The role of multiple high-risk human papillomavirus infections for cervical biopsies and findings in colposcopic procedures

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Abstract

Objective: The clinical outcome of high-risk HPV (hr-HPV) infection varies according to genotype(s). Patients may harbor either one single hr-HPV (s-HPV) or multiple HPV (m-HPV) genotypes. Recently, the relationship between m-HPV infections and high-grade dysplasia has been investigated, and controversial results have been obtained. Therefore, the clinical significance of m-HPV is not clear. This study aimed to evaluate which group is associated with higher grade dysplasia by analyzing colposcopic punch biopsies.

Material and Methods: A total of 690 patients who were scheduled for a diagnostic excisional procedure between April 2016 and January 2019 due to the detection of high-grade cervical intraepithelial neoplasia (CIN 2/3) in colposcopy were included. Patients who were not scheduled for colposcopic examination or cervical punch biopsy, or who were scheduled for an excisional procedure due to smear-biopsy incompatibility or persistent low-grade dysplasia were excluded. Patients with a negative HPV test and an unknown HPV genotype were also excluded.

Results: Among the patients scheduled for excision (n=404), 74.5% had a s-HPV and 25.5% had a m-HPV infection. The proportion of CIN 1, 2 and 3 per patient in the m-HPV group was significantly higher than the s-HPV group (p=0.017). When this analysis was made for the number of CIN 2+3 per patient in the s-HPV and m-HPV groups, it was 1.29 (389/301) and 1.36 (140/103), respectively, and no difference was found (p=0.491).

Conclusion: Patients in the m-HPV group, who underwent more colposcopic cervical biopsies, had higher numbers of CIN lesions, regardless of age and cytology results. (J Turk Ger Gynecol Assoc 2023; 24: 101-8)

Keywords: Cervical dysplasia, cervical intraepithelial neoplasia, colposcopy, HPV, multiple HPV infection

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Introduction

Every year, 570,000 patients are diagnosed with cervical cancer worldwide, and approximately 310,000 patients die (1). Although human papillomavirus (HPV), especially high-risk HPV (hr-HPV), infection is a prerequisite for cervical cancer, additional risk factors leading to viral persistence play an important role in the oncopathogenesis (2-4). More than 200 HPV genotypes have been identified and the relationship between some genotypes and cervical cancer has been well elucidated (5,6). Based on cervical cancer cases and control group studies, "the International Agency of Research on Cancer (IARC)" has reported that different HPV genotypes have different oncogenic risks.

The IARC divides HPV genotypes for all cancers that may be associated with HPV into four risk groups: carcinogens (group 1), probably carcinogenic (group 2A), possibly carcinogenic (group 2B), and unclassifiable (group 3) (7). Furthermore,

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the genotypes are classified with either sufficient and limited evidence. Twenty different hr-HPV genotypes were identified in these two groups: HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as having sufficient evidence, and HPV genotypes 26, 53, 66, 67, 68, 70, 73, and 82 as having limited evidence (8). Other HPV genotypes were defined as low or undetermined risk. Due to these risk differences, patients can be referred for colposcopy according to their HPV genotypes and cervical cytology results. Additionally, patients may harbor either one or multiple HPV genotypes. Recently, the relationship between multiple hr-HPV (m-HPV) infections and high-grade dysplasia has been investigated but, controversial results have been reported. Therefore, the clinical significance remains unclear. In a few studies, m-HPV infections are associated with cervical cancer, high-grade dysplasia, and larger cervical lesions (9-14). In contrast, Muñoz et al. (15) found no difference between the single hr-HPV (s-HPV) and m-HPV groups in terms of cervical cancer risk. However, the number of cervical punch biopsies performed in these groups was not compared.

The aim of the present study was to evaluate which group was associated with more diffuse or higher-grade dysplasia by analyzing the number of colposcopic cervical punch biopsies performed in the s-HPV and m-HPV groups.

