

HPV Infection in Women with ASC-US in Eastern Marmara, Türkiye: Genotype Prevalence and Clinical Implications

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Purpose: This study aimed to retrospectively evaluate the distribution of human papillomavirus (HPV) genotypes, age-specific prevalence, and their association with histopathological outcomes in women with cervical cytology results reported as atypical squamous cells of undetermined significance (ASC-US) and concurrent high-risk HPV (hr-HPV) positivity.

Patients and Methods: Among 39,189 records screened between October 2019 and October 2024, 705 patients with ASC-US and hr-HPV positivity were included. HPV genotyping was performed using real-time PCR, cytological evaluation followed the Bethesda System, and colposcopic biopsies were classified according to the Lower Anogenital Squamous Terminology (LAST) guidelines.

Results: HPV-16 (25.6%), HPV-51 (7%), and HPV-31 (6.5%) were the most prevalent genotypes, detected in 28.2% (n=199) of hr-HPV-positive cases. HPV-16 demonstrated the strongest association with high-grade lesions, accounting for 47.6% of cervical intraepithelial neoplasia grade 2 or worse (CIN2+). Multiple HPV infections correlated with an elevated risk of CIN2+. Age-stratified analysis revealed HPV-16 predominance in women aged 40–49 years, whereas HPV-51 was more frequent in those ≥60 years.

Conclusion: In ASC-US cases, HPV-16 is the predominant genotype and a significant predictor of high-grade cervical lesions. The unexpectedly high prevalence of HPV-51 and HPV-31 in this Turkish cohort underscores the importance of tailoring screening algorithms to regional genotype distributions. Furthermore, the observed age-specific variations in genotype prevalence highlight the need for age-adjusted management strategies.

Keywords: ASC-US, HPV genotyping, cervical intraepithelial neoplasia 2+, colposcopy, Turkish population

Introduction

Cervical cancer is the fourth most common cancer in women globally, and is a major public health problem, especially in low- and middle-income countries. According to GLOBOCAN 2022 data, cervical cancer has high morbidity and mortality rates in low- and middle-income countries, and its incidence is increasing worldwide.^{1,2} In developed countries, organized screening programs have led to significant reductions in the incidence of cervical cancer. For example, in 2008, the cervical cancer cytological screening service in the United Kingdom was implemented in all women aged 25 to 64 years and led to a decrease in cervical cancer.³ However, due to the limitations in the sensitivity and specificity of current cytology-based screening methods, atypical squamous cells of undetermined significance (ASC-US) pose a significant clinical challenge.⁴ ASC-US is one of the most common abnormalities encountered in cervical cytology and although it is considered as a low-risk abnormal cervical cytology, the underlying pathologies can vary widely from benign changes to high-grade squamous intraepithelial lesions (HSIL) and even cervical carcinoma.⁵ Although ASC-US is classified as a low-risk abnormal cervical cytology, approximately 5–10% of women diagnosed with this diagnosis have underlying

high-grade cervical lesions (CIN2+ and CIN3+), which requires careful follow-up.⁶ Furthermore, although most ASC-US cases heal spontaneously, 15–20% are histopathologically confirmed to be compatible with cervical epithelial neoplasia (CIN) during the follow-up period.⁴

The integration of molecular tests into screening protocols supports clinical decision-making processes and enables more effective planning of patient follow-up strategies. Comparing these molecular methods in terms of sensitivity and specificity parameters is of great importance for improving the performance of screening programs and expanding opportunities for early diagnosis.^{7,8}

The high-risk HPV (hr-HPV) positivity rate in women with ASC-US cytology varies between 30–70%.^{9,10} The 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines emphasize that hr-HPV testing plays a decisive role in the management of ASC-US. Accordingly, follow-up with co-testing is recommended after three years in hr-HPV negative ASC-US cases, while colposcopy is indicated in hr-HPV positive cases.^{9–11} The risk of CIN3+ is high in women with ASC-US cytology and HPV positivity, especially those associated with HPV 16, 18, 31 and 33/58 genotypes.¹² For example, in a study conducted in Thailand, HSIL or more advanced lesions were detected in 12.6% of ASC-US cases, and genotypes other than HPV 16 and 18 (HPV 58, 52, 33) were dominant in the majority of these cases.¹³ The oncogenic potential of HPV varies considerably due to genetic, geographic, socioeconomic and socio-cultural factors. Moreover, individual characteristics, including age, gender, anatomical features and health status, play an important role in the pathogenesis of HPV-associated conditions.¹⁴ In sub-Saharan Africa, high-risk HPV (hrHPV) genotypes include HPV16, 18, 35 and 52.¹⁵ In Asian populations, HPV genotypes include HPV16, HPV52 and HPV58, and their strong association with HSIL is striking.¹⁵ This heterogeneity highlights the need to support global guidelines with local epidemiological data.

