The histologic results in multiple-type HPV infections

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Abstract

Objective: To evaluate the rate, cytology and histopathological findings of multiple-type human papillomavirus (HPV) positive women referred to a tertiary colposcopy center. To compare the role of multiple- and single-type HPV infections in women with high-grade squamous intra epithelial lesion and cervical cancer (HSIL+).

Material and Methods: The cytological and histopathological results of 2070 HPV positive women were evaluated. Infection with more than one type was defined as multiple-type HPV infection. Patients were divided into single or multiple HPV groups and subgroups in terms of HPV types; and also examined in three age groups. Age-stratified HSIL and cervical cancer rates of the study groups were compared.

Results: The women with multiple HPV subtypes accounted for 24.9% of the study population. Multiple-type HPV infection rates in normal cytology, atypical glandular cells, low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells, cannot exclude HSIL and HSIL were 28.2%, 26.8%, 19.3%, 22.6%, and 21.8%, respectively. Age stratified multiple-type HPV infection rates in under 30 years, 30-49 years and ≥50 years were 27.8%, 24.1%, and 27.3%, respectively. The multiple-type HPV infection rates in LSIL, HSIL, and cancer patients were 31.4%, 19%, and 12.5%, respectively.

Conclusion: Multiple-type HPV infections were statistically less common in HSIL and cancer patients than single type HPV infection. However, multiple type infection rates were remarkable in older HSIL and cervical cancer patients. [J Turk Ger Gynecol Assoc. 2025; 26(2): 90-7]

Keywords: Multiple-type HPV infections, cervical cancer, cytology, histopathologic results

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Introduction

According to GLOBOCAN statistics in 2018, with 570,000 new cases and 311,000 deaths, cervical cancer is the fourth most common gynecological malignancy and human papilloma viruses (HPVs) are the main etiological factor in the development of cervical cancer (1). Analysis of cervical neoplasia lesions show the presence of HPV in of 99.7% of all cervical cancers (2).

Over recent years, pap smear and advanced HPV DNA testing have become more widely used in cervical cancer screening. Routine HPV screening have shown that there are over 200 HPV subtypes, and approximately 40 of them infect the genital tract (3-5). They are classified as high-risk (hr) and low-risk

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(lr) according to oncogenic potential. Infection with HPV is extremely common, but the majority of women infected with HPV do not develop cervical malignancy (6). In addition, the hr types of HPV are generally asymptomatic and, unless tested, women are unaware that they have an HPV infection.

Due to advances in HPV tests in secondary protection, there has been a transition from conventional pap tests, to combined screening or primary HPV-based screening methods (7,8). HPV-based screening systems and more sensitive HPV tests have shown the occurrence of multiple HPV infections and knowledge of these infections was limited (9,10). In addition, it was reported that positive HPV test results, particularly positivity for multiple types of HPV, cause serious fear and anxiety about cervical cancer in the patient group (11).

It is accepted that persistent infections with HPV 16 and HPV 18 types alone are responsible for 70% of cervical cancer (12). On the other hand the rate of infections caused by multiple HPV types is not definitely known. Moreover, their role in cytological abnormalities, cervical preinvasive lesions and cervical cancer is not fully elucidated. Many authors reported possible interactions between types (9,13-15). In contrast, some authors have reported that infections with multiple HPV types occur independently of one another (5,16).

There are still unanswered questions about multiple HPV infections. It is important that clinicians be informed correctly and they enlighten their patients. The aim of this study was to investigate the rate and role of multiple HPV positivity in patients diagnosed with cervical preinvasive lesions and cancer.