Material and Methods

Patients included in the study had attended the gynecological oncology department due to abnormal Pap smear and/or hr-HPV genotype(s) and were scheduled for a diagnostic excisional procedure between April 2016 and January 2019 due to the detection of high-grade cervical intraepithelial neoplasia (CIN 2/3) in colposcopy. If available, patient age, cervical cytology results, HPV test results, number of colposcopic cervical punch biopsies and histopathology results, endocervical curettage (ECC) results and diagnostic excisional procedure type were retrieved from patient files and computer records.

Written and verbal consent was obtained for a standardized colposcopic examination and punch biopsy ± ECC to be performed when necessary. All colposcopies were performed by the same team in the gynecological oncology department using the Olympus OCS 500 and Leisegang colposcopy devices. A biopsy was not performed in the cervical quadrant which had no abnormal findings on colposcopy. Colposcopic cervical sterile punch biopsies were performed under local anesthesia or sedation. The biopsies were then sent to the pathology department in formaldehyde, with a label indicating the patient name, file number, and biopsy clock dial. All punch biopsies and curettage materials were evaluated by gynecopathologists, and all histopathological results were reported according to the American Society for Colposcopy and Cervical Pathology guidelines.

Patients who were not scheduled for colposcopic examination or cervical punch biopsy, or who were scheduled for an excisional procedure due to smear-biopsy incompatibility or persistent low-grade dysplasia were excluded. Patients with microinvasive or invasive cancers detected on cervical cytology or colposcopic punch biopsy were also excluded. Pap smear results, reported using Bethesda (2014), were evaluated in five groups: benign (no dysplasia or cervicitis); atypical squamous cells-undetermined significance (ASC-US); atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesions (ASC-H); low-grade squamous intraepithelial lesions (LSIL); and high-grade squamous intraepithelial lesions (HSIL). In addition, no patients had cervical cytology reported as atypical glandular cells or adenocarcinoma in situ. Cytology was characterized into two groups; Cytology group A and B. Cytology group A consisted of “ASC-H” and “HSIL” while Cytology group B consisted of “ASC-US”, “LSIL” or “no dysplasia”. The results of HPV-DNA (Qiagen HC2) tests conducted in the community-based national HPV screening program conducted by the Ministry of Health, General Directorate of Public Health, Cancer Department, were evaluated. Patients with negative HPV test results or unknown HPV genotype, and those infected with HPV genotypes with low or undetermined risk for cervical cancer were excluded. Only patients infected with IARC hr-HPV genotypes with sufficient and limited evidence of cervical cancer were evaluated. According to the patient’s HPV genotypes, those with HPV-16 and/or HPV-18 positivity were classified as HPV group A, those with other hr-HPV genotype positivity were classified as group B, and those with HPV-16 and/or 18 and other hr-HPV genotype positivity were classified as group C. Patients infected with only one hr-HPV type were included in the s-HPV group, and those infected with at least two different hr-HPV genotypes were included in the m-HPV group. ECC results were characterized into two groups; ECC group A and B. ECC group A consisted of “CIN 2” or “CIN 3” while ECC group B consisted of “CIN 1” or “no dysplasia”. Written and oral consent was obtained from all patients before surgery. The study was reviewed by the University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital Local Ethics Committee and was performed under the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000 (approval number: 28, date: 05.02.2020).

Statistical analysis

IBM SPSS, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used for the statistical analysis. The mean, median, and standard deviation (SD) were used in the descriptive statistics of the data. Student’s t-test was used to compare the mean values. Non-parametric tests were used to analyze categorical and

dichotomous variables, whereas parametric tests were used to analyze continuous variables with a normal distribution. Significance was set at $p<0.05$.

