Journal of Research Administration
Society of Research Administrators International

ASSOCIATION BETWEEN TYPE OR NUMBER OF HPV INFECTIONS AND CYTOLOGICAL AND COLPOSCOPY-DIRECTED BIOPSY ANALYSIS OF CERVIX UTERI

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ABSTRACT

Aim: The association of high-risk (hHPV) genotypes with discordance between cytological and histopathological analysis of cervix uteri has been reported in a limited number of studies. We aimed to compare the cytological and histopathological analysis of cervix uteri and association of these with HPV genotypes and clinical factors in HPV-positive adult patients.

Materials and Methods: Patients who underwent cervical screening, HPV genotype analysis, and colposcopy-directed biopsy after cervical screening were included in this study. Patients were excluded when the time between the cytology screen and cervix uteri biopsy was longer than six months, patients were excluded. Smoking status, gravida, history of previous childbirth, route of previous delivery, and oral contraceptive [O.C.] or intrauterine device [I.U.D.] use were recorded from electronic and written patient files. Cytological findings were classified as negative for intraepithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LGSIL), and histopathological findings as benign, LGSIL, or high-grade squamous intraepithelial lesions (HGSIL). In the cytological analysis, HGSIL was not detected, and we omitted the atypia of the glandular cells. HPV-positive samples were typed using by Anyplex II 28 (Seegene;VR, Seoul, South Korea). We grouped the patients with positive for HPV 16 or 18 as Group I and those positive for other HPV types as Group II. The number of positive HPV types in each patient was assessed and analyzed. We defined it as under call if NLIM on cytology was changed to LGSIL, or HGSIL, or ASCUS on cytology was changed to HGSIL on histopathological analysis. We analyzed the risk factors for undercall. If ASCUS cytology changed to a benign finding on biopsy, it was defined as a minor variance. If cytological and histopathological findings were consistent, or if ASCUS cytology was changed to LGSIL on biopsy, we defined it as an agreement. If LGSIL on cytology was changed to benign on histopathological analysis, it was defined as an: overcall.

Results: The median age of the patients was 36 (16-70) in Group I (n=29) and 33 (21-65) in Group II in Group II (n=38) (p=0.405). In Group I, HPV type 16 was detected in 23 patients and HPV type 18 in 10 patients, with both types detected in 4 patients. In Group II, more than one HPV type was detected in 29 patients. Of the patients with discordance between cytology and histopathology (n=25), undercall was detected in 19 patients. When compared with agreement, undercall was associated with Group I HPV genotypes (p=0.013), number of HPV types (p=0.018), history of normal spontaneous vaginal delivery (p=0.023), I.U.D. (p=0.036), or O.C. use (p=0.036). After

adjustment for age, Group I HPV genotype (p=0.028, OR: 4.693) and multiple HPV types (p=0.029, OR: 20.587) predicted undercall.

Conclusion: We observed a relatively higher discordance ratio between cytological and histopathological results. Analysis of HPV 16 and 18 seems to be, important for reducing the possibility of undercalling. Besides, undercall was an important finding in those patients with other HPV types. This study aims to analyze the identication of multiple HPV genotypes predicted undercall.

Funding Statement: The study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data Access Statement: Research data are not publicly available due to privacy and ethical restrictions.

Conflict of Interest declaration: The author declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Author Contributions: The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Keywords: HPV, cervical dysplasia, cervix, cytology, colposcopy, biopsy.

INTRODUCTION

Cervical cancer is one of the most common types of cancer detected in women, and more than 600,000 new cases of cervical cancer were diagnosed in 2020 worldwide, with more than 340,000 deaths (1). Cervical cancer is responsible for 7.7% of cancer deaths and, together with breast cancer, is the leading cause of cancer-related mortality in females. Mortality and incidence of cervical cancer were found to be significantly higher in transitioning countries than in transitioned ones (18.8 *vs.* 11.3 per 100,000 and 12.4 *vs.* 5.2 per 100,000, respectively) (1). The decreasing incidence of cervical cancer and the associated mortality rates in developed countries may be explained by screening and HPV vaccination programs.