Material and Methods

Patients who were referred to our colposcopy clinic from the national screening program and other centers where opportunistic screening was performed, and who underwent colposcopy for the first time between January 2015 and August 2018 were included in the study. All the data of the patients were obtained from Hospital Data Management System and from the colposcopy records. Ethical permission for a retrospective study was granted by the Etlik Zübeyde Hanım Women's Health Education and Research Hospital's Institutional Review Board (approval number: 17, date: 15.11.2019). Patients who had previous colposcopy, conization, a history of hysterectomy, colposcopy due to vulvar diseases, and those who were pregnant were excluded from the study. The HPV tests of patients who were referred from national screening program were made with the Hybrid Capture 2 and genotypings were performed with the CLART kit (Genomica), while the tests of patients referred from opportunistic screening were examined by polymerase chain reaction. Conventional pap test constituted most of the cytological studies. Cytological results were classified according to the Bethesda System. All patients were informed about colposcopy and follow-up procedures and informed consents were obtained. Samples obtained by colposcopic biopsy, loop electrosurgical excision procedure (LEEP) and cold knife conization were evaluated and reported by pathology specialists experienced in Gynecologic Oncology according to the 2011 LAST classification system. For each women the worst cervical histology was defined as the diagnostic end point.

For analysis, the study population was divided into single-type HPV (women with single HPV positivity), and multiple-type HPV (women with multiple HPV positivity). In order to evaluate the effect of single and multiple infections in HPV 16-18 and other types of HPV groups in more detail in the HSIL and cancer patients, HPV subgroups were formed and compared. The term HSIL+ is used hereafter to describe women with HSIL, adenocarcinoma *in situ* or invasive adenocarcinoma, microinvasive or invasive squamous cell carcinoma; carcinoma other than cervix. Four HPV subgroups were formed as follows: group 1: patients with single HPV 16 or 18 positivity; group 2: patients with single other hr (non-HPV 16 and 18) HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68); group 3: patients with multiple HPV 16, 18, and other HPV types; and group 4: multiple other hrHPV types Figure 1. The patients were further evaluated by stratifying into three age groups: under 30 years; 30-49 years; and ≥50 years. HSIL and cancer rates in subgroups were compared after age-stratification.

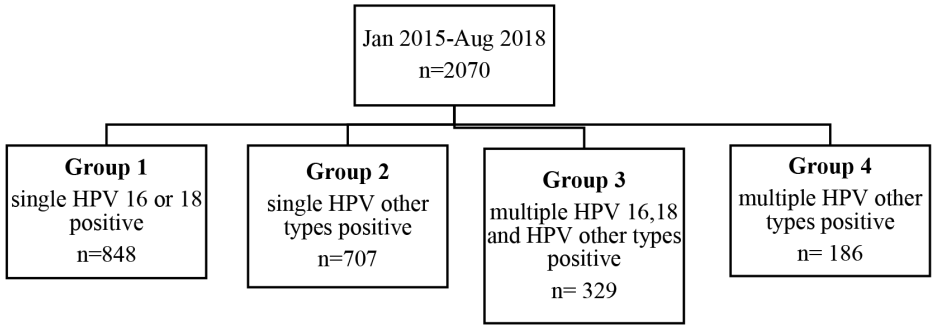

Figure 1. Flowchart of included cases
HPV: Human papillomavirus

Statistical analysis

SPSS, version 17.00 was used for statistical analyses (IBM Inc., Armonk, NY, USA). Quantitative data are expressed as means and standard deviations, and categorical data are described using counts and percentages. Chi-square and Kruskall Wallis tests were used to compare the categorical results of study groups. A $p<0.05$ was accepted statistically significant.

Results

The mean age of the 2070 women included in the study was 44.59±9.24 years. Table 1 summarizes the general characteristics of women entered the study.

The six most common HPV types were HPV16, HPV18, HPV51, HPV31, HPV 52, HPV 45 and the rates were 48.9%, 10.4%, 8.2%, 6.8%, 6.5% and 0.4% respectively. Although it constituted about half of the study group, the type present at the lowest rate in multiple infections was HPV 16.

Multiple HPV rates, based on age groups, are presented in Table 2. Multiple HPV rates were higher in the young (<30 years) and, surprisingly, the over 50 year-old age group, but the difference was not statistically significant ($p>0.05$).