Results

Of the 690 patients included at baseline, 203 patients did not meet the HPV criteria (167 patients with HPV-negative or unknown HPV genotype and 36 patients with low or unclassified risk), 42 patients with persistent low-grade dysplasia, 24 patients without colposcopy (gross cervical lesion, cervical cancer), and 17 patients scheduled for excision due to smear-biopsy incompatibility were excluded. In total, 404 patients with high-grade dysplasia (CIN 2/3) were included (Figure 1). The HPV genotypes, cervical cytology results, colposcopic punch biopsy counts, ECC results, and excisional procedure types are shown in Table 1. Among the patients scheduled for excision, 74.5% (301/404) had a s-HPV and 25.5% (103/404) had a m-HPV infection. The mean age of the patients was 40.5±0.4 years and the mean age of the m-HPV group was significantly lower than that of the s-HPV group (p=0.032). There was no significant difference between the percentage distribution of

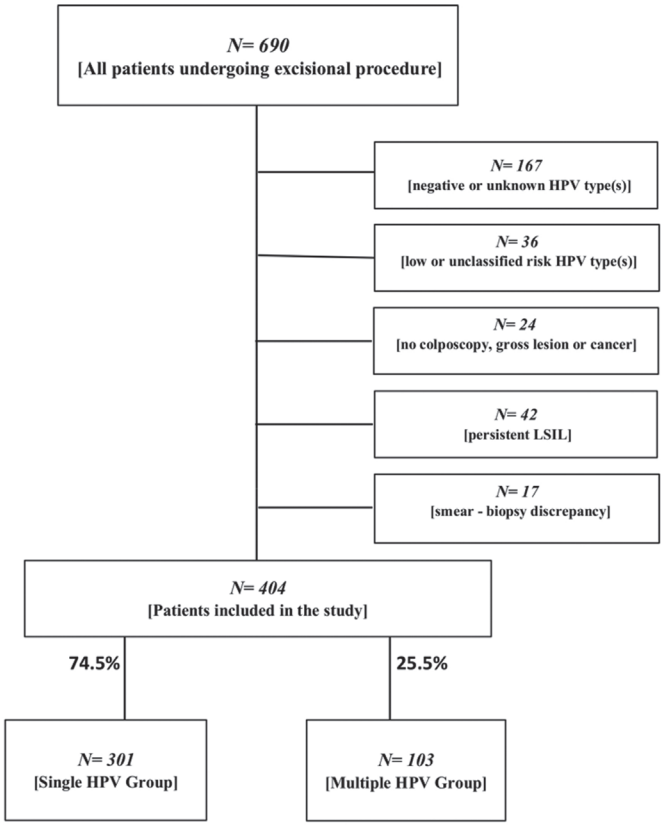

Figure 1. Inclusion and exclusion criteria are shown in the flow chart of this study
HPV: Human papillomavirus, LSIL: Low-grade squamous intraepithelial lesion

Table 1. Pathological data and HPV genotype related characteristics (n=404)

		%	n
HPV genotyping			
↗	HPV 16 positivity	54.7 ↘	221
HPV group A →	HPV 18 positivity	4.2 → 60.6	17
↘	HPV 16 and 18 positivity	1.7 ↗	7
HPV group B →	HPV others high risk (not including 16 or 18)	19.1	77
HPV group C →	HPV others high risk and HPV 16 and/or 18	20.3	82
Cervical cytology			
No lesion or cervisitis		54.2	219
ASC-US		12.6	51
ASC-H		6.2	25
LSIL		12.4	50
HSIL		5.4	22
Inadequate sampling		9.2	37
Single or multiple HPV status			
s-HPV group		74.5	301
m-HPV group		25.5	103
Colposcopic cervical biopsy count			
One biopsy		25.0	101
Two biopsies		35.2	142
Three biopsies		27.7	112
At least four biopsies		12.1	49
ECC results			
No lesion or cervisitis		54.2	219
CIN 1		6.4	26
CIN 2		11.9	48
CIN 3		24.0	97
Inadequate sampling		2.5	10
Not performed		1.0	4
Excisional procedure type			
LEEP		10.6	43
CKC		74.0	299
Other center or refuse the treatment		15.4	62

HPV: Human papillomavirus, ASC-US: Atipical squamous cells-undetermined significance, ASC-H: Atipical squamous cells-cannot exclude high grade squamous intraepithelial lesions, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, LEEP: Loop electrosurgical excision procedure, CKC: Cold knife conization, s-HPV: Single HPV, m-HPV: Multiple HPV, ECC: Endocervical Curettage, CIN: Cervical intraepithelial neoplasia

the ECC groups (p=0.214) and cytology groups (p=0.710) in the s-HPV and m-HPV groups. All statistical analyses are presented in Table 2.