Human Papillomavirus (HPV) is the most prevalent sexually transmitted infection and is responsible for cervical cancer and precancerous lesions. More than 200 HPV types, which may be subclassified as cutaneous or mucosal types, have been detected using D.N.A. sequencing (2, 3). They can also be divided based on their association with cervical cancer: high-risk genotypes (carcinogenic), of which HPV 16 is the most common, and low-risk genotypes, most often HPV types 6 and 11 (3, 4). HPV DNA has been found in 95% of patients with cervical cancer, with HPV 16 and 18 accounting for approximately 70% of these cases (5).

Cervical cancer may be entirely preventable by both primary (HPV vaccine) and secondary (screening) prevention measures. Most high-income countries have implemented a national HPV vaccination programs. Cervical cancer screening detects precancerous changes in the cervix uteri and is recommended for toall asymptomatic, immunocompetent patients agedbetween 30 and 65 years of age (6, 7). Different strategies may be applicable, such as primary HPV testing, a

combination of cervical cytology (Papanicolaou [Pap]) and HPV testing, or a Pap test alone. Several types of HPV tests may detect at least 11 HPV types based on D.N.A. or R.N.A. assay (6).

Colposcopy is a diagnostic procedure used to evaluate patients positive for high-risk HPV types and those with abnormal cervical smear results or a suspicious cervical appearance (8). A Ccolposcopy-directed biopsy is principally performed to differentiate high-grade from low-grade lesions and to prevent unnecessary excisions. Previous reports have showned that colposcopy-directed biopsy revealed different results differfrom those obtained usingcervical screening methods (9, 10). In one study, HPV types 16 and 18 were found to be responsible for the discrepancies between cytological and histopathological results (9). They showed that HPV 16 or 18 genotypes were found to increased the risk of cervical cancer in patients who were underestimated before biopsy. Similar results have been were also reported in several studies (11, 12). We aimed to analyze the cervical cytological and histopathological findings, the discordance between them, and the association between of HPV genotypes.

MATERIALS AND METHODS

Study Design

This retrospective study was conducted at Tınaztepe University Hospital and wasapproved by the local Ethics Committee of Tınaztepe University Hospital (with approval number 422-104). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all of the participants.

Patients older than 16 years who had undergone cervical screening and HPV genotype analysis between January 2017 and December 2021 in Gynecology and Obstetrics Clinics at Tınaztepe University Hospital were analyzed retrospectively. We included those patients who underwenthad undergonecolposcopy-directed biopsy after cervical screening in the study. The data were obtained from electronic and written patient files, which were obtained during routine gynecological examinations. Patients with a history of hysterectomy or for whom cervical cytological or histopathological findings were missing were excluded.

Data Collection

Demographic parameters (age), smoking status, and clinical parameters (gravida, history of previous childbirth, route of previous delivery, oral contraceptive [O.C.], andintrauterine device [I.U.D.] use were recorded from electronic and written patient files. The route of theprevious delivery was defined as either normal spontaneous vaginal delivery (NSVD) or cesarean section (C/S).

All cervical cytology specimens were obtained with a cervical brush and were collected in buffer for lysisbuffer. The specimens were also stored under the appropriate conditions for HPV testing. Thermo Scientific™ Cytospin™ 4 Cytocentrifuge System (Fisher Scientific, part of Thermo

Fisher Scientific Industry, U.S.A.) was used for thin-layer cell preparation. The cytocentrifugation technique provides flat cells and better visualization of cell nuclear images and uniform cell groups with a clear study of cells (13). We analyzed thecervical cytological findings and classified them based on the Bethesda system as follows: NLIM (negative for intraepithelial lesion or malignancy), atypical squamous cells of undetermined significance (ASCUS), or low-grade squamous intraepithelial lesion (LGSIL) (14,15). The atypia of glandular cells was not analyzed. No patients with HGSIL (high-grade squamous intraepithelial lesion) or ASC-H (atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion) were detected in our study.

