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FULL PAPER

High-Risk Human Papilloma Virus Genotype Distribution and Correlation with Cervical Cytomorphological Data in Turkish and Immigrant Women in Mersin Province

Murat Yaman[1], Hacer Ece Arslan Özcan [2], Ayfer Bakir[3]

[1]Marmara University Pendik Training and Research Hospital, Medical Microbiology and Virology Laboratory, Istanbul, Turkey; [2]Mersin City Training and Research Hospital, Department of Medical Pathology, Mersin-Turkey; [3]University of Health Science Gulhane Training and Research Hospital, Clinical Microbiology, Turkey

SUMMARY

Human papilloma virus (HPV) is the most common sexually transmitted viral agent in the world and the most common cause of cervical cancer. HPV prevalence and genotype distribution vary by region and demographic data. In a province in the south of Turkey that constantly receives immigration, we aimed to determine the prevalence of high-risk HPV (HR-HPV) genotypes, evaluate the compatibility between cervical Pap smear cytology results patients and HR-HPVs, and make an up-to-date contribution to the elucidation of epidemiological data. In this single-centre study, a total of 12,641 women aged 18 and over were evaluated retrospectively from January 2019 to July 2022. HPV detection and genotyping were analysed by the PCR method. Bethesda scoring was used for Pap smear cytological evaluation. The overall prevalence of HR-HPV was 12.6% (12.7% in Turkish women, 11.2% in foreign women). Among the typed HPVs that were detected, HPV-16 (31%) was found first, followed by HPV-18 (8%). The prevalence of HR-HPV was higher in women with abnormal cytology (977/1762, 55.4%) than in women with normal cytology (620/10879, 5.7%) ($p<0.001$). Turkey doesn't yet have a national HPV immunisation program. We think that determining the specific regional frequency of other HR-HPVs separately will be useful in the follow-up of the natural course of the type-specific infection and in vaccine studies in the future.

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INTRODUCTION

The cervix has a complex microbiota and rich progenitor-stem cell content, and many different lesions ranging from infection to carcinoma can be observed in it (Sato *et al*., 2017; Mendoza *et al*., 2019). Cervical cancer ranks fourth among women and seventh among all cancers worldwide, with an estimated 604,127 new cases and 341,831 deaths in 2020 (WCRFI, 2023). Cervical cancer ranks ninth among the most common cancer types in all age groups in Turkey and ranks fourth (3.5%) in women aged 25-49 (Turkey Cancer Statistics, 2023).

It is known that the most important viral agent causing cervical cancer is the human papillomavirus (HPV). HPV is the most common cause of sexually transmitted infections. There are more than 200 types of HPV identified today (Szymonowicz *et al*., 2020). In cervical cancer cases, there are 12 high-risk HPV (HR-HPV) genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and other rare HR-HPV types (26, 53, 66, 67, 68, 70, 73, 82) that have persistent infection and oncogenic potential (NCI, 2023). It has been reported that HPV genotypes 16, 18, 33, 45, 52, and 58 are responsible for approximately 90% of cervical cancers (Nn Arbyn *et al*., 2014). Persistent infection of HPV-infected epithelium is an important risk factor for cervical cancer. The incidence of precancerous lesions with malignant potential can be lowered by up to 50% by using cytological screening in the diagnosis of cervical cancer (Sung H. *et al*., 2021).

Cervical cancer usually does not cause symptoms in the early stages, and symptoms appear in the advanced stages. The aim of cervical cytological screening is the early detection and treatment of abnormal changes and precursor lesions in the cervix and the identification of at-risk populations. The updated Bethesda system is widely used in the evaluation of cervical cytology preparations. In this system, abnormal Pap smear

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Corresponding author:
Murat Yaman
E-mail: drmrtyaman@gmail.com

results are classified as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and squamous cell carcinoma (Nayar *et al*., 2017). Instead of the three levels (CIN I, II, and III) of cervical intraepithelial neoplasia (CIN), it is also defined as LSIL and HSIL (Alrajjal *et al*., 2021). According to the American Society of Colposcopy and Cervical Pathology (ASCCP) guide, since ASCUS may have the potential to cause cervical cancer, it is stated that imaging with an HPV DNA test or colposcopy is necessary in addition to cytological follow-up (ASCCP, 2023). The incidence and mortality of cervical cancer in developed countries have decreased by approximately half with sustainable screening programmes (Cohen *et al*., 2019).