A total of 17 different genotypes were available (except for HPV-26, -67, and -73) from 20 different HPV genotypes that were reported as high risk. In the s-HPV (73.4%) and m-HPV (68.9%) groups, there was no difference in the percentage of existing HPV-16 infection, which is the most common genotype (p=0.380). In the s-HPV group, the second most common genotype was HPV-31 (6%) and the third was HPV-18 (5.6%). In the m-HPV group, the most common types, excluding HPV-16, were HPV-18 (31.1%), HPV-51 (20.4%), HPV-31 (16.5%), and HPV-39 (16.5%). In the m-HPV group, 62.1% (64/103) of the patients were infected with two, 22.3% (23/103) with three, 10.7% (11/103) with four, and 4.9% (5/103) with five HPV genotypes. All hr-HPV genotypes in this study, except for HPV-16, were present at a higher rate in the m-HPV group than in the s-HPV group (Table 3).

At least one and at most five cervical biopsies were performed for all patients. When the rates of having ≥3 biopsies and having ≤2 biopsies were analyzed, the rate of having ≥3 biopsies was higher in the m-HPV group (p=0.003). In the m-HPV group, 52.5% of the patients had ≥3 biopsies (Figure 2). Furthermore, the mean number of biopsies was significantly higher in the m-HPV group tha the s-HPV group (2.53 vs 2.21, respectively; p=0.005). The calculated mean biopsy numbers of HPV groups A, B and C were 2.27, 2.14, and 2.50, respectively, with no statistical differences between the groups (p=0.061).

The total number of punch biopsies was 665 for 301 patients in the s-HPV group and 261 for 103 patients in the m-HPV group. The detection rates of CIN 1, 2 and 3 in punch biopsies taken in the s-HPV and m-HPV groups were calculated as 72.3% (481/665) and 73.2% (191/261), respectively and in terms of detecting dysplasia, there was no statistical difference between the groups (p=0.794) (Figure 3). The detection rates of CIN 1, 2, and 3 in the cytology A and B groups were calculated as 75.4% (101/134) and 72.1% (505/700), respectively, and again there was no difference between the groups (p=0.442). However, the

Table 2. Pathological data and HPV genotype related characteristics in single (s) or multiple (m) high-risk HPV groups

	HPV groups		
	s-HPV	m-HPV	p-value
Ages (n=404)			
Mean of age	41.06	38.97	p^a=0.032
Referral cytologies (n=367*) (%)			
No dysplasia	158 (57.9)	61 (64.9)	p=0.725
ASC-US	41 (15.0)	10 (10.6)	
ASC-H	20 (7.3)	5 (5.3)	
LSIL	38 (13.9)	12 (12.8)	
HSIL	16 (5.9)	6 (6.4)	
Cytology group A	36 (13.2)	11 (11.7)	p=0.710
Cytology group B	237 (86.8)	83 (88.3)	
ECC results (n=390) (%)**			
ECC group A	113 (39.0)	32 (32.0)	p=0.214
ECC group B	177 (61.0)	68 (68.0)	
Number of colposcopic biopsies (n=404) (%)			
One biopsy	84 (27.9)	17 (16.5)	
Two biopsies	110 (36.5)	32 (31.0)	
Three biopsies	73 (24.3)	39 (37.9)	
Four biopsies	28 (9.3)	12 (11.7)	
Five biopsies	6 (2.0)	3 (2.9)	
One or two biopsies	194 (64.5)	49 (47.6)	p=0.003
At least three biopsies	107 (35.5)	54 (52.4)	
Mean number of biopsies	2.21	2.53	p^a=0.005

Cytology group A: Consist of ASC-H and HSIL, Cytology group B: Consist of no dysplasia, ASC-US or LSIL, ECC group A: Consist of CIN 2 or CIN 3, ECC group B: Consist of CIN 1 or no dysplasia. N: Number of patients, N*: Inadequate sampling excluded, N**: Unknown ECC results excluded, %: Percent, p: Obtained by Pearson χ^2, p^a: Obtained by Independent Samples t-test

