

Do Human Papilloma Virus and Cytological Testing Results Before Colposcopy Alter the Pathological Grading of Colposcopy Acetic Acid Visual Examination?: A Retrospective Study

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Objective: To understand whether human papillomavirus (HPV) and cytological testing (TCT [ie, “thinprep” cytological testing]) results can provide more information beyond visual information for vaginal colposcopy examinations to upgrade or downgrade the visual diagnosis of vaginal colposcopy.

Patients and Methods: Data from 519 patients, who underwent vaginal colposcopy at the Beijing Obstetrics and Gynecology Hospital (Beijing, China) between January and June 2020, were included. Preoperative HPV and TCT results were statistically analyzed, and were divided into 3 groups according to postoperative cervical tissue pathological diagnosis: negative; low-grade squamous intraepithelial (LSIL); and high-grade squamous intraepithelial lesion (HSIL). Positive and negative predictive values for cervical inflammation, LSIL, and HSIL in patients diagnosed using vaginal colposcopy, based on cervical pathological grouping, and differences in HPV and TCT results among patients who underwent vaginal colposcopy, were analyzed.

Results: The age of patients diagnosed with cervicitis, LSIL, and HSIL using colposcopy gradually decreased, and the proportion of HPV16/18 infection in the HSIL group was significantly higher than the other 2 groups. There were significant differences in TCT results among the groups. According to pathological results from cervical tissue specimens, among all groups diagnosed using colposcopy, the age of the HSIL group was significantly younger than that of the other groups, and the proportion of patients with a TCT greater than LSIL was significantly higher than that of the other groups.

Conclusion: HPV did not provide additional information for vaginal colposcopy. Young(er) patients and those with a TCT greater than LSIL may consider upgrading the vaginal colposcopy diagnosis based on imaging information.

Keywords: vaginal colposcopy acetic acid visual diagnosis, cervical precancer, HPV, TCT

Introduction

Cervical cancer is the fourth most common cancer worldwide, accounting for 6.5% of all malignant tumors among women worldwide.¹ Approximately 100,000 patients undergo treatment for precancerous cervical lesions annually.² Cytological testing (TCT [ie, “thinprep” cytology testing]), visual inspection with acetic acid (VIA), and human papilloma virus (HPV) testing are the main screening methods for colposcopy.³ Digital colposcopy uses microscopy to examine the cervix and vagina, and can, to a certain extent, magnify and finely identify the junctions of scales and blood vessels. VIA during colposcopy is a simple, safe, and effective diagnostic method.⁴ However, it requires a specific level of physician skill/experience.⁵ Currently, the positive predictive rate of colposcopy is approximately 50%. Preoperative HPV and TCT results can provide additional textual information to supplement colposcopy imaging data, which can provide a basis for upgrading or downgrading colposcopy diagnosis. However, there is currently a lack of relevant

research investigating the relationship between HPV and TCT results and colposcopy diagnosis results. As such, this study collected data from patients who underwent vaginal colposcopy examination in our hospital and their preoperative HPV and TCT results, to clarify whether preoperative examination can provide specific reference information for vaginal colposcopy visual diagnosis.

Materials and Method

This retrospective study included data from 933 patients who underwent vaginal colposcopy at Beijing Obstetrics and Gynecology Hospital (Beijing, China) between January and June 2020. The inclusion criteria were as follows: underwent vaginal colposcopy and multipoint cervical biopsy during surgery; pathology confirmed grading of cervical precancerous lesions; and underwent preoperative HPV and TCT examinations, with relevant results recorded in the medical record system. Patients who underwent surgery, including cervical resection (such as Manchurian surgery or total hysterectomy for various reasons), before undergoing colposcopy, and pregnant women were excluded from the study.