Single and multiple HPV rates based on age-stratified cytology results of the study groups are shown in the Table 3. Multiple HPV rate was 28.2% in patients with normal cytology, 23.5%, 19.3%, 22.6% and 21.7% in atypical squamous cells (ASC) of undetermined significance, low-grade squamous intraepithelial lesion (LSIL), ASC-H, HSIL, respectively. In women with cytology and ≥50 years old, 60% had multiple HPV types, while the rate of multiple HPV infection in patients with normal cytology was 41.7% under 30 years of age and 32.8% in over 50 years of age.

Single and multiple HPV rates based on age-stratified histopathological results of the are shown in Table 4. Based on definitive histopathological results of cervical biopsy, cold knife conization and LEEP interventions, multiple HPV infection rates were 31.4% in LSIL, 32.8% in SIL, 19% in HSIL, and 12.5% in cervical cancer.

Based on age-stratified histopathological results, multiple HPV infection rates in women with LSIL were 33.3% under 30 years of age, and 38.7% in women over 50 years of age. Multiple HPV infection rate was 14% in women over 50 years old with HSIL, and 10% in women over 50 years old with cervical cancer.

Twenty four patients (1.2%) had cervical cancer. When the cervical cancer group was evaluated as adenocarcinoma or squamous cell type, multiple HPV infection rates were 33.5% and 5.6%, respectively. Multiple HPV positivity was detected in 2 of 3 patients (66.7%) diagnosed with non-cervical cancer.

Age-stratified histopathologically HSIL+ rates are presented in Table 5. None of the group under 30 years of age had cancer and only one patient had HSIL so this group was excluded from analysis. HSIL+ rates were significantly higher in group 1 than group 2, group 3 and group 4 in the other age groups ($p<0.001$).

Table 1. Patients characteristics (n=2070)

		n (%)
Age groups	<30	36 (1.7)
	30-49	1,432 (69.2)
	≥50	602 (29.1)
HPV groups	Group 1 (single type)	848 (41)
	Group 2 (single type)	707 (34.2)
	Group 3 (multiple type)	329 (15.9)
	Group 4 (multiple type)	186 (9)
Number of HPV	Single type	1,555 (75.1)
	2 types	354 (17.1)
	3 types	120 (5.8)
	4 types	31 (1.5)
	5 types	6 (0.3)
	6 types	4 (0.2)
Cytology	Normal	468 (22.6)
	Other†	912 (44.1)
	AGC	30 (1.4)
	ASCUS	328 (15.8)
	LSIL	233 (11.3)
	ASCH	53 (2.6)
	HSIL	46 (2.2)
Diagnostic procedure	Cervical biopsy	484 (18.7)
	ECC	365 (17.6)
	Cervical biopsy + ECC	844 (40.8)
	None	375 (18.2)
Intervention	Cold knife conization	266 (12.9)
	LEEP	43 (2.1)
	Other§	83 (4.0)
Histopathology	Benign	1023 (49.4)
	LSIL	287 (13.9)
	SIL	70 (3.4)
	HSIL	284 (13.7)
	Cancer (cervix)	24 (1.2)
	Cancer (non-cervical)	3 (0.1)

AGC: Atypical glandular cells, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, ASCH: Atypical squamous cells, cannot exclude high-grade intraepithelial lesion, SIL: Squamous intraepithelial lesion in which low and high-grade squamous intraepithelial lesion cannot be decided, HSIL: High-grade squamous intraepithelial lesion, HPV: Human papillomavirus, Other†: Infection, insufficient cytology, ECC: Endocervical curettage, LEEP: Loop electrosurgical excision procedure, Other§: Patients lost to follow-up after diagnostic procedure. Group 1: Single HPV16/18, group 2: Single HPV other types, group 3: Multiple HPV 16/18 and other HR types, group 4: Multiple other HPV types