HPV DNA testing and genotyping can also be performed simultaneously with the molecular method from the same patient sample used for the liquid-based cervical cytology method.

It has been reported that HPV-based screening provides 60% to 70% more protection against invasive cervical carcinomas compared to cytology alone (Ronco *et al*, 2014).

Due to Turkey's geographical and geopolitical position, we anticipate differences in the epidemiology of HR-HPV infection due to the significant increase in the migrant population in recent years. With this study, we aimed to determine the prevalence of HR-HPV in both Turkish and immigrant patients who applied to a Training and Research Hospital in Mersin province, located in southern Turkey and characterized by a dense migrant population, over the past four years. Additionally, we aimed to emphasize the importance of early diagnosis by evaluating the relationship between types of HR-HPV and the results of cervical Pap smear cytology screening tests and to contribute to epidemiological data.

MATERIALS AND METHODS

Study Population

This study is a single-centre, cross-sectional, retrospective study. In Turkey, at Mersin City Training and Research Hospital, from January 1, 2019 to July 31, 2022, 12,641 women aged 18 and over who came to gynaecology outpatient clinics for regular check-ups or were examined due to gynaecological complaints had a cervical swab sample taken and were included in the study. HPV DNA genotyping by PCR and cervical cytology tests from cervical swab samples were analysed in the Virology and Pathology Laboratory.

Analysis results were obtained from the archives in the Laboratory Information System (LIS), and demographic data (age, ethnicity, year) of female patients were obtained from the hospital information management database.

Our study excluded women under 18 years of age, pregnant women, those with a history of hysterectomy or cervical conization, results that did not include planned years, and duplicate reports.

Molecular Analysis

Collection and storage conditions of samples

Cervical samples collected by the endocervical brush scraping method were preserved in ready-made vials (BD SurePathTM Collection Vial, Ireland) and stored at 2-8°C until the time of study; HPV genotyping was analysed by the Multiplex PCR method.

HPV DNA testing

HPV was extracted from cervical swab samples using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) extraction kit according to the manufacturer's instructions.

Detection and typing of HPV DNA from the isolated DNA was performed on the Rotor-Gene® Q MDx (Qiagen, Germany) device using the QIAscreen HPV PCR Detection Kit (Qiagen, Germany). The QIAscreen HPV PCR HPV kit can detect HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68, and targets the viral genome region E7.

PCR testing detects HR genotypes, HPV-16 and HPV-18 separately, and other HR-HPV, including HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68. It can detect them in a single analysis by providing pooled results. The resulting viral nucleic acid products were briefly amplified by distributing 15 µl of QIAscreen mix (Qiagen, Netherlands) mixture into PCR tube strips and 5 µl of the extracted sample DNA into the tubes. A sample control (human β-globin) and a negative control were used to monitor the experimental process, identify potential inhibitory substances, and minimize sampling errors. The interpretation of the sample results was made according to the manufacturer's instructions.

Pathological Evaluation

Cytological and cytomorphological analysis

All cervical smear and biopsy samples were prepared in the pathology department of Mersin City Training and Research Hospital, and the results were evaluated by all pathologists.

Liquid-based preparation: Swab samples in ready-made vials were prepared by BD-Prepstain application (*Table 1*).

Cytomorphological examination: Prepared cervical smear samples were evaluated under a binocular light microscope (Nikon Eclips-Ci). Cytopathological findings were grouped within the framework of a systematized protocol specific to cervical lesions, based on Bethesda-2014, revised in 2015, and to which recommendations were added in 2017 (Nayar *et al*., 2017).

They are grouped as negative, suspected, or present for intraepithelial dysplasia and malignancy.