Table 3. Percentages of high-risk HPV genotypes found in single (s) or multiple (m) groups

HPV Genotypes	s-HPV	%	m-HPV	%
16	221	73.4	71	68.9
18	17	5.6	32	31.1
26	-	-	-	-
31	18	6.0	17	16.5
33	9	3.0	10	9.7
35	6	2.0	12	11.7
39	2	0.7	17	16.5
45	3	1,0	15	14.6
51	5	1.7	21	20.4
52	3	1.0	13	12.6
53	-	-	8	7.8
56	3	1.0	7	6.8
58	6	2.0	8	7.8
59	1	0.3	14	13.6
66	2	0.7	8	7.8
67	-	-	-	-
68	3	1.0	8	7.8
70	-	-	3	2.9
73	-	-	-	-
82	2	0.7	2	1.9

HPV: Human papillomavirus, s-HPV: Single HPV, m-HPV: Multiple HPV

number of CIN 1, 2, and 3 per patient in the m-HPV group was significantly higher than in the s-HPV group (p=0.017). All the statistical analyses are detailed in Table 4. The age distribution of the HPV groups is shown in Figure 4.

The power analysis of the study was calculated using OpenEpiPower to compare the two means calculators at www.openepi.com. The mean number of biopsies in the s-HPV and m-HPV groups was 79.8% at a 95% confidence interval with ± SD values.

Discussion

The prevalence of m-HPV in infected patients is reported to range between 18.5% and 46% (9,14,16). In the present study the m-HPV rate was 25.5% and significantly more punch biopsies were performed in this group. m-HPV infection is associated with larger lesions and more severe dysplasia (10,12,16,17). It has also been discussed in microdissection studies that s-HPV infection may cause dysplasia, and m-HPV infection may be associated with a greater number of cervical dysplasias (18,19). The findings of the present study support these previous reports that infection with more than one HPV genotype causes lesions that are more common and can involve more than one quadrant. However, Li et al. (20) reported that high-grade dysplasia and cancer lesions are more common in s-HPV infections. In the present study, the counts of CIN 2 and 3 lesions per patient did not differ between the s-HPV and m-HPV groups.

Lesion size is related to cervical cytology severity (21,22). However, Nam et al. (23) found no relationship between cytology results and quadrant involvement. Cytology and ECC results can be seen as confounding factors in these three previous studies. However, we believe that because these possible confounding factors did not show a significant distribution difference in the s-HPV and m-HPV groups, it makes our findings more robust. In addition, the present study provided an opportunity to examine age-related changes in a patient with high grade dysplasia and hr-HPV positivity. When the distribution of the population by age was examined, both the m-HPV and s-HPV groups showed a single peak between the ages of 30 and 40. Since the mean age of the m-HPV group was younger, it appears that infections with hr-HPV of more than one genotype decrease as patient age increases. This may be associated with an individual's immunity or a decreasing number of sexual partners with age.

Statistical analysis also allowed us to examine the HPV hierarchy in the group to be excised. Exception for HPV-16, 16 different hr-HPV genotypes had higher rates in the m-HPV group than in the s-HPV group. Furthermore, the similar distribution of the HPV-16 infection in the s-HPV and m-HPV groups was at least as valuable as the other confounding factors such as the ECC and cytology groups. The close relationship of the HPV-18 genotype with CIN 2, 3, and cancer lesions has been shown in previous studies (24-26). In our results, HPV-18 infection was found more frequently in the m-HPV group, suggesting that the mechanisms of oncopathogenesis in m-HPV infections may work differently than what has previously been suggested. Moreover, the fact that 54.2% of the patients had normal cytology results emphasizes the importance of cervical cancer screening using this co-test.

