14 (23.0) | 10 (16.4) | 22 (36.1) | 2 (3.3) |
| LSIL(n=6) | 0 (0.0) | 2 (33.3) | 4 (66.7) | 0 (0.0) | 0 (0.0) |
| HPV-(n=0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV+(n=6) | 0 (0.0) | 2 (33.3) | 4 (66.7) | 0 (0.0) | 0 (0.0) |
| HSIL(n=18) | 1 (5.6) | 1 (5.6) | 4 (22.2) | 9 (50.0) | 3 (16.7) |
| HPV-(n=0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV+(n=18) | 1 (5.6) | 1 (5.6) | 4 (22.2) | 9 (50.0) | 3 (16.7) |
| SCC(n=2) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (100) | 0 (0.0) |
| HPV-(n=0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV+(n=2) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (100) | 0 (0.0) |

WNL, within normal limit; HPV, human papillomavirus; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; CIN, cervical intraepithelial neoplasia; Ca, carcinoma.

cases continued to be classified as ASCUS.

In the LSIL category, biopsies confirmed koilocytotic changes and CIN1 in 2 of 2 cases (100%) and 4 of 4 cases (100%) with high-risk HPV, respectively. In the HSIL group in which high-risk HPV was also detected, 2 cases were confirmed as involving condyloma or koilocytotic changes, and 4 cases were diagnosed with CIN1. Three of the cases in the HSIL category were confirmed as having invasive SCC, and 2 of these showed microinvasive SCC. In the SCC cytologic category, two of the cases involving high-risk HPV were confirmed as also having CIS.

In the WNL cytologic category, 17 patients were confirmed with CIN; 16 of these were CIN1, and the remaining case was confirmed as CIN2. Reviews of the Pap smears of these patients showed that 1 case of CIN1

with high-risk HPV was associated with LSIL, and 1 of the cases of CIN2 had HSIL, but the other 15 cases remained in the WNL category, which might be attributable to sampling errors.

Colposcopic findings were correlated ordinarily (Jonckheere-Terpstra test, $P < 0.001$) with the degree of dysplasia on the biopsy-proven histologic diagnosis as shown in Table 4.

Table 5 shows the sensitivities and specificities of the Pap test, high-risk HPV test, colposcopy, and combined screening triages for the detection of all grades of CIN and carcinoma, predicated on the histological diagnoses. Although the results of Pap smears indicated a very high specificity when diagnosed above SIL, the finding of low sensitivity presents some problems. Sampling errors are believed to be the most important factor in this

Table 4. Relationship between histologic diagnosis and colposcopic findings

| Colposcopic findings | WNL (%) (n=61) | Flat condyloma (%) (n=38) | CIN1 (%) (n=36) | CIN2/3 (%) (n=35) | Ca (%) (n=6) |
|----------------------|-------------------|------------------------------|--------------------|----------------------|-----------------|
| Normal (n=31) | 14 (45.0) | 8 (25.8) | 5 (16.1) | 3 (9.7) | 1 (3.2) |
| SPI (n=53) | 26 (49.0) | 15 (28.3) | 9 (17.0) | 3 (5.7) | 0 (0.0) |
| HPV/LSIL (n=34) | 12 (35.0) | 5 (14.7) | 10 (29.4) | 7 (20.6) | 0 (0.0) |
| HSIL (n=45) | 18 (18.0) | 9 (20.0) | 12 (26.7) | 16 (35.6) | 0 (0.0) |
| Invasive ca (n=13) | 1 (8.0) | 1 (7.7) | 0 (0.0) | 6 (46.2) | 5 (38.5) |

WNL, within normal limit; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; SPI, suspicious for human papillomavirus infection; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; Ca, carcinoma.

* Jonckheere-Terpstra test, significance < 0.001.

Table 5. Sensitivities and specificities of Pap smear, HPV test, colposcopy, and combined triage for detection of CIN

| Screening test | Sensitivity | Specificity |
|---|-------------|-------------|
| Pap smear (above ASCUS) | 0.78 | 0.19 |
| Pap smear (above LSIL) | 0.29 | 0.96 |
| HPV test (HPV+) | 0.87 | 0.63 |
| Colposcopy (above SPI) | 0.88 | 0.22 |
| Colposcopy (above HPV/LSIL) | 0.72 | 0.64 |
| Pap smear with HPV (above ASCUS or HPV+) | 0.92 | 0.13 |
| Pap smear with Colposcopy (above ASCUS or SPI) | 0.99 | 0.03 |
| HPV with Colposcopy (HPV+ or above SPI) | 0.96 | 0.18 |
| Pap smear with HPV and Colposcopy (above ASCUS or HPV+or above SPI) | 0.99 | 0.02 |

HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; SPI, suspicious for human papillomavirus infection.

phenomenon. The ASCUS Pap smear category might result in the triage of more patients for further evaluation and markedly improve sensitivity, but this is associated with a concomitant drop in specificity. Any combination of triage using HPV tests or colposcopy for the detection of CIN was shown to improve sensitivity, but not

specificity, in comparison with cytology alone, or high-risk HPV tests alone. Therefore, the Pap smear should essentially remain the primary screening test, but could be coupled with the high-risk HPV test or colposcopy, which would effect improvements in the sensitivity of screening.

In addition, the mean RLU ratios (\pm SD) were determined to be 194.1 ± 450.8 in the histologically confirmed chronic cervicitis group, 401.0 ± 823.2 in the koilocytotic change group, 367.9 ± 490.8 in the CIN 1 group, 817.2 ± 1015.6 in the CIN 2/3 groups, and 820.3 ± 1036.4 in the invasive carcinoma group. The RLU ratios indicate a positive correlation according to disease severity (correlation coefficient 0.029, $p=0.001$, significant at the 0.01 level). The mean RLU ratios may also constitute supplementary information which would prove useful in the management of patients with ASCUS.

DISCUSSION

The Pap smear is the most popular and reliable screening test available for the detection of high-grade CIN and invasive carcinoma. In contrast with the high degree of specificity associated with the Pap smear with regard to the detection of high-grade CIN and invasive carcinoma, the accuracy of Pap test is rather poor, especially in terms of its ability to detect low-grade CIN.⁶ A practical strategy for the triage of patients

diagnosed as ASCUS is required in order to reduce the rates of unnecessary colposcopic examinations and biopsies.

The high-risk variants of HPV comprise 94% of all HPV associated with invasive cervical cancer.⁷ As the persistent infection of high-risk HPV appears to be related to the progression of low-grade CIN to high-grade CIN and the development of cervical carcinoma, the detection of high-risk HPV in patients who exhibit low-grade cytological abnormalities might be used as an additional method in selection strategies for further evaluation.

In our study, we demonstrated that 53.5% of histologically-confirmed patients within the ASCUS group also harbored high-risk HPV, and 55.7% of them also exhibited all grades of CIN and invasive carcinoma. After review of the Pap smears and reallocation of 8 of the ASCUS cases to the LSIL and HSIL categories, 42.6% of the ASCUS cases with high-risk HPV were confirmed to have CIN and invasive carcinoma. Taking into account these findings, it appears that the high-risk HPV test, when coupled with the Pap test, proved quite useful in the ASCUS group, in terms of the making of decisions regarding the referral of these cases to further colposcopic examinations and biopsies.

The high rate of high-risk HPV positivity (100%) in our LSIL and HSIL groups within the LSIL group, as compared to the rates observed within the ASCUS group, showed that the HPV test was not quite so useful in these cytological categories. This finding is comparable with the findings of the large NCI-sponsored ALTS (ACUS/LSIL Triage Study), in which 83% of the women diagnosed with LSIL on the Pap test were also determined, via the HCT II test, to harbor high-risk HPV DNA.⁸

In the WNL category, a relatively high rate of high-risk HPV positivity (47.2%) was found in this study, which might be attributable to selection strategy for concomitant HPV tests and colposcopies with Pap smears.

Our study indicated that the HPV HCT II test was highly sensitive (87.0%) for CIN detection. However, the HPV HCT II test alone was found to be unsuitable as an

initial screening method, due to its generally low specificity (62.6%) with regard to the detection of all grades of CIN. Colposcopy also showed high sensitivity (88%) but very low specificity (22%), which is not recommendable for initial screening test. Moreover, no combinations of the Pap test, the HPV HCT II test, and colposcopy were determined to effect overall improvements in both sensitivity and specificity with regard to the detection of CIN. This suggests that routine combination strategy (initial multiple tests) does not constitute a practical strategy for the screening of cervical cancer.

CONCLUSION

Precancerous cervical lesions should always initially be screened via the Pap test. Also, women allocated to the ASCUS category on a Pap test should be advised to take a high-risk HPV test and/or colposcopy, as this may markedly improve the rate at which any possible CIN can be detected.

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