Table 2. Multiple HPV rates in age-stratified HPV groups

Age groups	HPV groups		HPV subgroups				
	Single type n (%)	Multiple type n (%)	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	Total n (%)
<30	26 (72.2)	10 (27.8)	12 (33.3)	14 (38.9)	6 (16.7)	4 (11.1)	36 (100)
30-49	1.091 (76.2)	341 (23.8)	615 (42.9)	476 (33.3)	227 (15.8)	114 (8.0)	1.432 (100)
≥50	438 (72.8)	164 (27.2)	221 (36.8)	217 (36.0)	96 (15.9)	68 (11.3)	602 (100)
Total	1.555 (75.1)	515 (24.9)	848 (41.0)	707 (34.1)	329 (15.9)	186 (9.0)	2.070 (100)

HPV subgroups: Group 1: Single HPV16/18, group 2: Single HPV other types, group 3: Multiple HPV 16/18 and other HR types, group 4: Multiple other HPV types, HPV: Human papillomavirus

Table 3. Age-stratified cytology results of study groups

Cytology	Age groups	HPV groups		HPV subgroups				Total n (%)
		Single type n (%)	Multiple type n (%)	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	
n	<30	7 (58.3)	5 (41.7)	6 (50.0)	1 (8.3)	3 (25.0)	2 (16.7)	12 (100)
	30-49	241 (74.2)	84 (25.8)	164 (50.5)	77 (23.7)	67 (20.6)	17 (5.2)	325 (100)
	≥50	88 (67.2)	43 (32.8)	51 (38.9)	37 (28.2)	36 (27.5)	7 (5.3)	131 (100)
	Total	336 (71.8)	132 (28.2)	221 (47.2)	115 (24.6)	106 (22.6)	26 (5.6)	**468 (22.6)**
AGC	<30	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
	30-49	19 (79.2)	5 (20.8)	9 (37.5)	10 (41.7)	4 (16.7)	1 (4.2)	24 (100)
	≥50	2 (40.0)	3 (60.0)	2 (40.0)	0 (0.0)	1 (20.0)	2 (40.0)	5 (100)
	Total	22 (73.3)	8 (26.7)	11 (36.7)	11 (36.7	5 (16.7)	3 (10.0)	**30 (1.4)**
ASCUS	<30	8 (66.7)	4 (33.3)	2 (16.7)	6 (50.0)	3 (25.0)	1 (8.3)	12 (100)
	30-49	175 (78.5)	48 (21.5)	70 (31.4)	105 (47.1)	19 (8.5)	29 (13.0)	110 (100)
	≥50	68 (73.1)	25 (26.9)	28 (30.1)	40 (43.0)	8 (8.6)	17 (18.3)	93 (100)
	Total	251 (76.5)	77 (23.5)	100 (30.5)	151 (46.0)	30 (9.1)	47 (14.3)	**328 (15.8)**
LSIL	<30	8 (88.9)	1 (11.1)	2 (22.2)	6 (66.7)	0 (0.0)	1 (11.1)	9 (100)
	30-49	146 (80.2)	36 (19.8)	56 (30.8)	90 (47.5)	15 (8.2)	21 (11.5)	80 (100)
	≥50	34 (81.0)	8 (19.0)	14 (33.3)	20 (47.6)	4 (9.5)	4 (9.5)	42 (100)
	Total	188 (80.7)	45 (19.3)	72 (30.9)	116 (49.8)	19 (8.2)	26 (11.2)	**223 (10.7)**
ASCH	<30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)
	30-49	26 (76.5)	8 (23.5)	20 (58.8)	6 (17.6)	5 (14.7)	3 (8.8)	34 (100)
	≥50	15 (79.0)	4 (21.0)	3 (15.8)	12 (63.2)	1 (5.3)	3 (15.8)	19 (100)
	Total	41 (77.4)	12 (22.6)	23 (43.4)	18 (34.0)	6 (11.3)	6 (11.3)	53 (2.6)
HSIL	<30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)
	30-49	25 (73.5)	9 (26.5)	22 (64.7)	3 (8.8)	4 (11.8)	5 (14.7)	34 (100)
	≥50	11 (91.7)	1 (8.3)	6 (50.0)	5 (41.7)	1 (8.3)	0 (0.0)	12 (100)
	Total	36 (78.3)	10 (21.7)	28 (60.9)	8 (17.4)	5 (10.9)	5 (10.9)	**46 (2.2)**
Other	<30	2 (100)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)
	30-49	459 (75.2)	151 (24.8)	274 (44.9)	185 (30.3)	113 (18.5)	38 (6.2)	610 (100)
	≥50	220 (73.3)	80 (26.7)	117 (39.0)	103 (34.5)	45 (15.0)	35 (11.7)	300 (100)
	Total	681 (74.5)	231 (25.3)	393 (43.1)	288 (31.6)	158 (17.3)	73 (8.0)	912 (44.1)
Total		1,555 (75.1)	515 (24.9)	848 (40.96)	707 (34.15)	329 (15.9)	186 (8.98)	**2,070 (100)**

