Ann Med Res 2021;28(7):1366-70

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.07.764

Original Article

Comparison of colposcopic biopsy results of cervical cytology-negative and HPV 16/18 or other high-risk HPV subtypes

Zekiye Soykan Sert

Department of Gynecology and Obstetrics, Aksaray University Education and Research Hospital, Aksaray, Turkey

Abstract

Aim: This study aimed to compare the colposcopic biopsy results of patients with negative cervical cytology and positive human papillomavirus (HPV) tests and to investigate the necessity of colposcopy in patients with cytology-negative/other high-risk HPV (non-HPV 16/18) positive results.

Materials and Methods: The study included 126 patients aged 30–65 years who underwent HPV DNA testing between 2016 and 2019 and who underwent colposcopic biopsy at our hospital because of their positive results. The patients were divided into three groups: HPV16/18 positivity, other high-risk HPV positivity, and unclassified HPV positivity. Cytology and colposcopy-guided cervical biopsy results were compared with the HPV types.

Results: Approximately 44.4% of the patients had HPV 16/18 positivity, 23% had other high-risk HPV positivity, and 32.5% had unclassified HPV positivity. The cytology results revealed that 57.1% of patients were negative for intraepithelial lesions or malignancy (NILM), 22.2% had atypical squamous cells of undetermined significance, 17.5% had low-grade squamous intraepithelial lesions (LGSIL), and 2.4% had high-grade squamous intraepithelial lesions. Colposcopic biopsy results were normal in 15.1% of the patients, showed LGSIL in 15.9%, high-grade squamous intraepithelial lesions (HGSIL) in 10.3%, and cervical cancer in 0.8% of the patients. Evaluation of the biopsy results based on HPV type in the patients with NILM cytology revealed that 12.8% of those with HPV 16/18 positivity had LGSIL and 17.9% had HGSIL, whereas 7.1% of those with other high-risk HPV positivity had HGSIL and 7.1% had LGSIL.

Conclusions: The possibility of detecting dysplasia in the colposcopic biopsies of patients who are NILM and HPV 16/18-positive is higher than in the colposcopic biopsies of patients with other high-risk HPV types; therefore, these patients should be evaluated using colposcopic biopsy. Colposcopic biopsy is unnecessary in the presence of NILM and other high-risk HPV types.

Keywords: Biopsy; cytology; human papillomavirus

INTRODUCTION

Human papillomavirus (HPV), the most common sexually transmitted viral infection worldwide, is the most important cause of cervical cancer (1). More than 200 HPV types that are known to infect epithelial cells, including the skin, the respiratory mucosa, or the genital tract, have been identified, and in excess of 40 HPV types cause genital infections (2). Based on prevalence in patients with cervical carcinoma, anogenital HPVs are divided into high-risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82/MM4) and low-risk (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) groups (2) types. Additionally, HPV 26, 53, and 66 may be carcinogenic for humans (2). Approximately 70% of all invasive cervical cancers occur due to HPV 16/18, whereas about 20% occur due to other high-risk HPVs: 31, 33, 35, 45, 52, and 58 (3,4).

Papanicolaou (Pap) smear and HPV tests play an important role in early intervention for precancerous lesions and cervical cancer. The most important method in the management of abnormal Pap smear test results is colposcopic examination. Biopsy performed during colposcopy is considered the standard method for the diagnosis of cervical intraepithelial lesions (5,6). The American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommend colposcopic examination for patients with cytology-negative/HPV 16/18-positive results and co-testing repeated after one year for patients with other high-risk HPV positivity (7). Co-testing is defined as simultaneous Pap smear and HPV testing (7). Routine colposcopic examination is not recommended for other high-risk HPV types; however, studies have reported that HPV 45 and HPV 31 and 33 are responsible for 6% and 4% of cervical cancer cases, respectively (4).

Received: 22.07.2020 **Accepted:** 03.09.2020 **Available online:** 09.07.2021
Corresponding Author: Zekiye Soykan Sert, Department of Gynecology and Obstetrics, Aksaray University Education and Research Hospital, Aksaray, Turkey **E-mail:** zekiyesoykan@hotmail.com

In the present study, we aimed to evaluate the cytology and colposcopy-guided cervical biopsy results of patients with HPV positivity and to compare the biopsy results with the HPV types in patients with negative cervical cytology for intraepithelial lesions or malignancy.