The ages of patients who underwent vaginal colposcopy and cervical biopsy, as well of HPV and TCT test results before the examination, were collected. All colposcopy examinations and cervical biopsies were performed by approved colposcopy examining physicians. Results of vaginal colposcopy examination and pathological diagnosis of postoperative multipoint cervical biopsy specimens were collected. Pathological results were obtained by 2 qualified pathologists. Based on the results of the vaginal colposcopy examination, patients were divided into 3 groups: normal; low-grade squamous intraepithelial (LSIL); and high-grade squamous intraepithelial lesion (HSIL). Based on pathological diagnosis of cervical biopsy specimens, patients in each group were further divided into negative (including inflammation and visible foam cells), LSIL, and HSIL groups. The vaginal colposcopy and cervical specimen pathological diagnoses of patients at different stages of cervical precancerous lesions, screened patients with different vaginal colposcopy and cervical specimen pathological diagnoses, were compared. The relationship between preoperative HPV test and TCT results, and differences in visual and pathological diagnosis, were analyzed.

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and approved by the Ethics Committees of Beijing Obstetrics and Gynecology Hospital, Capital Medical University (Beijing, China; No:2023-KY-021-01).

Test Methods

HPV Testing

Cervical scrape specimens were gently collected from the squamocolumnar junction of the cervix using a sampling brush. HPV genotypes were determined using a commercially available kit (23-HPV Genotyping Real-time PCR Kit, Hybribio, China), which detects 15 high-risk HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82), in accordance with manufacturer's instructions.

Liquid-Based TCTs

Cells were collected from the ectocervix and cervical canal using a cervical canal brush. The cells attached to the small brush were then eluted into vials containing a cell preservation solution and sent to the pathology department (Beijing Obstetrics and Gynecology Hospital, Capital Medical University, China). The laboratory physician provided the final report.

Colposcopy

Colposcopic biopsy was performed according to operating procedures for colposcopy using a German Lescon photo-electric integrated electronic colposcopy system. Colposcopy was performed in accordance with the diagnostic criteria of the International Federation of Cervical Pathology and Colposcopy (IFPCC) and ASCCP.^{6,7} The colposcopy images were graded as normal, bend/low-grade lesions (LSIL), high-grade lesions (HSIL), and invasive cancer.

Detection of Pathology

Serial sections of cervical tissue specimens were prepared and histology was performed by 2 senior physicians from the pathology department of the authors' hospital, who read the films and performed the examination, diagnosis, and treatment. The pathological classification was based on the 2014 World Health Organization Female Genitalia classification. According to the oncology classification criteria, LSIL included CIN1 and p16-negative CIN2, and HSIL included P16-positive CIN2 and CIN3.

Statistical Methods

All statistical analyses were performed using SPSS version 26.0 (IBM Corporation, Armonk, NY, USA), Sigmapstat version 3.5 (sigmapstat.software.informer.com/) and spreadsheet software (Excel, Microsoft Corp., Redmond, WA, USA). Grading data are described using the frequency and composition ratio. Differently grouped data were compared using chi-squared analysis, while continuous data were analyzed using Kruskal–Wallis one-way analysis of variance (ie, “ANOVA”). Differences with $p < 0.05$ were considered to be statistically significant. According to the Bonferroni method, the p value was corrected to 0.017 for the 3 groups in each experiment.

Results

Data from 519 patients were included in this study, including 158 diagnosed with cervicitis by colposcopy, 256 with LSIL, 97 with HSIL, and 9 with cervical cancer (Table 1). If cervical cancer lesions could be directly observed during gynecological examinations without further HPV and TCT testing, most patients with cervical cancer were excluded from the study. Among the patients screened for vaginal colposcopy diagnosis of cancer in this study, 4 had cervical tissue pathology indicating cancer, 3 had HSIL, and 1 had LSIL. Due to the small number of patients who underwent HPV testing and TCT, patients with cervical cancer detected by colposcopy were not included in this study.

Patients diagnosed with cervicitis according to colposcopy were significantly older than those diagnosed with LSIL ($P=0.050$), and both groups of patients were significantly older than those diagnosed with HSIL (normal: LSIL, $P=0.017$; LSIL, HSIL, $P=0.025$). The different subtypes of HPV infection in the normal group and LSIL group had no significant effect on vaginal colposcopy recognition ($P=0.779$), whereas the proportion of HPV16/18 in the HSIL group was significantly higher than that in the cervicitis and LSIL groups ($P=0.003$ and $P=0.001$, respectively).