N: Normal, AGC: Atypical glandular cells, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, ASCH: Atypical squamous cells, cannot exclude high-grade intraepithelal lesion, HSIL: High-grade squamous intraepithelial lesion, HPV: Human papillomavirus, Other: Infection, insufficient cytology, group 1: Single HPV16/18, group 2: Single HPV other types, group 3: Multiple HPV 16/18 and other HR types, group 4: Multiple other HPV types

HSIL+ rates in group 2 and group 4 were not significant being p=0.763 in <50 year-olds and p=0.317 in ≥50 year-olds. Single HPV types were more prevalent in HSIL+ patients. In older patients HSIL+ rates in group 2 and in group 3 were not different.

HPV type distribution in cases with HSIL and cancer are presented in Table 6. While the rate of multiple HPV was 28.1% in the older age group (age ≥50 years), in younger women (30-49 years age group) multiple HPV types were the causal agent in 19% of those with HSIL.

Table 4. Age-stratified histopathologic findings of study groups

Histopathology	Age groups	HPV groups		HPV subgroups				Total n (%)
		Single type n (%)	Multiple type n (%)	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	
Benign	<30	6 (60.0)	4 (40.0)	3 (30.0)	3 (30.0)	3 (30.0)	1 (10.0)	10 (100)
	30-49	493 (75.8)	157 (24.2)	253 (38.9)	240 (36.9)	105 (16.2)	52 (8.0)	650 (100)
	≥50	259 (71.3)	104 (28.7)	125 (34.4)	134 (36.9)	58 (16.0)	46 (12.7)	363(100)
	Total	758 (74.1)	265 (25.9)	381 (37.2)	377 (36.9)	166 (16.2)	99 (9.7)	**102 3 (49.4)**
LSIL	<30	6 (66.7)	3 (33.3)	1 (11.1)	5 (55.6)	0 (0.0)	3 (33.3)	9 (100)
	30-49	161 (70.3)	68 (29.7)	86 (37.6)	75 (32.8)	42 (18.3)	26 (11.4)	229 (100)
	≥50	30 (61.2)	19 (38.8)	17 (34.7)	13 (26.5)	11 (22.4)	8 (16.3)	49 (100)
	Total	197 (68.6)	90 (31.4)	104 (36.2)	93 (32.4)	53 (18.5)	37 (12.9)	**287 (13.9)**
SIL	<30	2 (100)	0 (0.0)	1 (50.0)	1 (50,0)	0 (0.0)	0 (0.0)	2 (100)
	30-49	32 (65.3)	17 (34.4)	26 (53.1)	6 (12.2)	13 (26.5)	4 (8.2)	49 (100)
	≥50	13 (68.4)	6 (31.6)	9 (47.4)	4 (21.1)	5 (26.3)	1 (5.3)	19 (100)
	Total	47 (67.1)	23 (32.9)	36 (51.4)	11 (15.7)	18 (25.7)	5 (7.1)	**70 (3.4)**
HSIL	<30	1 (100)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0,0)	1 (100)
	30-49	186 (79.8)	47 (20.2)	150 (64.4)	36 (15.5)	39 (16.7)	8 (3.4)	233 (100)
	≥50	43 (86.0)	29 (24.6)	29 (58.0)	14 (28.0)	5 (10.0)	2 (4.0)	50 (100)
	Total	230 (81.0)	54 (19.0)	180 (63.4)	50 (17.6)	44 (15.5)	10 (3.5)	**284 (13.7)**
Cevical cancer	<30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)
	30-49	12 (92.3)	1 (7.7)	10 (76.9)	2 (15.4)	0 (0.0)	1 (7.7)	13 (100)
	≥50	9 (81.8)	2 (18.2)	9 (81.8)	0 (0.0)	2 (18.2)	0 (0.0)	11 (100)
	Total	21 (87.5)	3 (12.5)	19 (79.2)	2 (8.3)	2 (8.3)	1 (4.2)	**24 (1.2)**
Non-cervical cancer	<30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)
	30-49	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)
	≥50	1 (33.3)	2 (66.7)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)
	Total	1 (33.3)	2 (66.7)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	**3 (0.1)**
Total		1,254 (74.2)	437 (25.8)	720 (42.6)	534 (31.6)	284 (16.8)	153 (9.0)	1,691 (100)