HPV genotype analysis was performed together with cervical cytological examination in all participants. HPV DNA was detected as previously described by polymerase chain reaction (PCR) using consensus primers that amplify highly conserved regions of the HPV L1 gene: GP5þ/GP6þ primers, which amplify a region of 150 bp (16,17). The detection of HPV DNA was detected performedin duplicate to evaluate the efficiency of the amplification. HPV-positive samples were typed by Anyplex II 28 (SeegeneVR, Seoul, South Korea), a semiquantitative assay divided into set A, comprising high-risk (H.R.) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and set B, comprising probably high-risk HPV types (type 26, 53, 66,69, 73, and 82) and low-risk (L.R.) HPV types (6, 11, 40, 42, 43, 44, 54, 61, and 70). H28 uses HPV-specific dual priming oligonucleotides (D.P.O.) for multiplex (real-time) PCR. Recordings and expositions were automated using Seegene Viewer software according to perthe manufacturer's instructions.

We grouped the patients positive for HPV 16 or 18 as Group I and those positive for other HPV types as Group II. The number of co-existing positive HPV types in each patient was assessed and analyzed.

All colposcopic analyses were performed at the same center by the same gynecologist using a Dr. Camscope Video Colposcope (United States of America). Colposcopy-directed biopsy from the squamocolumnar junction was performed during colposcopy in a quadrant with a lesion or randomly if no visible lesion was present. Pathological analysis revealed subtypes based on morphological findings using hematoxylin and eosin staining: benign, low-grade squamous inraepithelial lesions (LGSIL), and HGSIL (high-grade squamous intraepithelial lesions (HGSIL) (14,15).

We defined under call as if NILM on cytology was changed to LGSIL or HGSIL, or if ASCUS on cytology was changed to HGSIL on histopathological analysis. We analyzed the risk factors forof undercall. If ASCUS cytology was changed to benign findings on biopsy, it was defined as a minor variance. If cytological and histopathological findings were consistent, agreement was defined as agreement. If ASCUS cytology was changed to LGSIL on biopsy, it was also defined as an agreement. If LGSIL on cytology was changed to benign on histopathological analysis, it was defined as anovercall.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS 25.0 (I.B.M. Corporation, Armonk, New York, United States). The conformity of the data to anormal distribution was evaluated using the Shapiro-Wilk Francia test. The homogenity of variance was evaluated using with the Levene's test. When comparing two independent groups of quantitative data according to each other, we used the independent-samples t-test with Bootstrap results or the Mann-Whitney U test with Monte Carlo results. When comparing categorical variables, the Pearson Chi-Square, the Fisher Exact, and Fisher-Freeman-Halton tests with the Monte Carlo simulation technique were used. Comparison of column ratios was expressed by Benjamini-Hochberg-corrected p values. The Odds Ratio with a 95%confidence interval was used to detect the proportion of those with a risk factor compared with those who were excluded. A logistic regression test was used to determine the cause-effect relationship between categorical variables and explanatory variables. Quantitative variables are expressed as mean (Standard Deviation) and median (minimum–maximum) values, and categorical variables as numbers (n) and percentages (%) in the tables. Variables were evaluated at a 95% confidence level, and a p-value<0.05 was accepted as statistically significant.

RESULTS

A total of 67 female patients were included. HPV type 16 was detected in 23 patients, and HPV type 18 was detected in 10 patients (Table 1). In Group I, HPV types 16 and 18 were detected in 4 patients. In Group II, more than one HPV type was detected in 29 patients.

Table 1. Distribution of the number of patients with different types of HPV.