Table 1 Basic Information of Patients With Different Degrees of Cervical Lesions Diagnosed by Colposcopy

	Normal (n=158)	LSIL (n=256)	HSIL (n=97)	P
Age	49(40,56)	41(34,50.75)	33(30,41.25)	0.000
HPV				0.003
Negative	12(7.6%)	15(5.9%)	4(4.1%)	
16/18 positive	67(42.4%)	104(40.6%)	61(62.9%)	
Positive for other high-risk types	77(48.7%)	128(50.0%)	29(29.9%)	
Unknown	2(12.7%)	9(3.5%)	3(3.1%)	
TCT				0.000
Normal	70(44.3%)	78(30.5%)	19(19.6%)	
AUSCS	44(27.8%)	65(25.4%)	24(24.7%)	
ACS-H	4(2.5%)	8(3.1%)	10(10.3%)	
LSIL	37(23.4%)	95(37.1%)	23(23.7%)	
HSIL	3(19.0%)	9(3.5%)	20(20.6%)	
AGC	0(0%)	1(0.4%)	1(1.0%)	
Pathological results				0.000
Negative	88(55.7%)	102(39.8%)	15(15.5%)	
LSIL	57(36.1%)	104(40.6%)	25(25.8%)	
HSIL	13(8.2%)	49(19.1%)	55(56.7%)	
ca	0(0%)	1(0.4%)	2(2.1%)	

In the TCT results, there were significant differences among the 3 groups (ie, normal, LSIL, and HSIL: normal versus [vs] LSIL [P=0.016]; normal vs HSIL [P=0.000]; LSIL vs HSIL [P=0.000]). There was consistency between TCT results and vaginal colposcopy diagnosis. The consistency between vaginal colposcopy diagnosis and postoperative cervical tissue biopsy was the lowest in the LSIL group, while that in the other two groups was > 55%.

The cervical inflammation, LSIL, and HSIL groups were analyzed based on pathological results after cervical biopsy as diagnostic criteria. Among the patients diagnosed as normal according to colposcopy, 70 had cervical tissue pathology indicating LSIL or higher (44.3%) (Table 2). For patients with normal pathological indications, the positive predictive value of colposcopy examination was 55.41% (95% confidence interval:47.55%–63.27%) and the negative predictive value was 67.50% (95% confidence interval:62.64%–72.36%). The analysis results indicated that age had no significant impact on the accuracy of vaginal colposcopy diagnosis in the normal group. HPV infection status had no significant impact on the accuracy of vaginal colposcopy diagnosis (Table 3). When the TCT results of patients indicated LSIL or above, the possibility of pathological upgrading significantly increased (P=0.024); however, the analysis of all TCT result types did not reveal significant differences among the 3 groups.

Among patients diagnosed with LSIL using colposcopy, 105 had cervical tissue pathology confirmed as LSIL (41.0%) (Table 4). For patients with pathological indications of LSIL, the positive predictive value of colposcopy examination was 41.02% (95% confidence interval:34.95%–47.08%), and the negative predictive value was 68.44% (95% confidence interval:62.79%–74.09%). Results suggested that age was related to the degree of cervical lesions, and there were no significant differences in HPV types among the negative, LSIL, and HSIL groups. Similar to the normal group, HPV infection did not significantly affect the accuracy of vaginal colposcopy examination (Table 5). There was a significant difference in preoperative TCT results between patients with negative postoperative pathology and those with LSIL and HSIL (P=0.023), indicating that preoperative TCT examination of LSIL or higher suggests an increased possibility of upgrading vaginal colposcopy diagnosis results.