Benign, histopathologic results such as cervicitis, polyps, microglanduler hyperplasia. LSIL: Low-grade squamous intraepithelial lesion, SIL: Squamous intraepithelial lesion grade cannot decided low or high, HSIL: High-grade squamous intraepithelial lesion cervical cancer, SCC: Microinvasive or invasive squamous cell carcinoma, HPV: Human papillomavirus, Non-cervical cancer, carcinoma other than cervix, group 1: Single HPV16/18, group 2: Single HPV other types, group 3: Multiple HPV 16/18 and other HR types, group 4: Multiple other HPV types

Table 5. Age-stratified HSIL+ rates of HPV subgroups

HPV subgroups	Age groups	30-49		≥50	
		HSIL+ n (%)	p	HSIL+ n (%)	p
Group 1		161/627 (25.7)	<0.001	38/221 (17.2)	<0.001
Group 2*[a]		38/490 (7.8)		15/217 (6.9)	
Group 3[a]		39/233 (16.7)		8/96 (8.3)	
Group 4*		9/118 (7.6)		3/68 (4.4)	

HSIL+: High-grade squamous intraepithelial lesion, adenocarcinoma *in situ* or invasive adenocarcinoma, microinvasive or invasive squamous cell carcinoma; carcinoma other than cervix, Chi-square tests: HSIL+ rates were statistically significant higher in group 1 than group 2, group 3, and group 4 in both age groups (p<0.001). *The difference of HSIL+ rates between group 2 and group 4 was not statistically significant in both age groups (p>0.05). [a]HSIL+ rates in group 2 versus group 3 were not statistically significant in age ≥50 (p=0.657), HPV: Human papillomavirus

Table 6. Age stratified HPV type distribution in HSIL+

HSIL+ age groups, years	HPV groups		HPV subgroups				Total n (%)
	Single type n (%)	Multiple type n (%)	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	
30-49	200 (81.0)	47 (19.0)	161 (65.2)	38 (15.4)	39 (15.8)	9 (3.6)	247 (100)
≥50	46 (71.9)	18 (28.1)	38 (59.4)	15 (23.4)	8 (12.5)	3 (4.7)	64 (100)
Total	246 (79.1)	65 (20.9)	199 (64.0)	53 (17.0)	47 (15.1)	12 (3.9)	311 (100)