In the negative group, the presence of Trichomonas

Table 1 - *Liquid-based preparation formation method.*

Process	*Duration*
Vortexing	5 minutes / 3 speed levels
Automatic Pipetting (Premate-Tripath Imaging)	1-2 minutes / 3-4 times
1. Centrifuge stage (Hettich Rotina 380)	2 minutes,15 seconds/200rpm
Liquid extraction on the surface with vacuum pump (PrepStain easy aspirator)	–
2. Centrifuge stage	10 minutes / 800rpm
3. Surface liquid extraction	By turning 180 degrees in one move
PAP staining (BD set)	30 seconds
Alcohol (Tekki), Xylen (Tekkim) and closure (Dd Mount) (DDK, Italia)	30 seconds

vaginalis, which is frequently associated with HPV in the etiology of inflammation, was examined in Pap smear preparations using light microscopy (Nikon Eclips-Ci) in the pathology laboratory. Epithelial cells in suspicious and existing groups in terms of intraepithelial dysplasia and malignancy were evaluated in two basic groups: squamous and glandular.

Cases were typed according to atypia status in squamous cells: atypia of undetermined significance (ASC-US), high-grade atypia of undetermined significance (ASC-H), as a group that does not exclude high-grade dysplasia, low-grade dysplastic squamous cell lesion (LSIL/CIN-I), high-grade dysplastic squamous cell lesion (HSIL/CIN-II and CIN III) and classified as malignant. The presence of dyskeratotic-parakeratotic cells in the ASC-US group and the presence of koilocytic cells with the cytopathic effect of HPV in the LSIL group were definitely noted.

When examining atypia in glandular cells, reports prepared according to the latest Bethesda protocol were taken into account. Endocervical or endometrial typing of atypical glandular cells (AGC) and their status in favour of neoplasia were determined. Squamous cell dysplasia and glandular invasion were also examined. Additionally, the presence of endometrial glandular cells in postmenopausal women has been reported, albeit with an exfoliated appearance.

Statistical Analysis

The SPSS version 25 (IBM Corp.) package programme was used in the statistical evaluation of the data. Qualitative data were described using frequency and percentage (%), and quantitative data were described using median and interquartile range, or mean and standard deviation, and range (minimum and maximum). The normality distribution of the data set was evaluated with the Kolmogorov-Smirnow test. The Pearson chi-square or Fisher's exact test was used where appropriate to test frequency differences between groups, and the Mann-Whitney U test was used to compare numerical variables between two groups. Risk estimation (odds ratio, OR) for LSIL and HSIL was made based on age, ethnicity, and HR-HPV types with binary logistic regression analysis. A p value of 0.05 or less was considered statistically significant.

RESULTS

HR-HPV Prevalence and Distribution of Genotypes

Overall HR-HPV prevalence in the study was found to be 12.6% (95%CI: 12.1-13.2).

1766 HR-HPV types were detected in 1597 women infected with HR-HPV. HPV-16 infection was detected in 31% of the cases (8% with HPV-18 and 71.6% with other HR-13 HPV types). Among the typed HR-HPVs, HPV-16 was the most frequently detected HPV type in Turkish women (31%) and foreign women (28.9%) (*Table 2*). The prevalence of HR-HPV was determined to be 12.7% in women of Turkish origin and 11.2% in women of foreign origin (p=0.412).

Distribution of Multiple HPV Infections

A single type of HR-HPV was detected in 27.6% of the HR-HPV positive samples, HR-13HPV with an unknown specific HPV type (untypeable) in 62.5%, and multiple types of HR-HPV in 9.9%.

The most common type detected in multiple infections was HPV-16. There was no significant difference between the infection rates of single, multiple, and HR-13 HPV types in Turkish and foreign women (p=0.770) (*Table 2*).

Multiple infections were detected in 28.5% (141/495) of women infected with HPV-16. The most multiple infections with HPV-16 were detected with HR-13 HPV types (n=118, 23.8%). The multiple infection rate in women infected with HPV-18 was 31.5% (40/127) and multiple infections were detected mostly with HR-13 HPV types (13.4%). The multiple infection rate in women infected with HPV-18 was 31.5% (40/127) and multiple infections were detected mostly with HR-13 HPV types (13.4%).

While the prevalence of multiple HR-HPV types was 0.2% (20/10879) in women with normal cytology, it was detected at a significantly higher frequency of 6.2% (110/1762) in women with abnormal cytology (p=<0.001).