The main purpose of this study is to determine the prevalence and type distribution of HPV infection in patients who applied to our gynecologic oncology surgery clinic and whose cervical cytology result was reported as ASC-US and to evaluate the relationship of each HPV genotype with colposcopic findings (especially HSIL+). The findings are aimed to emphasize the role of HPV testing in ASC-US cases, to contribute to cervical cancer screening strategies, to optimize patient follow-up protocols and to provide scientific contribution to patient follow-up guidelines.

Materials Method

This cross-sectional, retrospective study was conducted at the Gynecologic Oncology Department of the Gynecology and Obstetrics Clinic, Sakarya University Training and Research Hospital, which is a tertiary referral center providing comprehensive healthcare services to a large regional population. The hospital serves as a major referral hub for gynecologic oncology cases, ensuring a wide and diverse patient base. The medical records of patients who applied to this department between October 1, 2019, and October 1, 2024, were systematically reviewed. A total of 705 women aged ≥ 21 years who underwent liquid-based cytology (LBC) testing during this period, had cytology results reported as Atypical Squamous Cells of Undetermined Significance (ASC-US), and tested positive for high-risk human papillomavirus (hr-HPV) were included in the study. Both colposcopic examinations and directed biopsies were performed by the same experienced gynecologic oncology specialist to ensure standardization and minimize inter-observer variability. Patients who had hysterectomized (n=15), conized (n=28), diagnosed cervical cancer patients (n=10), pregnancy status (n=8) and immunosuppressed (n=2) were excluded from the study. HPV result file records of 485 patients whose LBC results were evaluated as ASC-US could not be accessed. Although the primary study population included only ASC-US patients with available HPV test results, we additionally compared baseline characteristics between patients with and without HPV results to assess whether missing data might have introduced systematic differences. As a rule, we recommended colposcopy to women with HR-HPV positive ASC-US. None of the participants had been vaccinated. In this study, a total of 39,189 liquid-based cytology tests (LBC) and 30,746 hr-HPV tests were examined over a four-year period.

Ethical approval for this study was obtained from the Sakarya University Faculty of Medicine Clinical Research Ethics Committee (Date: 19/09/2024/Protocol No: E-43012747-050.04–399700. Informed consent was obtained from all individual participants included in the study. All procedures performed were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Demographic information and medical history were recorded at the time of enrollment. Age, body mass index (BMI), education level, marital status, age at first sexual intercourse, total number of partners, parity, number of curettages, number of living children, contraceptive methods, comorbidities, active drug use, history of sexually transmitted diseases (STDs), smoking, and anti-HPV vaccination status were recorded. Speculum examination was performed, during which LBC cervical specimen was collected using an endocervical swab according to the manufacturer's instructions. Cytological processing was sent to the pathology laboratory using BD SurePath™ (Becton, Dickinson and Company, Sparks, Maryland 21152 USA). Cytological evaluation and reporting were performed according to The Bethesda System for Reporting Cervical Cytology (2001 version). In summary, NILM was negative for intraepithelial lesion or malignancy, ASC-US indicated atypical squamous cells (of undetermined significance), and LSIL and HSIL indicated low- and high-grade squamous intraepithelial lesion, respectively.¹⁶ Cytological evaluation was performed without an automated screening system. HPV DNA testing was performed using the Digene® HC2 DNA Collection Device (Qiagen Inc., Germantown, MD, USA) for HPV detection during speculum examination or 2–4 months after the LBC result was sent to the laboratory. HPV DNA screening was performed using the NeuMoDx® 96 Molecular System (Qiagen GmbH, Hilden, Germany) based on real-time PCR technology. Positive results from HPV tests directed subjects to colposcopy. Colposcopy:

All women aged ≥ 21 years ($n = 155$) with HR-HPV and ASC-US cytology were selected for colposcopy/biopsy. Women selected for standard colposcopy underwent the procedure within 42 days of the enrollment visit. Colposcopic examinations were performed using the OLYMPUS OCS-500® colposcope (Olympus Corporation, Tokyo, Japan). During colposcopy/biopsy, the colposcopist performed the procedure without considering the cytology and HPV test results. Biopsy samples included any lesions or acetowhite areas and endocervical curettage (ECC). If no lesions or acetowhite areas were seen during colposcopy, a random biopsy was performed at the squamocolumnar junction and an ECC was collected. Histopathology:

The study used the Lower Anogenital Squamous Terminology Standardization Project (LAST) guidelines for HPV-Associated Lesions developed by the College of American Pathologists (CAP) and the American Society for Colposcopy and Clinical Pathology (ASCCP) for the evaluation of cervical intraepithelial neoplasia (CIN) cases.¹⁷ Histopathological samples were evaluated using hematoxylin-eosin (H&E) staining according to standard diagnostic criteria.