HPV types	n	%
Group I		
16	23	19.2%
18	10	8.3%
Group II		
56	11	9.2%
31	10	8.3%
39	10	8.3%
35	8	6.7%
51	8	6.7%
66	7	5.8%
68	6	5.0%
52	5	4.2%
53	5	4.2%
59	5	4.2%
11	2	1.7%
42	2	1.7%
45	2	1.7%

58	2	1.7%
40	1	0.8%
43	1	0.8%
54	1	0.8%
73	1	0.8%

The median age of the patients was 35 (16-70), and the total number of patients was 67 (n=67), 36 (16-70) years in Group I (n=29), and 33 (21-65) in Group II (n=38) (p=0.405). The median number of co-existent HPV types was 1(1-8) in total, and the same in Groups I and II (p=0.513). The distribution of cytological and histopathological findings was similar in Groups I and II. Other clinical findings did not differ between the groups (Table 2).

Table 2. Comparison of clinical, cytological, and histopathological findings between Groups I and II.

	Total (n=67)	**Group I (n=29)**	**Group II (n=38)**	**p**
	median (min-max)	**median (min-max)**	**median (min-max)**	
Age	35 (16-70)	36 (16-70)	33 (21-65)	0.405 [u]
Number of co-existent different HPV types	1 (1-8)	2 (1-4)	1 (1-8)	0.513 [u]
Number of biopsy samplings	3 (2-7)	4 (2-7)	3 (2-7)	0.722 [u]
Gravida	1 (0-3)	1 (0-3)	1 (0-3)	0.230 [u]
	n (%)	**n (%)**	**n (%)**	
Number of co-existent different HPV types				0.677 [f]
1	34 (50.7)	13 (44.8)	21 (55.3)	
2	22 (32.8)	11 (37.9)	11 (28.9)	
≥3	11 (16.4)	5 (17.2)	6 (15.8)	
I.U.D. use				0.108 [c]
Absent	55 (82.1)	21 (72.4)	34 (89.5)	

	Present	12 (17.9)	8 (27.6)	4 (10.5)	
Smoking					0.225 [c]
	Absent	38 (56.7)	19 (65.5)	19 (50.0)	
	Present	29 (43.3)	10 (34.5)	19 (50.0)	
O.C. use					0.783 [c]
	Absent	49 (73.1)	22 (75.9)	27 (71.1)	
	Present	18 (26.9)	7 (24.1)	11 (28.9)	
Cytological finding					0.642 [f]
	NILM	50 (74.6)	20 (69.0)	30 (78.9)	
	ASCUS	12 (17.9)	6 (20.7)	6 (15.8)	
	LGSIL	5 (7.5)	3 (10.3)	2 (5.3)	
Histopathological findings					0.357 [f]
	Benign	41 (61.2)	15 (51.7)	26 (68.5)	
	LGSIL	21 (31.4)	11 (37.9)	10 (26.3)	
	HGSIL	5 (7.5)	3 (10.3)	2 (5.3)	
Previous childbirth					0.214 [c]
	Absent	27 (40.3)	9 (31)	18 (47.4)	
	Present	40 (59.7)	20 (69)	20 (52.6)	
Route of the previous delivery					0.346 [c]
	Absent	27 (40.3)	9 (31.0)	18 (47.4)	
	NSVD	18 (26.9)	8 (27.6)	10 (26.3)	
	C/S	22 (32.8)	12 (41.4)	10 (26.3)	

[U] Mann Whitney U test (Monte Carlo). [c] Pearson Chi-Square Test (Monte Carlo). [f] Fisher Freeman Halton Test (Monte Carlo)

Agreement between cytological and histopathological analyses was observed in 42 patients (62.68%). Minor variance was observed in 4 patients. In Group I, one patient with ASCUS cytology was diagnosed with LGSIL and two patients were diagnosed with HGSIL after histopathological examination. In Group I, nine patients with NILM cytology were diagnosed with LGSIL and one patient was diagnosed with HGSIL after histopathological examination. In Group II, three patients with ASCUS cytology were diagnosed with LGSIL and two patients were diagnosed with HGSIL after histopathological examination. In Group II, five patients with NILM cytology were diagnosed with LGSIL after histopathological examination (Table 3). The proportion of undercalls was 28.35% (n=19) in total, 41.37% (n=12) in Group I, and 18.42% (n=7) in Group II.