Table 2 General Information of Cervical Inflammation Group Patients Grouped According to Pathological Results of Cervical Biopsy Tissue

	Negative* (n=88)	LSIL (n=57)	HSIL (n=13)	P
Age	46.5 (36.5,55.0)	49.0(41.75,55.25)	51.0(48.0,59.0)	0.063
HPV				0.356
Negative	9 (10.2%)	3 (5.3%)	0 (0%)	
16/18 positive	40 (45.5%)	21 (36.8%)	6 (46.1%)	
Positive for other high-risk types	38 (43.2%)	33 (57.9%)	6 (46.1%)	
Unknown	1 (1.1%)	0 (0%)	1 (7.7%)	
TCT				0.051
Normal	45 (51.1%)	22 (38.6%)	3 (23.1%)	
AUSCS	26 (29.5%)	16 (28.1%)	2 (15.4%)	
ASC-H	0 (0%)	3 (5.3%)	1 (7.7%)	
LSIL	16 (18.2%)	15 (26.3%)	6 (46.1%)	
HSIL	1 (1.1%)	1 (1.8%)	1 (7.7%)	

Note: *The negative group includes patients with cervicitis or foam cells diagnosed by pathology.

Table 3 P-values of HPV Infection in Different Pathological Groups of Normal Group

	Negative	16/18 positive	Positive for Other High-Risk Types
Negative			
16/18 positive	0.451		
Positive for other high-risk types	0.218	0.362	

Table 4 General Information of LSIL Group Patients Grouped According to the Pathological Results of Cervical Biopsy Tissue

	Negative (n=102)	LSIL (n=105)	HSIL (n=49)	P
Age	44(34,51)	42(33.75,52.25)	37.5(32.5,40.5)	0.009
HPV				0.186
Negative	8 (7.8%)	4 (3.8%)	3 (6.1%)	
16/18 positive	38 (37.3%)	39 (37.1%)	27 (55.1%)	
Positive for other high-risk types	51 (50.0%)	58 (55.2%)	19 (18.1%)	
Unknown	5 (4.9%)	4 (3.8%)	0 (0%)	
TCT				0.001
Unknown	39 (38.2%)	24 (22.9%)	15 (30.6%)	
AUSCS	27 (26.5%)	24 (22.9%)	14 (28.6%)	
ASC-H	4 (3.9%)	1 (0.1%)	3 (6.1%)	
LSIL	31 (30.4%)	53 (50.5%)	11 (22.4%)	
HSIL	0 (0%)	3 (2.9%)	6 (12.2%)	
AGC	1 (0.1%)	0 (0%)	0 (0%)	

Table 5 P-values of HPV Infection in Different Pathological Groups of LSIL Group

	Negative	16/18 Positive	Positive for Other High-Risk Types
Negative			
16/18 positive	0.457		
Positive for other high-risk types	0.42	0.118	

Fifty-four individuals in the HSIL group were diagnosed with cervical tissue pathology of HSIL (56.8%) (Table 6), and there was no significant age difference among the 3 groups. For patients with pathological signs of HSIL, the positive predictive value of colposcopy examination was 55.67% (95% confidence interval:45.61%–65.73%), and the negative predictive value was 85.2% (81.92%–88.70%).

Compared with the HSIL group, patients with a mild pathological degree diagnosed by colposcopy exhibited no significant changes in HPV typing (P=0.114). Pathological confirmation revealed that patients with HSIL had more severe TCT results (P=0.000) than those with pathological results, indicating a decrease in lesion severity and a certain degree of consistency between TCT and pathological biopsy results for vaginal colposcopy diagnosis of patients with HSIL.

Table 6 General Information of Patients in the HSIL Group Grouped According to the Pathological Results of Cervical Biopsy Tissue

	Negative (n=15)	LSIL (n=26)	HSIL (n=54)	P
Age	32(29,47)	37(31,51)	33(30,39)	0.366
HPV				0.089
Negative	0(0%)	3(11.5%)	1(1.9%)	
16/18 positive	8(53.3%)	13(50%)	38(70.3%)	
Positive for other high-risk types	7(46.7%)	9(34.6%)	13(24.1%)	
Unknown	0(0%)	1(3.7%)	2(3.7%)	
TCT				0.007
Normal	5(33.3%)	8(30.8%)	6(11.1%)	
AUSCS	5(33.3%)	10(38.5%)	9(16.7%)	
ASC-H	0(0%)	1(3.8%)	9(16.7%)	
LSIL	4(26.7%)	6(23.1%)	13(24.1%)	
HSIL	1(6.7%)	1(3.8%)	16(29.6%)	