HSIL+: High-grade squamous intraepithelial lesion, adenocarcinoma *in situ* or invasive adenocarcinoma, microinvasive or invasive squamous cell carcinoma; carcinoma other than cervix, Group 1: Single HPV16/18, Group 2: Single HPV other types, Group 3: Multiple HPV 16/18 and other HR types, Group 4: Multiple other HPV types, HPV: Human papillomavirus

Multiple HPV infections were detected in only three of the 24 cervical cancer cases (13%) and 18 (78%) had HPV 16 infection alone. Single HPV 18 infection was detected in one patient (4%) and single HPV of another type was detected in two patients (HPV 56, HPV 59). When the cytology results of the patients diagnosed with cancer were reviewed, it was reported as HSIL in three patients, ASC, cannot exclude high-grade intraepithelal lesion in one patient, and suspicious cytology in two patients. Furthermore, 14 (60%) of the patients with cancer were over 50 years old.

Discussion

Multiple HPV positivity is becoming more frequently detected and reported due to improvements in HPV testing methods and widespread use of HPV testing in cervical cancer screening (4,10,17). Identifying the role of multiple-type HPV infections in cervical preinvasive disease and cervical cancer is important for improving screening, follow-up strategies and HPV vaccine policy (15).

In the literature, multiple HPV positivity has been examined from different aspects including the prevalence, age distribution, cytological relations, histopathological correlations, and treatment response (4,5,9,14,15,18-29). Initial studies into multiple HPV are usually related to prevalence, and in the majority of the studies lr and hr types have been studied together. The rate varies widely from 20-100% (4,14,18-20). In the current study, only hrHPV types were studied and the rate of multiple-type HPV was 24.9%. All these studies show that multiple-type HPV infections are a common phenomena in sexually active women, infection rate varies according to the sensitivity of the test used and the age distribution of the group screened (24). However, knowing the multiple HPV rate alone is not sufficient for optimal management of women infected with HPV. In Türkiye the prevalence of hrHPV was reported as 3.5% and the commonest type was HPV 16 with 20.5% of HPV positive women (0.7% of all women). It was followed by types 51, 31, 52, 18 with rates of 10.5%, 8.3%, 7.8%, and 4.5%, respectively. No data related to multiple HPV infection were presented (30).

It is noted in many publications that multiple HPV positivity is more common in young women and in the older postmenopausal group. Impaired immune system and multiple sexual partners have been reported as the main factors (5,21). Safaeian and Rodriguez (20) reported that the distribution of concurrent infections varied by age and cervical abnormality, being highest in persons with abnormal lesions. Yang et al. (9) reported that multiple HPV infections were more common among young women with low-grade cytology and also among older women. Aro et al. (24) reported that multiple HPV types were most common in women <30 years of age. Resende et al. (23) also reported the prevalence of multiple type infections followed a bimodal distribution, peaking in women younger than 29 years and again in those aged 50 to 59 in Brazilian women.

Türkiye's national cervical cancer screening program covers women aged 30-65 years (30). In the present study, when HPV-positive patients were examined by age group, the prevalence of multiple HPV positivity in the below 30 years and above 50 years groups were found to be non-significantly higher (27.8 and 27.2%) than in the 30-49 years-old age group.

Many studies have reported that multiple HPV positivity is higher in women with abnormal cytology (5,9,15,20,25). Due to the low sensitivity of cervical cytology, its relationship with multiple HPV infections cannot be clearly demonstrated. Insufficient cytology rates are frequently encountered, especially when using the conventional pap smear test. Based on cytology reports, the rate of multiple HPV was 28.2% in those with normal cytology, 19.3% in LSIL, and 21.5% in HSIL cytology in the present study. Of note, 44.1% of cytology results were reported as "infection" or "insufficient".