Pathological evaluation of cervical specimens employs a standardized diagnostic framework comprising four principal categories: (1) Negative for malignancy, encompassing benign reactive/inflammatory alterations, squamous metaplasia, and epithelial changes without significant atypia; (2) Epithelial cell abnormalities, including ASC-US, ASC-H, and AGC, which constitute cytological atypia necessitating further clinical investigation; (3) Premalignant lesions, stratified as CIN1 (LSIL, characterized by basal third epithelial involvement) and CIN2-3 (HSIL, demonstrating moderate to severe dysplasia with two-thirds or full-thickness epithelial involvement), alongside adenocarcinoma in situ (AIS) representing non-invasive glandular neoplasia; and (4) Invasive carcinomas, comprising squamous cell carcinoma (SCC), adenocarcinoma (AC), and adenosquamous carcinoma (ASC). This tiered classification system facilitates accurate histopathological diagnosis and informs evidence-based clinical management.

Statistical Methods

The Kolmogorov–Smirnov test was used to evaluate the normal distribution of the numerical data used in the study. In cases where normal distribution was not provided, the nonparametric Mann–Whitney *U*-test was preferred for comparisons between two independent groups. Numerical data were presented as mean $\pm$ standard deviation, median [interquartile range] and minimum-maximum values. In the study, the Pearson chi-square test was applied to examine the independence relationship between categorical variables. Categorical data were presented in tables as number (*n*) and percentage (%). The significance level was accepted as $p < 0.05$ to determine statistically significant differences. All statistical analyses were performed using the IBM SPSS Statistics 23.0 statistical system program. (Armonk, NY: IBM Corp)

Results

The total number of patients who had LBC tests between October 1, 2020 and October 1, 2024 was determined as 39,189, and the total number of patients who underwent HPV tests was determined as 30,746. A total of 1253 (3.2%) patients had smear results as ASC-US. Of the 30,746 patients who underwent HPV tests, 2176 HPV results were positive (7.1%), while 28,140 patients had negative HPV results (91.5%). The HPV test results of 430 patients (1.4%) were insufficient, and retesting was deemed appropriate. Of the 1253 patients whose LBC test results were ASC-US, the HPV results of 485 patients were not in the file records or could not be reached. When comparing ASC-US patients with available HPV test results to those without, there were no statistically significant differences in age ($p = 0.075$), BMI ($p = 0.056$), or educational level ($p = 0.062$). A total of 63 patients met the exclusion criteria. Patients who did not have an HPV result and had one of the exclusion criteria were not included in the study. A total of 705 patients were included in our study (Figure 1).

When 705 ASC-US cases included in the study were examined; mean age was 44.56 ± 11.38 years, BMI was 29.37 ± 4 kg/m², age at first coitus was 20.99 ± 2.3 years and mean duration of marriage was 21.35 ± 10.74 years. 33.8% of the patients ($n=238$) were concentrated in the 40–49 age group. According to the education status, more than half of the cases were primary school