Table 3. Demonstration of discordance between cytological and histopathological findings in total, Groups I and II.

	Total (n=67)			**Group I (n=29)**			**Group II (n=38)**		
	Cytological Findings			**Cytological Findings**			**Cytological Findings**		
	NILM	**ASCUS**	**LGSIL**	**NILM**	**ASCUS**	**LGSIL**	**NILM**	**ASCUS**	**LGSIL**
	n (%)								
Histopathological Findings									
Benign	35 (70)	4 (33.3)	2 (40)	10 (50)	3 (50)	2 (66.7)	25 (83.3)	1 (16.7)	0 (0)
LGSIL	14 (28)	4 (33.3)	3 (60)	9 (45)	1 (16.7)	1 (33.3)	5 (16.7)	3 (50)	2 (100)
HGSIL	1 (2)	4 (33.3)	0 (0)	1 (5)	2 (33.3)	0 (0)	0 (0)	2 (33.3)	0 (0)
Comparison									
Minor Variance	0	4	0	0	3	0	0	1	0

Overall	0	0	2	0	0	2	0	0	0
Undercall	15	4	0	10	2	0	5	2	0
Agreement	35	4	3	10	1	1	25	3	2

We compared patients with agreement between cytological and histopathological findings and those undercall. The number of HPV types was higher in the undercall group (p=0.018). The proportion of patients in Group I was 63.2%, which was higher in the undercall group than in the agreement group (p=0.013). I.U.D. and O.C. use were higher in the undercall group than in the agreement group (p=0.036 and p=0.036, respectively). The incidence of NSVD was higher in the undercall group (p=0.023) (Table 4).

Table 4. Comparison of clinical, cytological, and histopathological findings between agreement and undercall groups.

Parameters	**Comparison of cytological and histopathological findings**		**p** [2]
	Agreement	**Undercall**	
	(n=42)	**(n=19)**	
	mean (S.D.)	**mean (S.D.)**	
Age	33.7 (9.9)	39.8 (13.1)	0.071 [t]
	median (min-max)	**median (min-max)**	
Number of co-existent different HPV types	1 (1 / 8)	2 (1 / 4)	**0.018** [u]
Gravida	1 (0 / 3)	2 (0 / 3)	0.062 [u]
	n (%)	**n (%)**	
Group			**0.013** [c]
I	12 (28.6)	12 (63.2) [A]	4.3 (1.4-13.5) [OR]
II	30 (71.4) [B]	7 (36.8)	
Number of co-existent different HPV types			**0.046** [ff]
1	26 (61.9) [B]	6 (31.6)	
2	13 (31.0)	8 (42.1)	
3≤	3 (7.1)	5 (26.3) [A]	7.2 (1.3-38.9) [OR]
IUD use			**0.036** [f]
Absent	37 (88.1)	12 (63.2)	4.3 (1.2-16.1) [OR]
Present	5 (11.9)	7 (36.8)	
Smoking			0.999 [c]
Absent	22 (52.4)	10 (52.6)	
Present	20 (47.6)	9 (47.4)	

O.C. use			**0.036** [c]
Absent	26 (61.9)	17 (89.5) [A]	5.2 (1.1-25.7) [OR]
Present	16 (38.1) [B]	2 (10.5)	
Previous childbirth			0.088 [c]
Absent	20 (47.6)	4 (21.1)	
Present	22 (52.4)	15 (78.9)	
Route of the previous delivery			**0.023** [c]
Absent	20 (47.6) [B]	4 (21.1)	
NSVD	8 (19.0)	10 (52.6) [A]	6.3 (1.5-25.9) [OR]
C/S	14 (33.3)	5 (26.3)	

[t] Independent Samples t Test (Bootstrap), [u] Mann Whitney U test Monte Carlo), [c] Pearson Chi-Square Test (Monte Carlo), [ff] Fisher Freeman Halton Test (Monte Carlo),[OR] Odds Ratio (95% Confidence interval), [A] SD: Standard Deviation

After adjusting for age, logistic regression analysis showed that Group I (HPV 16 and 18) predicted undercall (p=0.028; OR: 4.693 [1.181-18.648]). The higher number of co-existent HPV types was also an important predictor of undercall (p=0.029). The co-existence of ≥3 different types of HPV increased 20.587 (2.225-190.510) times the risk of undercall according to the existence of only 1 HPV type (p=0.008).