The relationship between multiple HPV infection and histopathological results is the most important factor for selecting optimal patient management. However, they are conflicting results. Some publications related to multiple HPV infections with histological results show an increase in high-grade cervical preinvasive disease, while others do not. Carrillo-García et al. (25) reported that their data suggested that the presence of more than one hrHPV type increased the

risk of developing high-grade cervical lesions and invasive cervical cancer. Chaturvedi et al. (21) also reported that multiple oncogenic types of α9 species increased the risk of both cervical intraepithelial neoplasia 2 (CIN2) and HSIL. Akış et al. (22) reported that the multiple HPV group had a high rate of CIN lesions. Senapati et al. (19) reported that women infected with multiple genotypes with phylogenetically related clad had the higher risk of cervical carcinoma as compared to the population infected with phylogenetically unrelated clad. Spinillo et al. (26) reported among women with cervical cytological abnormalities that infection by multiple hrHPVs increased the risk of CIN3+ in both HPV16-positive and HPV16-negative women. De Brot et al. (6) reported that HPV coinfections was present in the majority of HSIL cases.

In contrast, Aguilar-Lemarroy et al. (27) reported that, when considering HPV-positive samples only, coinfections occurred most often in controls (63%) and were less frequent in those with cervical cancer (26%). Wu et al. (28) reported HPV16/18 co-infection with other hrHPVs to be a common phenomenon and they also found that HPV16 co-infected with other hrHPVs appeared to have a lower associated risk of CIN3+ in ≥30 year-old women. In serological studies, a tendency for antagonistic interactions between HPV16 and HPV18 were demonstrated. Seropositivity for HPV18 reduces the risk of HPV16-related cervical cancer (31).

In the present study, only 2.46% of patients with HSIL had HPV 16/18 coexistence, but no HPV 16/18 positivity was found in patients diagnosed with cervical cancer. The results of this study also showed that multiple type HPV rates were less common in HSIL and cancer cases.

Study Limitations

The study has some limitations. This study was cross-sectional. Follow-up results were not available. Furthermore, solely results related to hrHPV types are presented. The study center is a referral center and so the study population may introduce some selection bias.

The strengths of the study include an estimated large population. This is because hrHPV positivity in Türkiye was reported as 3.5%, and of that 20% was HPV16 (0.7%) so that the number of patients participating in this study roughly represents about 150,000 Turkish women. Moreover, we present the histological data of multiple hrHPV positive patients, as well as prevalence and cytological data. Our hospital provides services for gynecological cancers and our pathologists are highly experienced in this field. Also experienced gyneco-oncologists work in our colposcopy outpatient clinic. In addition, our colposcopy clinic is one of the colposcopy reference centers of the national HPV based screening system.

Conclusion

In the present study multiple-type HPV infections were less common than single-type infections in high-grade cervical lesions and cervical cancer. Our results suggest that multiple HPV positivity may be related to shorter duration of infection and this may be the main reason for low HSIL+ rates. However, the rate of multiple type infections in HSIL and cervical cancer patients in women older than 50 years was remarkable, although not statistically significantly different from the other age group (28.1%). However, the age of the women should be taken into account in the triage of Turkish women and possibly in other populations too, but this suggestion requires data from other populations before widespread implementation.

To gain more insights into the natural history and dynamics of multiple HPV infections, more sensitive assays and longitudinal studies with long-enough follow-up periods are necessary. Comprehensive and long follow-up results of national HPV screening programs will be particularly important because of the regional HPV distribution. In the light of these results, more efficient strategies will need to be developed to prevent preinvasive disease and cervical cancer.

Ethic

Ethics Committee Approval: *Ethical permission for a retrospective study was granted by the Etlik Zübeyde Hanım Women's Health Education and Research Hospital's Institutional Review Board (approval number: 17, date: 15.11.2019).*

Informed Consent: *All patients were informed about colposcopy and follow-up procedures and informed consents were obtained.*

Footnotes

Author Contributions: *Surgical and Medical Practices: S.K., D.Y., F.K., Concept: S.K., F.K., Design: S.K., F.K., Data Collection or Processing: S.K., D.Y., E.Ü., Analysis or Interpretation: S.K., F.K., Literature Search: S.K., E.Ü., T.K., Writing: S.K.*

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