Table 2 - *HPV prevalence and genotype distribution.*

Parameter	*Total*		*Turkish*		*Foreign National*		*p-valuea*
Positivity (n=1597)	*n/T*	*% (95%Cl)*	*n/T*	*% (95%Cl)*	*n/T*	*% (95%Cl)*	
HPV +	1597/12641	12.6 (12.1–13.2)	1559/12301	12.7 (12.1–13.3)	38/340	11.2 (8.0–15.0)	0.412
HPV –	11044/12641	87.4 (86.8–87.9)	10742/12301	87.3 (86.7–87.9)	302/340	88.8 (84.9–91.9)	
Genotypes (n=1766)							
HPV-16	495/1597	31 (28.7–33.3)	484/1559	31.0 (28.8–33.4)	11/38	28.9 (15.4–45.9)	0.921
HPV-18	127/1597	8.0 (6.7–9.4)	121/1559	7.8 (6.5–9.2)	6/38	15.8 (6.0–31.3)	0.117
13 HR-HPV	1144/1597	71.6 (69.4–73.8)	1117/1559	71.6 (69.3–73.9)	27/38	71.1(54.1–84.6)	0.936
Distribution of HPV genotypes in single, multiple and untyped infections (n=1597)							
Single (n=441)	441/1597	27.6 (25.4–29.9)	431/1559	27.6 (25.4–29.9)	10/38	26.3 (6.0–31.3)	0.770
Multiple (n=158)	158/1597	9.9 (8.5–11.5)	153/1559	9.8 (8.4–11.4)	5/38	13.2 (4.4–28.1)	
13HRHPVs *w*	998/1597	62.5 (60.1–64.9)	975/1559	62.5 (60.1–65.0)	23/38	60.5 (43.4–76.0)	
HR-HPV types							
HPV-16	354/1597	22.2 (20.2–24.3)	348/1559	22.3 (20.3–24.5)	6/38	15.8 (6.0–31.3)	0.447
HPV-18	87/1597	5.4 (4.4–6.7)	83/1559	5.3(4.3– 6.6)	4/38	10.5 (2.9–24.8)	0.149
13 HR-HPVs	599/1597	37.5 (35.1–39.9)	975/1559	62.5 (60.1–65.0)	23/38	60.5 (43.4–76.0)	0.933
HPV-16&18	12/1597	0.8 (0.4–1.3)	11/1559	0.7 (0.4–1.3)	1/38	2.6 (0.1–13.8)	0.252
HPV-16&13HR-HPV	118/1597	7.4 (6.2–8.8)	115/1559	7.4 (6.1–8.8)	3/38	7.9 (1.7–21.4)	0.757
HPV-18&13HR-HPV	17/1597	1.1 (0.6–1.7)	17/1559	1.1(0.6–1.7)	0/38	0.0 (0.0–9.3)	1.000
HPV-16&18&13HR-HPV	11/1597	0.7 (0.3–1.2)	10/1559	0.6 (0.3–1.2)	1/38	2.6 (0.1–13.8)	0.233
Years							
2019	267/2098	12.7 (11.3–14.2)	262/2054	12.8 (11.3–14.3)	5/44	11.4 (3.8–24.6)	
2020	308/2287	13.5 (12.1–14.9)	306/2227	13.7 (12.3–15.2)	2/60	3.3 (0.4–11.5)	0.017*
2021	738/5637	13.1 (12.2–14.0)	710/5464	13.0 (12.1–13.9)	28/173	16.2 (11.0–22.5)	
2022[a]	284/2619	10.8 (9.7–12.1)	281/2556	11.0 (9.8–12.3)	3/63	4.8 (1.0–13.3)	

Abbreviations: n: number of cases; T: total number of cases; 13 HR HPVs : high-risk HPV types: HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68. HPV, Human papillomavirus; 13 HRHPVs, high-risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67 and 68); p-valuea : Pearson's $\chi 2$ test (less than 0.05 is statistically significant), * statistical analysis was calculated for overall prevalence by year, [a] the first six months of 2022 have been included.

Prevalence of HR-HPV During the Time Period Studied

HPV prevalence by year (2019 to 2022) is shown in *Table 2*. Although the prevalence of HPV-16 decreased from 2019 to 2022 (from 6.0% to 2.5%), it was the most common HPV type in the years analysed (p 0.001). At the same time, a decrease in the prevalence of HPV-18 (from 1.5% to 0.3%) was detected from 2019 to 2022 (p 0.001). Differently, the prevalence of other HR-13 HPV types increased (from 6.8% to 9.4%) over the studied years (from 2019 to 2022[a]) (*Figure 1*) (p=0.001). [a]The first six months of 2022 have been included.