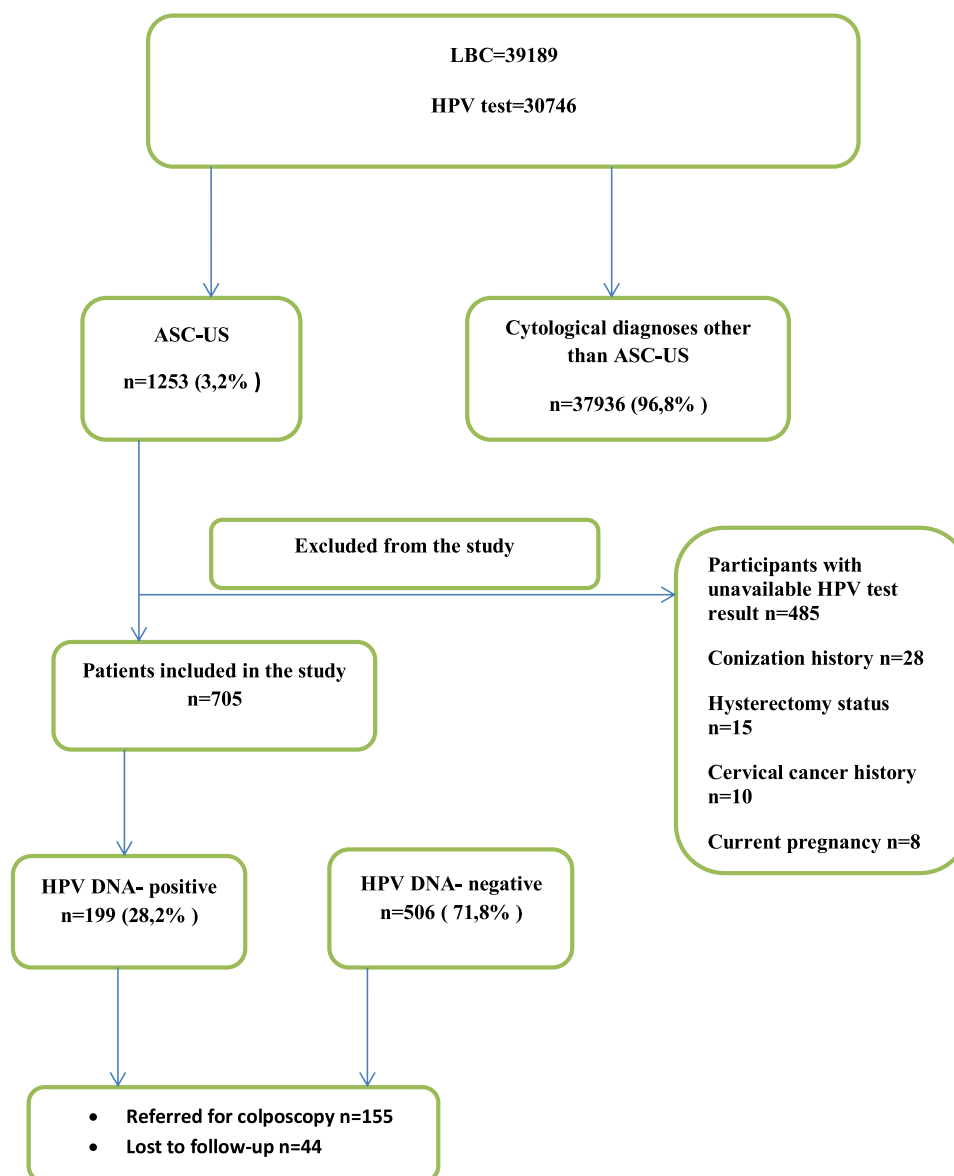


Figure 1 Study Flowchart of HPV Genotype Distribution Analysis in Women with ASC-US Cytology.

graduates (%51.2, n=361). While the prevalence of smoking was determined as 47.5% (n=335), the majority of the cases had a single partner history (%79.1, n=558) and STD negativity (%97.6, n=688). Among contraceptive methods, intrauterine devices (28.2%, n=199) and oral contraceptives (20%, n=141) are prominent. Multiparity (71.2%, n=502) was found to be dominant in parity distribution, and normal spontaneous delivery was recorded as the mode of delivery in 63% (n=444) (Table 1).

In the analysis of 199 HPV positive patients, the most common finding was single genotype positivity, with HPV 16 (25.6%, n=51) dominating this group. This was followed by HPV 51 (7%, n=14), HPV 31 (6.5%, n=13), HPV 45 (6%, n=12), HPV 18 (3.5%, n=7) and HPV 56 (3.5%, n=7), respectively. These six genotypes represented 52.3% (n=104) of single genotype positive patients. The most common combination in multiple infections was HPV 16 and 33 (2%, n=4). Other significant combinations included HPV 16/52 (n=3), HPV 31/51 (n=1) and HPV 16/18/51 (n=2). The total prevalence of multiple infections was calculated as 15.6% (n=31) (Table 2).

Table 1 Distribution of Socio-Demographic and Clinical Characteristics (n=705)

Features	n (%)
Age	
<30	69 (9.8)
30–39	170 (24.1)
40–49	238 (33.8)
50–59	158 (22.4)
≥60	70 (9.9)
Age (Mean±Standard Deviation)	44.56±11.38
Vaccination status	
Not vaccinated	705 (100.0)
Vaccinated	0 (0.0)
BMI (Mean±Standard Deviation)	29.37±4.0
Educational Status	
Illiterate	11 (1.6)
Primary School	361 (51.2)
Secondary School	20 (2.8)
High School	254 (36.0)
University	59 (8.4)
Smoking	
No	370 (52.5)
Yes	335 (47.5)
Age of First Intercourse (Mean±Standard Deviation)	20.99±2.3
Duration of Marriage (Mean±Standard Deviation)	21.35±10.74
Total Number of Partners	
One	558 (79.1)
More than One	147 (20.9)
Sexually Transmitted Disease	
No	688 (97.6)
Yes	17 (2.4)
Hepatitis B	15 (2.1)
HIV	2 (0.3)

(Continued)

Table 1 (Continued).

Features	n (%)
Protection Method	
Not Protected	159 (22.6)
Protected	546 (77.4)
Intrauterine Device	199 (28.2)
COC	141 (20.0)
Tubal Ligation	99 (14.0)
Coitus Interruptus	65 (9.2)
Condom	42 (6.0)
Parity Type	
Nulliparous	59 (8.4)
Primiparous	144 (20.4)
Multiparous	502 (71.2)
Gravida	
No	54 (7.7)
One	118 (16.7)
Two	265 (37.6)
Three and above	268 (38.0)
Parity	
One	144 (22.3)
Two	307 (47.5)
Three and above	195 (30.2)
Abortion	
No	1 (0.7)
One	92 (66.2)
Two	38 (27.3)
Three and above	8 (5.7)
Ectopic	
Yes	2 (0.3)
Living	
One	145 (22.5)
Two	308 (47.8)
Three and above	192 (29.7)
Stillbirth	
One	4 (80.0)
Two	1 (20.0)
Mode of Birth	
No	59 (8.4)
SVB	444 (63.0)
C/S	192 (27.2)
SVB ve C/S	10 (1.4)

Abbreviations: BMI, Body Mass Indeks; SVB, Spontaneous vaginal birth; C/S, C/Section.