MATERIALS and METHODS

This retrospective study was conducted between January 2016 and December 2019 at the Aksaray University Training and Research Hospital, Gynecology and Obstetrics Department. Patients aged 30–65 years who were referred to our hospital following an abnormal HPV test in the national cervical cancer screening program were evaluated. The study included 126 patients who underwent colposcopic examination and whose histopathological results could be accessed. The study was approved by the hospital ethics committee (2019/12-37).

The patients were divided into three groups: HPV 16/18 positivity, other high-risk HPV positivity, and unclassified HPV positivity. The cytology and colposcopy-guided cervical and/or endocervical biopsy results were compared with the HPV types.

Cytological evaluation was performed using a conventional Pap smear, and the results were evaluated using the Bethesda 2001 classification (8). Based on the cytology results, the patients were classified as follows: negative for intraepithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesions (ASC-H), low-grade squamous intraepithelial lesions (LGSIL), and high-grade squamous intraepithelial lesions (HGSIL).

Colposcopic evaluation was performed based on the International Federation for Cervical Pathology and Colposcopy Classification (9). After applying acetic acid to the cervix, acetowhite epithelium, atypical vascularization, mosaic, and punctuation areas were considered pathological. Biopsy samples were obtained from these areas using cervical punch biopsy and/or endocervical curettage. Colposcopy assessment for women with abnormal screening results was carried out by gynecologists. Histopathology revealed that LGSIL corresponded to cervical intraepithelial neoplasia (CIN) 1, and HGSIL corresponded to carcinoma in situ and CIN 2 and 3. The cytology and colposcopy-guided cervical biopsy results of the patients were recorded.

The patients with multiple HPV positivity, a history of known cervical dysplasia and/or surgery performed for this purpose, the presence of a disease that may have an effect on the immune system, HIV positivity, and presence of known gynecological malignancies as well as those whose files could not be accessed were excluded.

Statistical Analysis

Data were analyzed using Statistical Package Social Sciences (SPSS), version 22.0 (SPSS Inc., Chicago, IL, USA). The study data were evaluated using descriptive statistical methods (percentage calculations, median, mean, and standard deviation). Data were evaluated for normal distribution by Kolmogorov–Smirnov test. In the comparisons between groups, chi-squared test or Fisher's exact test were used for categorical variables. Kruskal–Wallis variance analysis was used for sequential variables. A p value of <0.05 was considered statistically significant.

RESULTS

As part of the national cervical cancer screening program, 325 women who were found to be HPV DNA-positive between 2016 and 2019 referred to our hospital. Colposcopy-guided cervical biopsy samples were obtained from 126 (42.5%) patients. The mean age of the patients was 45.5 ± 9.4 years. Sixty-nine (54.8%) patients were between 30 and 45 years old, and 57 (45.2%) patients were over 45 years old. Of the patients, 105 (83.3%) patients were multipara. Furthermore, 56 (44.4%) of the patients had HPV 16/18 positivity, 29 (23%) had other high-risk HPV (31, 33, 35, 39, 45, 51, 52, 58, or 68) positivity, and 41 (32.5%) had unclassified HPV positivity. Low-risk HPV genotypes were not detected in women who presented with HPV positivity. Other than HPV 16/18, the most common subtype was HPV 52.

Table 1. Distribution of HPV types, cytology and histopathological diagnoses of patients

Age, years	
30-45	69(54.8%)
>45	57(45.2%)
Parity	
0	4(3.1%)
1	17(13.4%)
≥2	105(83.3%)
HPV types	**n (%)**
HPV16/18	56(44.4%)
Other high-risk HPV	29(23%)
Unclassified HPV	41(32.5%)
Cytology diagnosis	
NILM	72(57.1%)
ASC-US	28(22.2%)
LGSIL	22(17.5%)
HGSIL	3(2.4%)
ASC-H	1 (0.8%)
Histopathology diagnosis	
Normal	19(15.1%)
Cervicitis	70(55.6%)
Metaplasia	2(1.6%)
LGSIL	20(15.9%)
HGSIL	13(10.3%)
Atypical cell	1 (0.8%)
Cervical cancer	1 (0.8%)

HPV: Human papillomavirus; NILM: Negative for malignancy and intraepithelial lesion; ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low grade squamous intraepithelial lesion; HSIL: High grade squamous intraepithelial lesion; ASC-H: Atypical squamous cells, cannot exclude HSIL