Table 5. Logistic regression analysis demonstrating the predictors of undercall.

	Not Adjusted for age				**Adjusted for age**			
			95% CI for OR				**95% CI for OR**	
	P	**OR**	**Lower Bound**	**Upper Bound**	**P**	**OR**	**Lower Bound**	**Upper Bound**
Group (I)	**0.029**	4.631	1.175	18.254	**0.028**	4.693	1.181	18.648
IUD use	0.277	0.438	0.099	1.941	0.281	0.440	0.099	1.955
OC use	0.080	4.763	0.832	27.264	0.097	5.087	0.746	34.698
Route of the previous delivery	0.444				0.506			
(Absent vs. C/S)	0.901	0.891	0.144	5.505	0.937	0.927	0.141	6.090
(NSVD vs. Absent)	0.255	2.454	0.522	11.529	0.310	2.693	0.398	18.223

(NSVD vs. C/S)	0.768	1.304	0.224	7.593	0.359	2.012	0.452	8.963
Number of co-existent different HPV types	**0.011**				**0.029**			
(3≤ vs. 1)	**0.003**	22.244	2.913	169.880	**0.008**	20.587	2.225	190.510
(3≤ vs. 2)	**0.028**	8.968	1.265	63.587	0.052	8.340	0.983	70.797
(2 vs. 1)	0.783	1.212	0.309	4.749	0.071	34.055	0.739	1569.291

Multiple Logistic Regression (Method = Enter) OR: Odds Ratio CI: Confidence Interval

DISCUSSION

Undercall was detected in approximately one-half of the patients in Group I but in less than one-fifth of those in Group II. Approximately 40% of patients in Group I, 80% of those in Group II, and more than 60% in total were consequent with the fcytological and histopathological findings. Group I (HPV 16 and 18) co-existence of multiple HPV genotypes, I.U.D. or O.C. use, and NSVD were associated with undercall. Logistic regression showed that the presence of Group I HPV types (16 and 18) increased approximately 4.7 times the risk of undercall, with the co-existence of multiple HPV genotypes (≥3 vs. 1) increasing it by approximately 20.6 times.

Cytological analysis of cervix uteri has been recommended for screening all asymptomatic, immunocompetent women with a cervix between the ages of 30 and 65 years (6). In clinical practice, not all women for whom a Pap smear is obtained undergo pathological analysis. Possible discrepancies between cytological and histopathological analyses may explain under or over diagnosis and, hence, may result in under treatment or over treatment of abnormalities of the cervix uteri. Recently, several studies have investigated the discordance between the findings of cytological and pathological results of the uterine cervix (18, 19). In one study, negative Pap smear tests in HPV-positive patients in whom HGSIL was detected by pathological analysis were reviewed (18). They observed atypical features in 63.1% of patients on a review of smear preparations. In some studies, discrepancies between cytology and histopathology were defined and categorized as either major or minor discrepancies, but there was no consistency in the literature (19, 20). In the guidelines, minor variations and minor and major discrepancies are clearly defined (21, 20). We did not subdivide the discordance into major or minor categories. In our study, there was no patient in whom HGSIL was detected on cytology in any of the patients. This might have precluded the correct assessment of overcalls in our study. In one study, which included 323 cytological and histopathological analyses of cervix uteri, major and minor discrepancies were detected in 5.8% and 18.8% of cases, respectively, according to routine methods (19). They found minor variance in 2.3%, major discordance in 6.4%, and minor