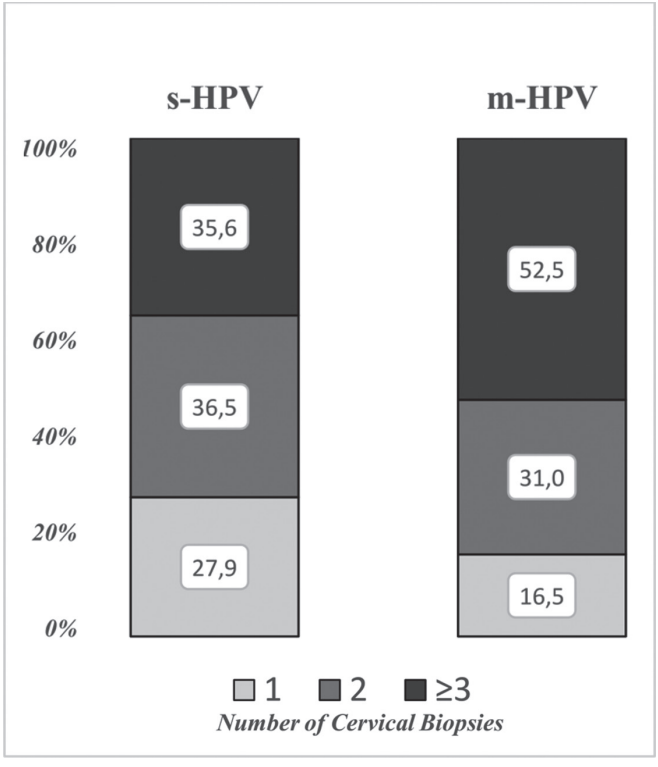

Figure 2. Percentage distributions of cervical punch biopsy count according to single or multiple high-risk HPV groups
m-HPV: Multiple HPV, s-HPV: Single HPV, HPV: Human papillomavirus

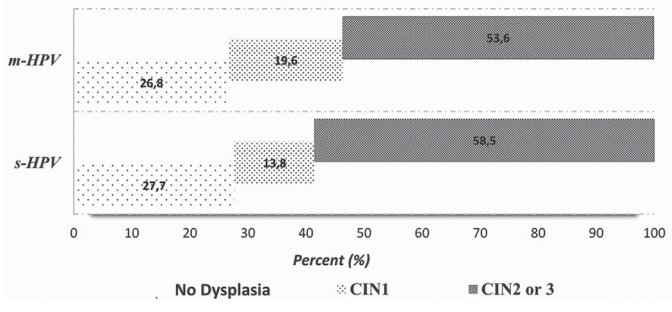

Figure 3. Percents of high-risk s-HPV and m-HPV groups according to no dysplasia, CIN 1 or CIN 2-3
m-HPV: Multiple HPV, s-HPV: Single HPV, CIN: Cervical intraepithelial neoplasia, HPV: Human papillomavirus

Table 4. Distribution of biopsy results in high-risk single (s) or multiple (m) HPV groups

		s-HPV				m-HPV				
	Punch biopsy count	**Patient (n)**	**Punch biopsies (Total)**	**Punch biopsies (DD)**	**%**	**Patient (n)**	**Punch biopsies (total)**	**Punch biopsies (DD)**	**%**	**p-value**
Normal/cervisitis	Total	301	665	184	27.7	103	261	70	26.8	p=0.794
CIN 1	Total	301	665	92	13.8	103	261	51	19.6	p=0.031
CIN 2, 3	Total	301	665	389	58.5	103	261	140	53.6	p=0.179
	DD Punch Biopsy per patient (DD/N) (CIN 2-3)									
	1.29					1.36				p^b=0.491
	1	84	84	77	91.7	17	17	16	94.1	p^a=1.000
	2	110	220	164	74.5	32	64	50	78.1	p=0.559
	3	73	219	144	65.8	39	117	78	66.7	p=0.866
CIN 1, 2, 3	≥4	34	142	96	67.6	15	63	47	74.6	p=0.314
	Total	301	665	481	72.3	103	261	191	73.2	p=0.794
	DD Punch biopsy per patient (DD/N) (CIN 1-2-3)									
	1.60					1.85				p^b=0.017

P: Obtained by Pearson χ^2, P^a: A obtained by Fisher's exact test, p^b= Obtained by Independent samples t-test, n: Number, DD: Dysplasia detected, CIN: Cervical intraepithelial neoplasia