Table 2 Distribution of HPV Gene Types According to Patient Age Groups

HPV Gene Types	Groups of Age n(%)				
	<30 (n=26)	30-39 (n=49)	40-49 (n=72)	50-59 (n=34)	≥60 (n=18)
16	7 (26.9)	14 (28.6)	19 (26.4)	9 (26.5)	2 (0.9)
18	2 (7.7)	2 (4.1)	3 (4.2)	-	-
31	1 (3.8)	3 (6.1)	6 (8.3)	2 (5.9)	2 (0.9)
33	1 (3.8)	2 (4.1)	-	3 (8.8)	-
35	1 (3.8)	1 (2.0)	4 (5.6)	-	1 (0.5)
39	1 (3.8)	1 (2.0)	2 (2.8)	2 (5.9)	-
43	-	1 (2.0)	-	-	-
45	3 (11.5)	3 (6.1)	4 (5.6)	1 (2.9)	1 (0.5)
51	2 (7.7)	5 (10.2)	2 (2.8)	1 (2.9)	4 (1.9)
52	-	-	3 (4.2)	-	1 (0.5)
53	-	-	-	1 (2.9)	-
56	-	1 (2.0)	5 (6.9)	1 (2.9)	-
58	-	-	2 (2.8)	-	-
59	2 (7.7)	-	2 (2.8)	1 (2.9)	-
6	-	3 (6.1)	-	-	-
66	-	-	2 (2.8)	-	-
68	-	-	1 (1.4)	-	1 (0.5)
70	-	-	1 (1.4)	-	-
81	-	2 (4.1)	1 (1.4)	-	-
82	-	-	1 (1.4)	-	-
87	1 (3.8)	-	-	-	-
16, 18	-	-	-	1 (2.9)	-
16, 31	-	-	-	1 (2.9)	1 (0.5)
16, 33	2 (7.7)	-	2 (2.8)	-	-
16, 51	-	-	1 (1.4)	-	-
16, 52	-	1 (2.0)	-	2 (5.9)	-
16, 56	-	-	1 (1.4)	-	-
16, 68	-	-	-	-	1 (0.5)
31, 33	1 (3.8)	1 (2.0)	-	-	-
31, 51	-	1 (2.0)	-	-	-
31, 52	-	-	-	1 (2.9)	-

(Continued)

Table 2 (Continued).

HPV Gene Types	Groups of Age n(%)				
	<30 (n=26)	30-39 (n=49)	40-49 (n=72)	50-59 (n=34)	≥60 (n=18)
31, 56	-	1 (2.0)	-	-	-
31, 58	-	-	-	-	1 (0.5)
31, 59	-	-	1 (1.4)	1 (2.9)	-
33, 58	-	1 (2.0)	-	-	-
35, 45	-	1 (2.0)	1 (1.4)	-	-
35, 52	-	-	1 (1.4)	1 (2.9)	-
39, 45	-	1 (2.0)	1 (1.4)	-	-
39, 51	-	-	1 (1.4)	-	-
39, 59	-	-	-	1 (2.9)	-
39, 81	-	-	-	1 (2.9)	-
45, 51	-	1 (2.0)	-	-	-
45, 56	-	1 (2.0)	-	-	-
45, 68	-	-	1 (1.4)	-	-
51, 33	-	-	-	-	1 (0.5)
51, 52	-	1 (2.0)	-	1 (2.9)	1 (0.5)
51, 56	-	-	-	2 (5.9)	-
52, 56	1 (3.8)	-	-	-	-
6, 16	1 (3.8)	-	-	-	-
6, 51	-	-	1 (1.4)	-	-
16, 18, 31	-	1 (2.0)	-	1 (2.9)	-
16, 18, 39	-	-	1 (1.4)	-	-
16, 18, 51	-	-	2 (2.8)	-	-
16, 59, 52	-	-	-	-	199 (92.1)

In total, HPV 16 from the high-risk HPV (hrHPV) group was the most dominant genotype, detected in 35.7% (n=71) of the patients. Other hrHPV types in order of frequency: HPV 51 (13.7%, n=27), HPV 31 (12.6%, n=25), HPV 45 (9.5%, n=19), HPV 33 (7%, n=14), HPV 18 (6.5%, n=13), HPV 56 (6.5%, n=13). These seven genotypes cover 84.9% (n=169) of all HPV-positive cases (Table 2).

HPV 16 infections were seen at the highest rate in the 40–49 age group (37.3%, n=19). HPV 18 positivity peaked in the 40–49 age group (42.9%, n=3). HPV 51 showed a higher prevalence than expected in the ≥60 age group (28.6%, n=4) (Table 2).