Discussion

Screening methods for cervical cancer include HPV and cervical TCT, and the results of HPV and TCT are important factors in determining whether to perform vaginal colposcopy.⁸ If necessary, vaginal colposcopy and cervical tissue biopsy are necessary to detect precancerous lesions of the cervix early and ensure timely intervention and ensuing treatment.⁹ Physicians can make a visual diagnosis during vaginal colposcopy and acetic acid administration. Cervical tissue sampling should be performed if the physician deems it necessary to confirm the diagnosis via pathology. However, visual diagnosis relies on the sensitivity, specificity, and experience of the physician.¹⁰

Colposcopy is a highly subjective examination method, and different physicians may obtain different results when performing colposcopy on the same patient, mainly depending on their experience.¹¹ In many countries and, especially, in primary hospitals, colposcopy physicians have poor abilities to identify, diagnose, and biopsy transitional bands.^{12,13}

Improving the accuracy of visual diagnosis with colposcopy is beneficial for reducing the selection of subsequent cervical biopsy locations and providing timely disease information to physicians and patients.¹⁴ A Swedish study¹⁵ suggested that colposcopy has a weak ability to distinguish between normal and atypical lesions, and that simply increasing the number of times physicians perform colposcopy does not necessarily improve accuracy. Advances in technology have improved the accuracy of colposcopy, and Booth et al¹⁶ found that dynamic spectral imaging can help locate biopsy sites. Ferris et al¹⁷ suggested no significant difference in the area under the receiver operating characteristic curve (AUC) between polarized and unpolarized colposcopy. Patel et al¹⁸ used fluorescein sodium under a blue filter to assist in the observation of cervical lesions, which can effectively improve diagnostic accuracy. Tsampazis et al¹⁹ demonstrated that electrical impedance spectroscopy was effective in diagnosing CIN2+ and significantly increased the number of CIN2+ cases missed by vaginal colposcopy alone, especially in women referred for LSIL cytological examination. Artificial intelligence-based vaginal colposcopy image recognition systems are gradually being developed, with their sensitivity increasing from 70% in 2015 to > 90% currently.²⁰

Reid and Scalzi proposed the Reid Colposcopic Index (RCI) in 1985 to ensure standardization and accuracy of colposcopy.²¹ New scoring systems for vaginal colposcopy results have been proposed, including the modified RCI²² and Swede Score.²³ However, these scores have not been well correlated with pathology in some studies.^{24,25} Strander et al²³ proposed the Swede score in 2005, which included acetouptake, margins and surface, vessels, locomotion size, and iodine staining. In a subsequent study by Alfonzo et al,²⁶ this scoring criterion was able to distinguish patients with CIN2+ in clinical settings, with an AUC of 0.88 when combined with cytology and HPV diagnosis. In a study by Kushwah et al,²⁷ Reid scores of 3–4 were mainly associated with CIN1-2; however, the management of these 2 grades was significantly different. Sweden scores ≥ 5 exhibited a sensitivity of 100% and specificity of 91.3% in distinguishing CIN2+ lesions. However, there is currently no consensus regarding the diagnosis of vaginal colposcopy.²⁸ The International Federation of Cervical Pathology and Colposcopy (IFCPC), which is currently the authoritative international organization for cervical pathology and colposcopy, proposed 4 versions of vaginal terminology in 1975, 1990, 2002, and 2011, with the aim of promoting the harmonization of colposcopy terminology and practice. The American Society for Colposcopy and Cervical Pathology (ASCCP) proposed colposcopy standards in 2017 based on colposcopy practices in the United States; this terminology and evaluation method is commonly used in China for vaginal colposcopy image assessment.