Table 2. Cytological and histopathological results according to HPV types

	HPV 16/ 18 positive n=56	Other high-risk HPV positive n=29	Unclassified HPV positive n=41	p value
Age (years)	46(38-51.8)	45(38.5-56.5)	43(37.5-47.5)	0.32
Cytology diagnosis				
NILM (n%)	39(69.6%)	14(48.3%)	19(46.3%)	
ASC-US (n%)	10(17.9%)	9(31.0%)	9(22.0%)	
LGSIL (n%)	6(10.7%)	4(13.8%)	12(29.3%)	
HGSIL (n%)	1(1.8%)	1(3.4%)	1(2.4%)	
ASC-H (n%)	0	1(3.4%)	0	
Histopathology diagnosis				
Normal (n%)	7 (12.5%)	6(20.7%)	6 (14.6%)	
Cervicitis (n%)	28(50%)	17(58.6%)	25(61%)	
Metaplasia (n%)	1(1.8%)	0	1(2.4%)	
LGSIL (n%)	9(16.1%)	4(13.8%)	7(17.1%)	
HGSIL (n%)	9(16.1%)	2(6.9%)	2(4.9%)	
Atypical cell (n%)	1(1.8%)	0	0	
Cervical cancer(n%)	1(1.8%)	0	0	

NILM: Negative for malignancy and intraepithelial lesion; HPV: Human papillomavirus; ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low grade squamous intraepithelial lesion; HSIL: High grade squamous intraepithelial lesion; ASC-H: Atypical squamous cells, cannot exclude HSIL

The cytology results revealed that 72 (57.1%) of the patients were NILM. The colposcopic biopsy results revealed that 19 (15.1%) were normal. The cytology and colposcopic biopsy results are shown in Table 1. The cytology and colposcopic biopsy results based on HPV types are shown in Table 2. Based on the biopsy results, the HGSIL rate in the HPV 16/18-positive group was higher than in the other high-risk HPV and the unclassified HPV groups (16.1%). The LGSIL rates were 9 (16.1%), 4 (13.8%), and 7 (17.1%) in the HPV 16/18-positive, other high-risk HPV, and unclassified HPV groups, respectively, according to HPV type.

Table 3. Comparison of cytology results with HPV types

	Cytology group		p value
	NILM (n:72)	≥ ASC-US (n:54)	
HPV types			0.04
HPV 16/18 (n%)	39(%54.2)	17(%31.5)	
Other high-risk HPV (n%)	14(%19.4)	15(%27.8)	
Unclassified HPV (n%)	19(%24.4)	22(%40.7)	

NILM: Negative for malignancy and intraepithelial lesion; HPV: Human papillomavirus; ASC-US: Atypical squamous cells of undetermined significance

Table 4. Histopathological diagnosis of patients with cytology NILM and ≥ASC-US according to HPV types

			Histopathology diagnosis						
Cytology group	**No. of cases**	**Age Mean±SD**	**Normal**	**Cervicitis**	**Metaplasia**	**LGSIL**	**HGSIL**	**Atypical cell**	**Cervical cancer**
NILM-HPV16/18	39(54.2%)	47±8	3(7.7%)	22(56.4%)	1(2.6%)	5(12.8%)	7(17.9%)	1(2.6%)	0
NILM-other high-risk HPV	14(19.4%)	50±9	4(28.6%)	8(57.1%)	0	1(7.1%)	1(7.1%)	0	0
NILM-unclassified HPV	19(24.4%)	46±9	3 (15.8%)	13(68.4%)	1(5.3%)	1(5.3%)	1(5.3%)	0	0
≥ ASC-US-HPV16/18	17(31.5%)	44±10	4(23.5%)	6(35.3%)	0	4(23.5%)	2(11.8%)	0	1(5.9%)
≥ASC-US-other high-risk HPV	15(27.8%)	42±10	2(13.3%)	9(60%)	0	3(20%)	1(6.7%)	0	0
≥ASC-US-unclassified HPV	22(40.7%)	41±6	3(13.6%)	12(54.5%)	0	6(27.3%)	1(4.5%)	0	0

NILM: Negative for malignancy and intraepithelial lesion; HPV: Human papillomavirus; ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low grade squamous intraepithelial lesion; HSIL: High grade squamous intraepithelial lesion

Approximately 39 (54.2%) of the patients with NILM cytology had HPV 16/18 positivity, 14 (19.4%) had other high-risk HPV positivity, and 19 (24.4%) had unclassified HPV positivity. The HPV types were significantly different between the groups with NILM and lesions of ASC-US and above (p = 0.04) (Table 3). Evaluation of the biopsy results based on the HPV types in the patients with NILM-HPV positivity revealed that five (12.8%) of those with HPV 16/18 positivity had LGSIL and seven (17.9%) had HGSIL, whereas one (7.1%) of those with other high-risk HPV positivity had HGSIL and one (7.1%) had LGSIL (Table 4). The biopsy results revealed that the HGSIL and LGSIL rates were higher in the patients with HPV 16/18 positivity than in those with other high-risk HPV and unclassified HPV positivity.