Distribution of HR-HPV Infection Prevalence by Age Groups

The women's ages ranged from 18 to 97 years, with a median age of 43 (interquartile range [IQR]: 36-51). The median age was 43 years in HPV-negative women (IQR: 36-51), and was 44 years in HPV-positive women (IQR: 36-51).

The prevalence of HPV infection was highest in the 18–30 age group (14.1%) and lowest in the ≥70 age group (11.7%) (p=0.262).

The highest prevalence of HPV-16 was recorded in the ≥70 age group (4.2%) and for HPV-18 in the 18-30 age group (1.7%) (p=0.999 and p=0.014, respectively) (*Table 3*). No significant difference was detected in HPV prevalence due to ethnic differences in all age groups (p=0.261 and p=0.221, respectively). HPV positivity rates in different ethnicities and age groups are presented in *Figure 1*.

Risk Status for Increased Cytology Score of Age, Ethnicity, and Presence of HR-HPV Types

For this purpose, Pap smear cytology results were scored (Benign cytology=1, AGCUS=2, ASCH=3, ASCUS4, LSIL=5, HSIL= 6). While a cytology score ≥5 creates a significant risk in HR-HPV positive patients compared to HPV negative patients, this situation was not determined for age and ethnicity (*Table 4*).

Table 3 - *Prevalence of HR-HPV types by age group and cytology result.*

Variables	*HPV status*	*Genotyping (n=1766)*			*Distribution of HPV genotypes in single, multiple and untyped infections (n=1597)*						
Age groups	*HPV +*	*HPV16*	*HPV18*	*13HR-HPV*	*HPV16*	*HPV18*	*13HRHPV*	*HPV16,18*	*HPV16, 13HRHPV*	*HPV18, 13HR-HPV*	*HPV16,18, 13HRHPV*
	n(%)	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*	*n(%)*
18–30 (n=1635)	230 (14.1)	66 (4.0)	27 (1.7)	162 (9.9)	48 (2.9)	19 (1.2)	139 (8.5)	1 (0.1)	16 (1.0)	6 (0.4)	1 (0.1)
31–40 (n=3307)	393 (11.9)	129 (3.9)	24 (0.7)	279 (8.4)	95 (2.9)	15 (0.5)	247 (7.5)	4 (0.1)	27 (0.8)	2 (0.1)	3 (0.1)
41–50 (n=4478)	557 (12.4)	172 (3.8)	35 (0.8)	406 (9.1)	124 (2.8)	26 (0.6)	354 (7.9)	1 (0.0)	44 (1.0)	5 (0.1)	3 (0.1)
51–60 (n=2170)	291 (13.4)	85 (3.9)	28 (1.3)	209 (9.6)	57 (2.6)	20 (0.9)	185 (8.5)	5 (0.2)	21 (1.0)	1 (0.0)	2 (0.1)
61–70 (n=812)	98 (12.1)	33 (4.1)	10 (1.2)	69 (8.5)	23 (2.8)	5 (0.6)	57 (7.0)	1 (0.1)	8 (1.0)	3 (0.4)	1 (0.1)
≥70 (n=239)	28 (11.7)	10 (4.2)	3 (1.3)	19 (7.9)	7 (2.9)	2 (0.8)	16 (6.7)	0 (0.0)	2 (0.8)	0 (0.0)	1 (0.4)
Total	1597 (12.6)	495 (3.9)	127 (8.0)	1144 (9.0)	354 (2.8)	87 (0.7)	998 (7.9)	12 (0.1)	118 (0.9)	17 (0.1)	11 (0.1)
*p–value**	*0.262*	*0.999*	*0.014*	*0.477*	*0.994*	*0.054*	*0.525*	*0.134*	*0.976*	*0.036*	*0.445*
Cytological evaluation											
Normal (n=10879)	620 (5.7)	167 (1.5)	47 (0.4)	462 (4.2)	122 (1.1)	30 (0.3)	420 (3.9)	6 (0.1)	31 (0.3)	3 (0.0)	8 (0.1)
Abnormal (n=1762)	977 (55.4)	328 (18.6)	80 (4.5)	682 (39.7)	232 (13.2)	57 (3.2)	578 (32.8)	6 (0.3)	87 (4.9)	14 (0.8)	3 (0.2)
–ASGUS	2 (0.2)	2 (0.6)	0 (0.0)	0 (0.0)	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
–ASCH	35(3.6)	23 (7.0)	1 (1.3)	18 (2.6)	16 (6.9)	1 (1.8)	11 (1.9)	0 (0.0)	7 (8.0)	0 (0.0)	0 (0.0)
–ASCUS	755 (77.3)	222 (67.7)	61 (76.3)	541 (79.3)	160 (69)	50 (87.7)	477 (82.5)	4 (66.7)	57 (65.5)	6 (42.9)	1 (33.3)
–LSIL	163 (16.7)	65 (19.8)	16 (20.0)	114 (16.7)	42 (18.1)	5 (8.8)	86 (14.9)	2 (33.3)	19 (21.8)	7 (50)	2 (66.7)
–HSIL	22 (2.3)	16 (4.9)	2 (2.5)	9 (1.3)	12 (5.2)	1 (1.8)	4 (0.7)	0 (0.0)	4 (4.6)	1 (7.1)	0 (0.0)
*p–value**	*<0.001*	*<0.001*	*0.362*	*0.006*	*<0.001*	*0.639*	*0.071*	*0.394*	*<0.001*	*0.004*	*0.170*