Among the 705 patients included in the study, single or multiple HPV gene positivity was detected in 199 (28.2%) patients. Single gene positivity was present in 150 (75.4%) of these cases, while multiple gene positivity was observed in 49 (24.6%). When the distribution according to age groups was examined, it was seen that single gene positivity was dominant in all groups (p=0.459). However, multiple gene positivity was observed with the highest prevalence in the

50–59 age group (38.2%; n=13), and secondarily in the ≥ 60 age group (33.4%; n=6). Single gene positivity was detected most frequently in the <30 age group (80.8%; n=21), followed by the 40–49 (80.6%; n=58) and 30–39 age groups (77.6%; n=38), respectively. No statistically significant difference was found between age groups in terms of the distribution of HPV positive gene numbers ($p>0.05$) (Table 3).

In histopathological examinations performed in HPV-positive patients, CIN2+ lesions were detected at a rate of 28.4% (44/155). Single gene positivity was found to be dominant in both CIN1 (81.3%) and CIN2+ (79.5%) lesions. While HPV-16 was detected alone at a rate of 45.5% (20/44) in the CIN2+ group, this rate decreased to 13.6% (6/44) in cases with multiple genotypes. The second most common genotypes were single HPV-31, HPV-33 and HPV-45 (6.8% each); In multiple infections, HPV-31 (13.6%) and HPV-33 (9.1%) were prominent.

The contribution of HPV-18 in the CIN2+ group was limited (4.5%), and it was observed in the third place together with HPV-56. On the other hand, in CIN1 lesions, the most common was HPV-16 (28.1%), followed by HPV-51 (12.5%) and HPV-6/39/56 (6.3% each) (Table 4).

Table 3 Distribution of the Number of HPV Genotypes by Age Groups

Groups of Age	HPV Genotype Positivity			p-değeri
	1 Genotype n=150	2 Genotype n=43	≥ 3 Genotype n=6	
<30	21 (14.0)	5 (11.6)	-	0.459
30-39	38 (25.3)	10 (23.3)	1 (16.7)	
40-49	58 (38.7)	11 (25.6)	3 (50.0)	
50-59	21 (14.0)	12 (27.9)	1 (16.7)	
≥ 60	12 (8.0)	5 (11.6)	1 (16.7)	

Table 4 Distribution of Patients' HPV Gene Types According to Histopathological Results (With Column Percentages)

HPV Gene types	Histopathological Result			
	CIN I (n=32)	CIN 2+ (n=44)	Squamous Cell Carcinoma (n=3)	Other (n=76)
16	9 (28.1)	20 (45.5)	2 (66.7)	11 (14.5)
18	–	2 (4.5)	–	3 (3.9)
31	–	3 (6.8)	–	10 (13.1)
33	–	3 (6.8)	–	2 (2.6)
35	1 (3.1)	–	–	3 (3.9)
39	2 (6.2)	–	–	2 (2.6)
45	1 (3.1)	3 (6.8)	–	5 (6.6)
51	4 (12.5)	–	–	5 (6.6)
52	1 (3.1)	–	–	3 (3.9)
53	1 (3.1)	–	–	–
56	2 (6.2)	2 (4.5)	–	2 (2.6)

(Continued)

Table 4 (Continued).

HPV Gene types	Histopathological Result			
	CIN I (n=32)	CIN 2+ (n=44)	Squamous Cell Carcinoma (n=3)	Other (n=76)
58	–	1 (2.3)	–	1 (1.3)
59	1 (3.1)	–	–	4 (5.3)
6	2 (6.2)	–	–	1 (1.3)
68	1 (3.1)	–	–	–
70	1 (3.1)	–	–	–
81	–	–	–	2 (2.6)
82	–	1 (2.3)	–	–
16,18	–	1 (2.3)	–	–
16,31	–	–	1 (33.3)	1 (1.3)
16,33	1 (3.1)	1 (2.3)	–	1 (1.3)
16,51	–	–	–	1 (1.3)
16,52	1 (3.1)	1 (2.3)	–	1 (1.3)
16,56	–	1 (2.3)	–	–
16,68	–	–	–	1 (1.3)
31,33	–	–	–	1 (1.3)
31,51	–	1 (2.3)	–	–
31,52	–	1 (2.3)	–	–
31,58	1 (3.1)	–	–	–
31,59	–	–	–	1 (1.3)
35,45	–	–	–	1 (1.3)
35,52	–	–	–	1 (1.3)
39,45	–	–	–	1 (1.3)
39,51	–	–	–	1 (1.3)
39,59	–	1 (2.3)	–	–
45,51	1 (3.1)	–	–	–
45,56	–	–	–	1 (1.3)
45,68	1 (3.1)	–	–	–
51,52	1 (3.1)	–	–	1 (1.3)
51,56	–	–	–	2 (2.6)
52,56	–	–	–	1 (1.3)
6,16	–	1 (2.3)	–	–

(Continued)

Table 4 (Continued).