DISCUSSION

Currently, HPV tests and cytology examinations using Pap smears are widely used for screening and early diagnosis of preinvasive cervical lesions (10,11). Reportedly, the cytology results of some high-risk HPV-positive patients may be negative (10,12). The Pap smear test's sensitivity is less than 50%, and the potential for missing CIN or invasive cancer is more than 35% (13). In the present study, we aimed to determine the risk of developing cervical precancerous lesions in other high-risk HPV-positive patients with NILM cytology (non-HPV 16/18). The colposcopic biopsy examination revealed that the rate of LGSIL or HGSIL lesions among the NILM patients was higher in the HPV 16/18-positive patients than in the other high-risk HPV-positive patients (16.6% vs. 2.7%). HGSIL was detected in seven patients in the NILM-HPV 16/18-positive group and in one patient in the group with NILM cytology and other high-risk HPV positivity.

Based on the ASCCP guidelines, the management of patients with high-risk HPV positivity varies according to whether the cytology result is positive or negative (14). The ASCCP recommends two methods for the management of cytology-negative/high-risk HPV-positive women (14): co-testing repeated after one year or HPV genotyping for HPV 16/18. HPV 16/18-positive patients are directly referred for colposcopy, whereas repeating the co-testing after 12 months is recommended for HPV 16/18-negative patients (14). The cytology results of patients with high-risk HPV positivity are critical in management because the risk of developing cervical preinvasive lesions or cancer is directly proportional to persistent HPV infection and transition time. Prospective observational studies have reported that the risk of short-term CIN 3 in cytology-negative/HPV-positive patients is significantly lower than that in ASC-US-LGSIL/HPV-positive patients (15). In a study of nearly one million cervical cytology samples in the USA, a five-year cervical preinvasive lesion or cancer risk was evaluated. In that study, 3.6% of women were reported to be cytology-negative/HPV-positive with a five-year positive CIN 3 risk of 4.5% (16). In the present study, the biopsy results revealed LGSIL or HGSIL in 16.6% of the NILM-HPV 16/18-positive patients, 2.7% of the patients with NILM cytology and other high-risk HPV positivity, and 2.7% of the NILM-unclassified HPV-positive patients. We found that other high-risk HPV types (non-HPV 16/18) were associated with a low rate of cervical precancerous lesions. Consistent with the literature, the present study suggests that colposcopic biopsy is unnecessary in the presence of NILM cytology and other high-risk HPV positivity.

Fujiwara et al., in a study of cytology-negative/HPV-positive patients, detected LGSIL or HGSIL risk to be 3% in co-testing performed after one year (17). In the study by Castle et al. including 1,156,387 patients, 5% cotesting positivity was detected every year during a four-year co-testing follow-up (18). Sasaki et al. found that the rate of LGSIL or HGSIL was 7% in the follow-up of cytology-negative/high-risk HPV-positive patients (19). In the present study, 12.5% (9) of patients with the final diagnosis of HGSIL had NILM cytology; seven of these patients had HPV 16/18 positivity, one had other high-risk HPV positivity, and one had unclassified HPV positivity. Based on these results, we conclude that Pap smear is a screening test in the presence of HPV 16/18 and the diagnosis should be confirmed by colposcopy-guided biopsy. In the case of other HPV types, follow-up is required in accordance with the cervical screening program.

Previous studies have suggested that colposcopic examination is an appropriate management technique in women with NILM cytology and other high-risk HPV positivity (20,21). Kececioglu et al. performed a cost-effectiveness analysis to determine whether the HPV DNA test is a cost-effective alternative to immediate colposcopy or conservative treatment. The results confirmed that triage based on a positive HPV DNA test can detect more CIN 3 cases and is cheaper than urgent colposcopy (22). Preinvasive lesions can be effectively treated because the natural course of the disease is slow, and invasive cervical cancers usually have a long preinvasive disease stage. Therefore, considering the necessity of invasive methods such as colposcopy or biopsy used in the management of lesions, increasing demand, and the cost-effectiveness of personnel and equipment, we believe that patients with NILM cytology and other high-risk HPV positivity would benefit from annual cotesting follow-up.