Abbreviations: HPV:Human papillomavirus; 13 HR-HPV: high risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68); AGCUS, Atypical glandular cells of unknown significance; ASC-H, HSIL cannot be excluded, atypical squamous cells; ASC-US: Atypical squamous cells of undetermined significance; LSIL, Low-grade squamous intraepithelial lesion; HSIL, High-grade squamous intraepithelial lesion; a Pearson chi-square test (statistically significant if <0.05).

Figure 1 - *HPV positivity rates in different ethnicities and age groups.*

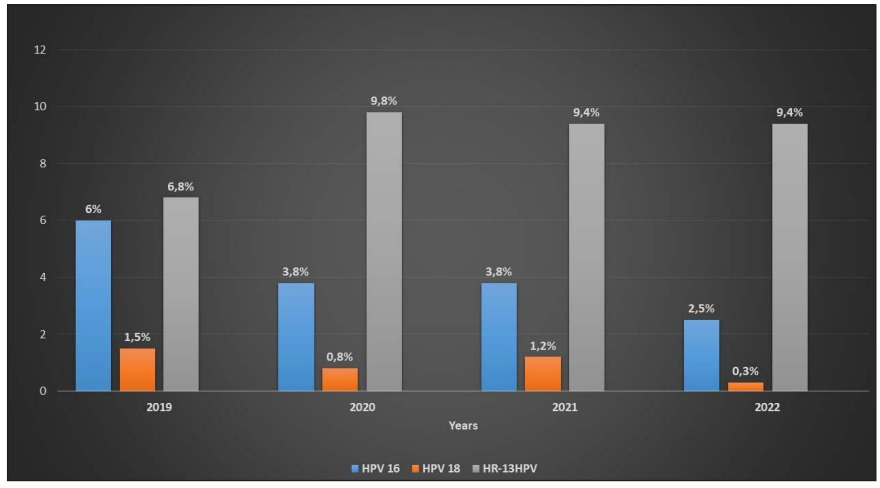

Table 4 - *Multivariate analysis of risk factors (age, ethnicity, and HPV genotypes) with cervical cytological scoring.*

	Cervical Cytology Score		OR	95%Cl		p–value
Risk Factors	*<5*	*≥5*		*LL*	*UL*	
Age Groups	*n (%)*	*n (%)*				
18–30	1600 (97.9)	35 (2.1)				
31–40	3238 (97.9)	69 (2.1)	0.97	0.65	1.47	0.901
41–50	4384 (97.9)	94 (2.1)	0.98	0.66	1.45	0.920
51–60	2120 (97.7)	49 (2.3)	1.06	0.68	1.64	0.806
61–70	789 (97.2)	23 (2.8)	1.33	0.78	2.27	0.292
>70	233 (97.5)	6 (2.5)	1.23	0.51	2.96	0.715
Ethnic Groups						
Turkish woman	12034 (97.8)	267 (2.2)				
Others	331 (97.4)	9 (2.6)	1.23	0.63	2.24	0.553
HPV Results						
HPV negative	10953 (99.2)	91 (0.8)				
HPV-16	300 (84.7)	54 (15.3)	21.67	15.18	30.92	<0.001
HPV-18	81(93.1)	6 (6.9)	8.92	3.79	20.95	<0.001
13 HR-HPVs	908 (91.0)	90 (9.0)	11.93	8.85	16.09	<0.001
HPV-16&HPV-18	10 (83.3)	2 (16.7)	24.07	5.20	111.41	<0.001
HPV-16&13 HR-HPV	95 (80.5)	23 (19.5)	29.14	17.67	48.05	<0.001
HPV-18&13 HR-HPV	9(52.9)	8 (47.1)	106.99	40.38	283.49	<0.001
HPV-16&HPV-18&13 HR-HPV	9(81.8)	2 (18.2)	26.75	5.70	125.52	<0.001