discrepancy in 18.2% according to the criteria reported in the previous guidelines, in which atypical features of glandular cells were also evaluated (21). They demonstrated that undercall might result from screening, sampling, or interpretative errors in cervical smears and overcall due to interpretative or sampling errors. We could not analyze the factors that might have led to overcall or undercall. In our study, the results of cytological and pathological analyses were retrospectively compared and analyzed; however, but they were not re-examined by the pathologists. We found that HPV 16 and 18, co-existence of multiple HPV genotypes, O.C. or I.U.D. use, and NSVD were associated with undercall. In the studies conducted before the guidelines were reported, A.G.C. lesions were not included in the interpretation of the discordance (21). We did not include atypical features of glandular cells.

Pap smear tests failed to detect clinically significant precancerous lesions and cancers at a rate of approximately 50% (22). The rate of discordance between cytological and histopathological findings was approximately 37% in our study, which is higher than that previously noted in the literature(30%) (19,20,23). Heterogeneity may be due to cervical biopsy type, choice of study population, inadequate sampling, biopsy errors, or cytological errors, which were reported as significant reasons for discrepancies. In our study, the rate of discordance between cytological and histopathological findings was higher in those patients with ASCUS (66.7%) than in those with NILM or LGSIL on cytological examination. In one study, the rate of concordance between cytological and histopathological findings was approximately 100% for HGSIL cytology (24). No patients were diagnosed with HGSIL in our study. In another study, the LGSIL group was shown to have the highest rate of discrepancy (20).

The relationship betweenof HPV infections and the development of cervical cancer has been previously documented (25). The prevalence of HPV was reported as 70-90% in C.I.N. 2-3 (26). High-risk HPV positivity was found in 98% of the patients with high-grade cervical neoplasia (25). Given the relatively high rate of discrepancy between cytology and histopathology, and the association of HPV with cervical neoplasia, the determination of high-risk HPV genotypes has become important in detecting high-grade squamous intraepithelial neoplasia or cervical cancer.

The number of studies analyzing the effect of high-risk HPV genotypes on the ratio of the discordance between cytological and histopathological examinations of cervix uteri was limited. We showed that HPV 16 and 18 increased about 4.7 times the risk of under call. We analyzed HPV 16 and 18 in a different group from the other high-risk HPV genotypes because they possess the highest risk for cervical cancer and are responsible for more than 70% of the cases of cervical cancer cases. In a large retrospective study analyzing patients with ASCUS cytology, high-risk HPV positivity was found to be associated with undercall (12). Another study analyzing the discrepancy between cytological and histopathological examinations in 52 women found that an increase in the degree of cytological atypia decreased this discrepancy (20). They found that a significant discrepancy (undercall) was reported in approximately about 40% of the patients. High-risk HPV positivity was shown in nearly all patients with ≥ C.I.N. 2 in that study and was found to be associated with a significant discrepancy (undercall). They included only those patients with documented discordance between cytological and histopathological findings, which

was a significantly different from our study. We included those women for HPV analysis was performed. Using logistic regression analysis, we demonstrated that HPV 16 and 18 are predictors of undercall. In one study analyzing 3,798 women, approximately 80% were positive for HPV, and the ratio of undercall was approximately 10% (9). Logistic regression analysis showed that HPV 16 and 18 increased the risk of undercall by approximately two times and four times the risk of under call when comparing HPV negative and other high-risk HPV genotypes, respectively, in logistic regression analysis. They also showed that patients with negative result for HPV or other high-risk HPV genotypes were found to be at a high risk for overcall. We did not include HPV-negative patients. These findings suggest that the determination of high-risk HPV genotypes together with cytological sampling is very important in women's's further management strategies. Colposcopy and, if necessary, colposcopy-directed biopsy for high-risk HPV genotypes are precise strategies. Given the relatively higher ratio of discrepancy between the cytological and histopathological analyses, especially concerning undercall, the high-risk HPV test has acquired greater prominence. The Australian Society for Colposcopy and Cervical Pathology recommends colposcopy in patients with HPV 16 or 18 positivity, regardless of cytological findings (27).