A similar relationship was observed between age and the presence of cervical lesions is very close. A study from Brazil reported that women 25–29 years of age had the highest vaginal colposcopy referral rates after undergoing HPV testing and TCT. In terms of the diagnosis rate of cervical precancerous lesions, the odds ratio for CINII-III lesions diagnosed by HPV in the 25–29 years' age group was 2.1–3.0 compared with the 30–64 years' age group.²⁹ In a big data study, among women 24–29 years of age who underwent HR-HPV testing, 26.9% tested positive, and 10.4% were directly transferred for colposcopy after cytological triage, with CIN2+ accounting for 47%. Among the population 50–64 years of age, these proportions were 5.3%, 1.2%, and 27%, respectively.³⁰ Similar to previous studies, in our study, age was closely related to the severity of disease(s) diagnosed using colposcopy. Studies have suggested that females with CIN III < 50 years of age have a sensitivity of 85.9% in vaginal colposcopy, while women > 50 years of age have a slightly lower sensitivity (78%).³¹ Some researchers have suggested that this phenomenon is associated with age-

related squamous epithelial regression.³² This study investigated the influence of age on the accuracy of vaginal colposcopy. In the normal and HSIL groups, our results indicated that age was not related to the results of colposcopy diagnosis. Younger patients have a higher probability of a pathological diagnosis corrected to HSIL only when they are diagnosed with LSIL using colposcopy. Based solely on the results of the vaginal colposcopy examination, young patients may have been missed or misdiagnosed.

In a previous prospective study, Petousis et al³³ suggested that the positive predictive value of colposcopy was high, but the negative predictive value was low. Our results indicated that the negative predictive value was also low for patients with LSIL or cervicitis, suggesting that colposcopy cannot effectively distinguish between LSIL and cervicitis. However, the negative predictive value of the HSIL group was high, similar to that reported in previous studies, and colposcopy may be an effective diagnostic method for patients with HSIL.

The combination of HPV and TCT is considered to be a method for distinguishing between HSIL and non-HSIL.³⁴ However, the diagnostic performance of colposcopy may be affected by the method and results selected during the initial screening. Compared with cytological abnormalities, cervical lesions with only HPV infection may be more difficult to identify because they are milder.¹² A study by Liu et al³⁵ found that vaginal colposcopy was more accurate in patients who were positive for HPV16/18 or had a high viral load. In the present study, we focused on patients classified as having other degrees of cervical lesions according to vaginal colposcopy. The results revealed no significant difference in HPV test results between the groups, indicating that it was not possible to correct the vaginal colposcopy results based on HPV results. However, the proportion of TCT results greater than or equal to LSIL was significantly different among the groups, suggesting that TCT results greater than or equal to LSIL can be used as a reference for upgrading vaginal colposcopy image diagnosis.

New detection indicators have also been used to triage patients with cervical intraepithelial neoplasia. Clarke et al³⁶ used p16/Ki67 double staining to triage patients with HPVs other than those positive for HPV16/18. Stoler et al³⁷ found that extended HPV genotyping (ie, “xGT”) is similar in efficacy to HPV combined screening and p16/Ki67 double staining for the detection of high-grade CIN. The detection of the HvpE7 protein and AX1/ZNF582 methylation is also considered to be a new diversion method applied in clinical studies.³⁸

The present study aimed to provide information beyond imaging data for vaginal colposcopy diagnosis. One limitation of this study was the small number of participants and the large number of HPV subtypes; as such, quantitative analysis of TCT and HPV data was not performed, and inclusion factors were also limited. Due to the retrospective design of this study, the results of some patient examinations were unknown, which may have led to biased results. More targeted research is required to systematically develop relevant standards for upgrading or downgrading colposcopy.

Abbreviations

ASC-H, Atypical squamous cells: cannot exclude high-grade squamous intraepithelial lesion; ASC-US, Atypical Squamous Cells of Undetermined Significance; HR HPV, High-risk human papilloma virus; HPV, human papilloma virus; TCT, Thin prep cytologic test; LSIL, Low-grade Squamous Intraepithelial Lesion; HSIL, High-grade Squamous Intraepithelial Lesion.

Ethical Approval

The study was carried out in accordance with the ethical standards laid down in the Declaration of Helsinki, and was approved by the ethics committees of Beijing Obstetrics and Gynecology Hospital, Capital Medical University(No:2023-KY-021-01).As the experiment was a retrospective study and did not have any impact on patient treatment or prognosis, the ethics committee agreed to exempt informed consent from the patients. Patient data will be safeguarded and kept strictly confidential.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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