Abbreviations: OR: Odds Ratio, CI: confidence interval, LL: lower limit, UP: upper limit, n:Number of cases, HPV: Human papillomavirus, Cervical Cytological Scoring: Benign Cytology: 1, AGCUS: 2, ASCH: 3, ASCUS: 4, LSIL: 5, HSIL: 6

HR-HPV Prevalence and Distribution of Genotypes According to Cervical Biopsy Findings

Of the 93 patients who underwent cervical biopsy, CIN I was detected in 78, CIN II in 7, and CIN III in 8. HPV positivity was found in 16.1% of biopsy samples, and the most detected were HR-13 HPV types (9.6%). Distributions of HPV-16, HPV-18, and HR-13 HPV types according to histological diagnosis are shown in *Table 5*.

DISCUSSION

In this study, the overall prevalence of HR-HPV in women aged 18 years and over in Turkey's Mersin province was between 11.2% and 12.7%, depending on ethnicity, and the overall prevalence in the study population was determined to be 12.6%. Regardless of ethnicity, the highest prevalence rates according to age groups were found in the 18-30 age group (14.1%), 51-60 age group (13.4%), and 41-50 age group (12.4%). HPV prevalence and genotype distribution vary depending on region and age (Zhu *et al*., 2021). Globally, the prevalence of HPV was estimated to be 9.9% among women with normal cervical cytology as of 2019, and it has been reported that HPV carriage has the highest prevalence on the Asian (45.5%) and African (29.6%) continents (Kombe *et al*., 2021). It is reported that it generally peaks in adolescence and in the 20s in European women, and the prevalence of HPV infection is estimated to be 14% (Bruni L *et al*., 2010). In a meta-analysis study conducted on the African continent in 2015, it was reported that the prevalence of HPV infection varies according to geographical regions (from 12.8% to 57.3%), and the highest prevalence was found in the 25-34 age range (Ogembo *et al*., 2015). In a 20-year meta-analysis including women in China and the Asian continent, the overall HR-HPV prevalence was found to be 19%, and it was reported that this rate was highest in women under 25 years of age, 24.3% (Li *et al*., 2019). In a study investigating the prevalence of HR-HPV types in women aged 25-65 in Hungary, the overall prevalence was found to be 11.15%, and was most commonly detected in the 25-29 age range (19.43%) (Fogarasi *et al*., 2022). In the most comprehensive HPV surveillance screening covering approximately 4 million women over the age of 30 in Turkey, the prevalence was found to be 4.39% (Gultekin *et al*., 2020). The prevalence of HR-HPV we found in our study was higher (12.6%). We think this is due to the fact that we worked hospital-based, the specificity of our patient population, and the close cooperation between the gynaecology