HPV Gene types	Histopathological Result			
	CIN I (n=32)	CIN 2+ (n=44)	Squamous Cell Carcinoma (n=3)	Other (n=76)
6,51	–	–	–	1 (1.3)
16,18,31	–	1 (2.3)	–	1 (1.3)
16,18,39	–	–	–	1 (1.3)
16,18,51	–	–	–	2 (2.6)
16,59,52	–	–	–	1 (1.3)

After LEEP applied to 44 HPV-positive patients, CIN2+ was detected in 17 (85%) of 20 patients who were single-gene HPV-16 positive and CIN1 was detected in 2 (10%). CIN2+ was observed in all 3 patients (100%) who were single-gene HPV-31 and HPV-33 positive, while CIN2+ was recorded in all 8 patients (100%) with multiple genotypes. In other single-gene infections (HPV-45, –56, –58, –82), CIN2+ rates were determined as 66.7%, 100%, 100% and 100%, respectively (Table 5).

Table 5 Distribution of Post-Conization Pathology Results of Patients According to HPV Gene Types

HPV Gene types	Post-Conization Pathology Result		
	CIN I (n=3)	CIN 2+ (n=37)	Other (n=4)
16	2 (66.7)	17 (45.9)	1 (25.0)
18	-	-	2 (50.0)
31	-	3 (8.1)	-
33	-	3 (8.1)	-
45	-	2 (5.4)	1 (25.0)
51	1 (33.3)	-	-
56	-	2 (5.4)	-
58	-	1 (2.7)	-
82	-	1 (2.7)	-
16,18	-	1 (2.7)	-
16,33	-	1 (2.7)	-
16,52	-	1 (2.7)	-
16,56	-	1 (2.7)	-
31,52	-	1 (2.7)	-
39,59	-	1 (2.7)	-
6,16	-	1 (2.7)	-
16,18,31	-	1 (2.7)	-

Discussion

This cross-sectional, retrospective study aimed to analyze the distribution of cervical pathologies in women with ASC-US cytology and hr-HPV positivity and the correlation of HPV genotypes with histopathological findings. The obtained data revealed that HPV-16 (both in monoinfections and coinfections) showed a significant association with high-grade cervical intraepithelial neoplasia (CIN2+). This finding is consistent with global data emphasizing the dominant role of HPV-16 in cervical carcinogenesis. It has been reported in the literature that HPV-16 is responsible for more than half of cervical cancers,^{18–20} and in our study, this genotype was detected in 47.6% of CIN2+ cases, confirming its clinical importance once again.

In the community-based study conducted by Pham et al in Vietnam, the prevalence of high-risk HPV (hrHPV) infection was reported as 7.5%.²¹ In our present study, the overall HPV positivity rate among all patients who underwent HPV testing was found to be 7.1%, a finding which is consistent with general population screening data. However, due to the unique structure of our study cohort, the HPV positivity rate was significantly higher (28.2%) in the patient group whose cervical cytology was reported as ASC-US. It is evident that both studies accurately and consistently reflect the HPV prevalence within their respective target populations. This observation aligns with the well-established knowledge in the literature that individuals with cytological abnormalities naturally exhibit a higher prevalence of HPV infection.¹⁰

Meta-analysis data show that HPV-16 plays a role as an etiological agent in 50–60% of cervical cancers globally and is the most frequently detected high-risk genotype in ASC-US cases.⁹ These findings are also parallel to studies conducted in Türkiye. In fact, studies conducted by Begüm et al and Yaman et al^{22,23} confirmed that HPV-16 is the most dominant genotype in ASC-US and LSIL cases. In our study, HPV-16 was determined as the most common genotype by being detected in 25.6% of HPV-positive patients, and its oncogenic potential was confirmed once again by showing a significant association with CIN2+ lesions in particular. These results are consistent with international and national data emphasizing the central role of HPV-16 in cervical carcinogenesis. According to the results of our study, the most frequently detected HPV genotype in cervical pathologies was HPV-16, followed by HPV-51 (7%), HPV-31 (6.5%), HPV-45 (6%), HPV-18 (3.5%) and HPV-56 (3.5%). These findings are consistent with Kulhan et al, who demonstrated that HPV-51 and HPV-31 have higher prevalence in the Turkish population compared to global averages.²⁴ This indicates that the HPV genotype distribution in Turkey may exhibit significant differences from the general distribution in the world.