Besides the association of high-risk HPV genotypes with undercall, we showed that a considerable number of patients with HPV types other than those with the highest risk (HPV 16 or 18)] had discordance of cytological findings with histopathological analysis. In a large study, HPV 16 or 18 positivity was found to increased the risk of undercall, with an undercall rate of 7.4% shown in those patients with other high-risk HPV genotypes (9). It was seen that the ratio of undercall was 2.4% in HPV-negative patients; however, but no direct comparison was made between HPV-negative patients and with others. In another study analyzing those women with ASCUS cytology and high-risk HPV genotypes, cervical intraepithelial neoplasia two positivity was observed in 21.1% of HPV 16, 15.2% of HPV 33, 10% of HPV82, and 9.9% of HPV 18 genotypes (12). In our study, undercall was detected in approximately one-fifth of patients positive for HPV genotypes other than 16 or 18. Therefore, undercall still remains a considerable but debatable problem for those women with HPV genotypes other than types 16 or 18. We did not include the HPV-negative patients and could not predict the risk of undercall associated with other HPV genotypes. Future studies including HPV-negative patients may indicate if that patients with positive HPV genotypes other than 16 or 18 possess a higher risk for undercall than HPV negative ones.

Few studies have investigated multiple HPV infections. In one study that including a population with a low rate of vaccination against HPV, multiple HPV infections was detected in about half of the participants (28). H.I.V. infection has been demonstrated to increase the risk of infection with multiple different types of HPV (29). We found that the coexistence of multiple HPV types (≥3) of HPV infection significantly increased the risk of undercall. To our knowledge, no other reports in the literature previously revealed such a finding. Different types of HPV tests are available; however, but based on our findings, we propose that testing a higher number of different HPV genotypes may help with further management.

The method of cervical biopsy is also important in evaluating the discordance between cytological and histopathological analyses. We performed colposcopy-directed biopsy. One study compared the pathological results of colposcopy-directed biopsy and loop electrosurgical excisional procedure (LEEP) in women with cytological HGSIL (30). They also performed HPV testings in more than half of the patients. They revealed that the high-risk HPV genotype was not associated with the discrepancy between colposcopy-directed biopsy and LEEP. Relatively high undercall rates of colposcopy-directed biopsy compared to LEEP have been reported in several studies, including various patient baseline cytological results (30-32). It would be beneficial to evaluate the relationship between HPV genotypes and the discordance between cytological findings and histopathological examinations in subjects undergoing colposcopy-directed biopsy and LEEP.

Strength and Limitations

A limited number of studies have indicated the association of HPV types 16 and 18 with the discordance between cytological and histopathological findings. Our study is the first to analyze the coexistence of multiple HPV genotypes as an important predictor of undercall. We analyzed a broad spectrum of HPV genotypes. This study was conducted retrospectively. We could not re-examine the samples and, hence, could not determine whether any analytic or technical factors might underlie this discordance. We did not analyze the sexual preferences of the patients.

CONCLUSIONS

Discordance between cytological and histopathological findings was more frequent in our study than in previous reports and was higher in ASCUS cytology. HPV types 16 and 18 and the coexistence of multiple HPV types were predictors of undercall. Undercalls were also associated with a history of NSVD, I.U.D., or O.C. use. Based on the critical association between HPV with a high rate of discordance and cervical neoplasia, we suggest the identification of high-risk HPV genotypes. In addition, undercall also seems to be found in an important ratio of patients with HPV genotypes other than 16 or 18. If supported by new studies, management strategies may be changed based on a more extensive range of different HPV genotypes beyond just HPV types 16 or 18 alone. In future studies, it may be useful to analyze various cytological results, such as atypical glandular cells or HGSIL, and to include pathological findings obtained not only by colposcopy-directed biopsy but also with LEEP in a patient population with a broad spectrum of HPV genotypes, and also in HPV-negative patients.

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