LIMITATIONS

The main limitation of our study is that it was a single-center study with a limited number of patients. Another important limitation was that we failed to determine HPV type in all patients.

CONCLUSION

In contrast to studies suggesting that colposcopic examination can be an appropriate management method in patients with NILM cytology and other high-risk HPV-positivity, the present study revealed that colposcopic biopsy is unnecessary in the presence of NILM and other high-risk HPV types, which is consistent with the findings in the literature. Cervical cancer can be detected and prevented during the preinvasive period by continuous active monitoring at appropriate intervals. Thus, it would be possible to avoid missing an existing disease or performing unnecessary interventions.

Competing Interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical Approval: This study protocol was approved by Aksaray University Faculty of Medicine Clinical Research Ethics Committee with protocol number 2019/12-37.

REFERENCES

1. Zandberg DP, Bhargava R, Badin S, et al. The role of human papillomavirus in nongenital cancers. CA Cancer J Clin 2013;63:57-81.

2. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.
3. Clifford GM, Smith JS, Plummer M, et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003;88:63-73.
4. De Sanjose S, Quint WG, Alemany L, et al. Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048-56.
5. Poomtavorn Y, Suwannarurk K. Accuracy of visual inspection with acetic acid in detecting high-grade cervical intraepithelial neoplasia in pre- and post-menopausal Thai women with minor cervical cytological abnormalities. Asian Pac J Cancer Prev 2015;16:2327-31.
6. Kingnate C, Supoken A, Kleebkaow P, et al. Is Age an Independent Predictor of High-Grade Histopathology in Women Referred for Colposcopy after Abnormal Cervical Cytology? Asian Pac J Cancer Prev 2015;16:7231-5.
7. Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol 2013;121:829-46.
8. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287:2114-9.
9. Massad LS, Einstein MH, Huh WK, et al. 2012 ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol 2013;121:829-46.
10. Schiffman M, Burk RD, Boyle S, et al. A study of genotyping for management of human papillomavirus-positive, cytology-negative cervical screening results. J Clin Microbiol 2015;53:52-9.
11. Carozzi F, Ronco G, Confortini M, et al. Prediction of high-grade cervical intraepithelial neoplasia in cytologically normal women by human papillomavirus testing. Br J Cancer 2000;83:1462-7.
12. Kinney W, Fetterman B, Cox JT, et al. Characteristics of 44 cervical cancers diagnosed following Pap-negative, high risk HPV-positive screening in routine clinical practice. Gynecol Oncol 2011;121:309-13.
13. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.
14. Saslow D, Solomon D, Lawson HW, et al. ACS-ASCCP-ASCP Cervical Cancer Guideline Committee. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin 2012;62:147-72.
15. Kitchener HC, Almonte M, Thomson C, et al. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol 2009;10:672-82.
16. Katki HA, Kinney WK, Fetterman B, et al. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. Lancet Oncol 2011;12:663-72.
17. Fujiwara H, Suzuki M, Morisawa H, et al. The Impact of Triage for Atypical Squamous Cells of Undetermined Significance with Human Papillomavirus Testing in Cervical Cancer Screening in Japan. Asian Pac J Cancer Prev 2019;20:81-5.
18. Castle PE, Kinney WK, Xue X, et al. Role of Screening History in Clinical Meaning and Optimal Management of Positive Cervical Screening Results. J Natl Cancer Ins 2019;111:820-7.
19. Sasaki Y, Iwanari O, Arakawa I, et al. Cervical Cancer Screening With Human Papillomavirus DNA and Cytology in Japan. Int J Gynecol Cancer 2017;27:523-9.
20. Clifford GM, Smith JS, Aguado T et al. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer 2003;89:101-5.
21. Zhao XL, Hu SY, Zhang Q, et al. High-risk human papillomavirus genotype distribution and attribution to cervical cancer and precancerous lesions in a rural Chinese population. J Gynecol Oncol 2017;28:e30.
22. Kececioglu M, Seckin B, Baser E, et al. Cost and effectiveness comparison of immediate colposcopy versus human papillomavirus DNA testing in management of atypical squamous cells of undetermined significance in Turkish women. Asian Pac J Cancer Prev 2013;14:511-4.