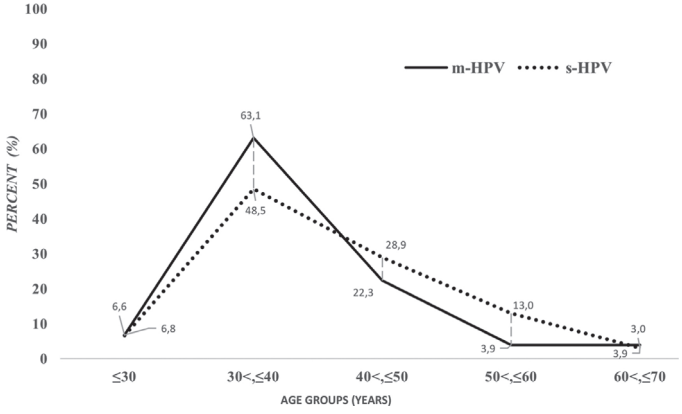

Figure 4. Proportional distribution of single and multiple high-risk HPV genotypes according to age groups
m-HPV: Multiple HPV, s-HPV: Single HPV, CIN: Cervical intraepithelial neoplasia, HPV: Human papillomavirus

The possibility of detecting lesions increases with a higher number of biopsies (21,27). In another study, the benefit of obtaining multiple biopsies, independent of cytology and HPV-16 status, was reported (28). In the current study, the number of dysplasias per patient in the s-HPV and m-HPV groups was 1.60 and 1.85, respectively, with a significant difference between these groups. These results support the hypothesis that because there are more colposcopic findings in the m-HPV group, more biopsies are performed.

This study is valuable because of the number of biopsies and the prevalence of dysplasia in light of HPV genotypes in a large group of patients with high-grade lesions, planned excision, and infection with hr-HPV genotypes. The possible limitations of this study include retrospective design, HPV and cervical cytology results were known before colposcopic procedures, two different colposcopes were used, and colposcopy was performed by four different experts. However, even if physicians feel the need to perform more biopsies in cytology reports, such as HSIL or ASC-H, the distribution of these cytologies in the s-HPV and m-HPV groups did not differ. By excluding IARC defined low-risk HPV genotypes from the study, patient groups were more homogeneous leading to more robust findings. Owing to the high rate of patients needing ECC, we were also able to analyze the ECC results in the s-HPV and m-HPV groups. The most important parameter that distinguishes this study from others is the examination of the colposcopy punch biopsy counts and HPV genotypes of patients who were scheduled for excision due to the detection of CIN 2/3. Therefore, we evaluated patients with persistent hr-HPV genotypes infections, that is, those diagnosed with CIN 2 or 3 in the colposcopic biopsy. As this study was not a colposcopy accuracy study, evaluating all patients who underwent colposcopy would lead to an increase in confounding factors. All patients had hr-HPV genotype(s) specified by the IARC and high-grade pre-invasive disease. In this specific population, if the relationship between colposcopic punch biopsies and dysplasia detected in these biopsies with HPV genotypes is analyzed, the most valuable and accurate result can be achieved. If we evaluated a newly infected population (insufficient virus persistence), it would be expected that there would be fewer abnormal findings on colposcopic examination. Therefore, the mean number of punch biopsies would be lower. This could lead to

misinterpretation of HPV genotypes found in newly infected patients as low-risk. We also had the opportunity to compared the distribution of hr-HPV genotypes and their hierarchies in this specific group.

Conclusion

Patients in the m-HPV group, who underwent more colposcopic cervical biopsies, had higher numbers of CIN lesions, regardless of age and cytology results. However, there was no relationship between the increase in the number of biopsies and the detection of high-grade lesions. The mechanisms by which m-HPV infections cause dysplasia, how they spread to different quadrants, and why they show more CIN lesions should be investigated.

Ethics Committee Approval: *The University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital Institutional Review Board approved the study (approval number: 28, date: 05.02.2020).*

Informed Consent: *Written and oral informed consents were obtained from all patients.*

Peer-review: *Externally peer-reviewed.*

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