The prominence of HPV-16 in both single and multiple infections once again confirms the central role of this genotype in the pathogenesis of cervical cancer. However, in our study, the prevalence of HPV-18 was relatively low, detected at a rate of 3.5% in single-genotype infections and 6.5% among all high-risk HPV (hrHPV) positive cases. The low frequency of HPV-18 reported in the study by Przybylski et al also supports our finding. Nevertheless, it should be considered that this low prevalence of HPV-18 may be associated with regional epidemiological variations or the potential impact of vaccination programs.²⁵

The effect of multiple HPV infections on the risk of cervical lesions is an important issue that continues to be discussed in the current literature. Meta-analyses and large-scale studies have shown that this relationship does not vary according to the combination of infecting genotypes and the histological grade of the cervical lesion.²⁶ Some studies (Su, et al) reported that multiple infections increase the risk of progressive lesions, but this is partially inconsistent with the findings of our study.²⁷ In our study population, the risk of CIN2+ multiple infections was found to be lower. This lower rate compared to other studies in the literature may be due to demographic characteristics of the population, genetic predisposition factors, or differences in regional HPV genotype distribution.

The data obtained from histopathological evaluations after conization are quite striking. While CIN2+ lesions were detected in 85% of single genotype HPV-16 positive cases, CIN2 positivity was found in all cases with multiple genotypes. These findings show that HPV-16 alone is an important risk factor for high-grade cervical lesions, and this risk increases even more in the presence of multiple infections. It is thought that multiple infections, especially those involving HPV-16, may have a synergistic effect on the development of cervical neoplasia.

In our series, where multiple infections showed a prevalence of 15.6%, it was observed that the combination of HPV-16 and other high-risk genotypes (such as HPV-33, HPV-52) caused a significant increase in the risk of CIN2+ lesions.

This finding is consistent with the study of Ye et al, which reported that multiple infections are associated with an increased risk of cervical lesions.²⁸ Similar results have been reported in studies conducted in the Turkish population. In the study of Alaçam et al, it was reported that multiple infections were detected at a rate as high as 40.7% and that high-risk genotypes such as HPV-16, -39 and -51 were detected in a significant portion of these cases.²⁹

Another striking finding of our study is that multiple infections are more common, especially in the 50–59 age group. This can be interpreted as a result of the decrease in the functional capacity of the immune system at older ages and/or long-term persistent HPV infections. It is thought that age-related immunosenescence processes may have an effect on the prevalence of multiple HPV infections and the risk of persistent infection.^{30,31}

Analysis of epidemiological data by age groups reveals significant differences in HPV genotype distribution. In our study, HPV-16 showed a high prevalence of 37.3% in the 40–49 age group, indicating that this age group is at high risk for cervical cancer. This finding suggests that the 40–49 age range should be considered as the priority target group in cervical cancer screening programs. Indeed, ASCCP guidelines recommend HPV testing every 5 years in women aged 30–65.³² However, another striking finding of our study is the unexpectedly high prevalence of HPV-51 in the ≥60 age group (28.6%). Although HPV-51 is classified in the high-risk HPV group, it has a lower risk than genotypes such as HPV-16, -18, -31, -33, -52, and -58 in terms of persistent infection and carcinogenic potential. Literature data show that HPV-51 infections carry a risk of <5% CIN2+ at 7-year follow-up and generally tend to regress spontaneously.^{33,34} However, the high rate of HPV-51 detection in the older age group may be related to immunological changes in the postmenopausal period or reactivation of long-term latent infections. This situation increases the importance of HPV screening in older age groups.

When the histopathological data of our study were analyzed, CIN2+ lesions were detected in 28.4% of HPV-positive patients. These findings are in significant agreement with current clinical guidelines. ASCCP guidelines require colposcopic evaluation especially in ASC-US cases with HPV-16 positivity, since it is known that the risk of CIN3+ lesions in this group is between 20–30%.³⁰ It is reported in the literature that the prevalence of invasive cancer in cases diagnosed with ASC-US varies between 1–1.8%.^{5,35} These rates observed in our patient population are in full agreement with current literature data.

Limitations of the Study

The retrospective design of this study has limited access to some data. In particular, the lack of access to HPV results for 485 patients may have affected the overall results of the study. In addition, since the study was conducted in a single center, the generalizability of the findings may be limited. Considering these limitations, multicenter, prospective studies that include vaccination status are needed to evaluate the applicability of the study results to larger populations.

Conclusion

This study has revealed the dominant role of HPV-16 in cervical pathologies in ASC-US and hr-HPV positive patients and the potential for multiple infections to increase lesion severity. The findings show that genotypes such as HPV-51 and HPV-31 have a higher prevalence in the Turkish population compared to global averages, and this supports the need to optimize screening protocols according to regional epidemiological data. The study results emphasize the clinical importance of including high-risk HPV genotypes in routine screening and expanding vaccination programs. In addition, the different genotype distribution observed in older age groups highlights the need to develop age-specific screening strategies. It is clear that large-scale prospective studies, especially those covering older age groups, are needed to reflect these findings in clinical practice.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Committee Approval

Although the study was in the category of noninterventional clinical research with its retrospective nature, we did apply for ethics committee approval. Our study was approved by the Local Ethics Committee of Sakarya University Faculty of Medicine (Project number: E-43012747-050.04-399700) and formal consent in addition to what the patients had given prior to hospitalization was waived.

Informed Consent

Informed consent forms were signed by all patients who participated in the study.

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Disclosure

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