Table 5 - *Distribution of HPV genotypes according to cervical biopsy results.*

	Histological Diagnosis of Patients				
	CINI (n=78)	*CINII (n=7)*	*CINIII (n=8)*	*Total (n=93)*	*p–value*
	n(%)	*n(%)*	*n(%)*	*n(%)*	
HPV PCR Result					
HPV PCR (–)	65(83.3)	6(85.7)	7(87.5)	78(83.9)	1.000[a]
HPV PCR (+)	13(16.7)	1(14.3)	1(12.5)	15(16.1)	
HPV Types					
HPV-16	5 (6.4)	1(14.3)	0(0.0)	6(6.5)	
HPV-18	0(0)	0(0)	0(0)	0(0)	
13 HR-HPVs	8(10.3)	0(0)	1(12.5)	9(9.6)	
HPV Types: Single/Multiple/Untypeable					
HPV- 16	5(6.4)	1(14.3)	0	6(6.5)	
HPV-18	0	0	0	0	
13 HR-HPVs	8(10.3)	0	1(12.5)	9(9.6)	
HPV-16&HPV-18	0	0	0	0	
HPV-16&13 HR-HPVs	0	0	0	0	
HPV-18&13 HR-HPVs	0	0	0	0	
HPV-16&HPV-18&13 HR-HPVs	0	0	0	0	
Cytological Diagnostic Categories					
Normal	68(87.2)	5(71.4)	8(100)	81(87.1)	
ASGUS	0(0)	0(0)	0(0)	0(0)	
ASC–H	0(0)	0(0)	0(0)	0(0)	
ASCUS	9 (11.5)	2(28.6)	0(0)	11(11.8)	
LSIL	1(1.3)	0(0)	0(0)	1(1.1)	
HSIL	0(0)	0(0)	0(0)	0(0)	

Abbreviations; n: Number of cases; HPV: Human papillomavirus; CIN: Cervical intraepithelial neoplasia; AGCUS: Atypical glandular cells of unknown significance; HSIL: High-grade squamous intraepithelial lesion; ASCH: HSIL cannot be ruled out atypical squamous cells; ASCUS: Atypical squamous cells of unknown significance; LSIL: Low-grade squamous intraepithelial lesion; [a]Pearson chi-square test (statistically significant if <0.05).

unit and microbiology and pathology diagnostic laboratories. The general prevalence rates we determined in this study are compatible with the prevalence rates reported for Europe but are lower than the rates reported for Asia and Africa.

Racial and ethnic differences in HPV infection have been demonstrated. Non-Hispanic blacks have been reported to have the highest prevalence of HPV, followed by Hispanics and non-Hispanic whites. It is thought that the differences detected in HPV prevalence according to race and ethnicity may be related to social norms and sexual attitudes (Lin *et al.,* 2015). When prevalence rates are examined in terms of ethnicity, they were found to be 12.7% in people of Turkish origin and 11.2% in people of foreign origin, and no significant difference was detected. Determining the prevalence of HPV infection in immigrant populations is important for the development of health policies. For this reason, it would be useful to conduct multi-centre studies, especially in areas receiving immigration.

It has been reported in the literature that HPV infection is detected at high frequency primarily in women in the young age group, and that a second peak is detected in post-menopausal age groups, resulting in a u-shaped distribution (Kaleli *et al*., 2021; Jarienė *et al*., 2012; Gupta *et al*., 2022). Another study reported that the prevalence decreased with advancing age and there was no second peak (Fogarasi *et al.,* 2022).

In our study, these two peaks could not be determined; the highest infection incidence was detected in the 18-30 age range, and different infection rates were detected in each age group. In this situation, we think it may be caused by differences in the age of onset of sexual activity, number of partners, behavioural differences, and changes in the immune response to HPV.

While HPV-16 and HPV-18 constitute 70% of cervical cancer cases in the world, HPV-31, HPV-58, and HPV-52 are the other most commonly detected types (Nn Arbyn *et al*., 2014; Bruni L *et al*., 2010). Since HR-HPV genotypes other than HPV types 16 and 18 are

detected in approximately 30% of cervical cancers, it has been reported that defining HR-HPV (non-HPV 16/18) types by pooling can provide sufficient information for HPV screening (Monsonego *et al*., 2015). In many studies conducted abroad and in different regions of our country, the most frequently detected HR-HPV type is HPV-16, and the second most frequent is HPV-18 (Szymonowicz *et al*., 2020; Kombe *et al*., 2021; Monsonego *et al*., 2015; Seneldir *et al*., 2019; Beyazit *et al*., 2018).

In our study, among the typed HR-HPVs, the most frequently detected type in both Turks (31%) and foreign nationals (28.9%) was HPV-16, in line with the literature. Due to the test kit design used in PCR analysis, specific HPV types other than HPV-16 and HPV-18 could not be differentiated. Therefore, data on the prevalence of HR-13 HPV types could not be obtained.

It has been reported in the literature that the rate of multiple HPV infection varies from 20% to 30% (Salazar *et al.* 2015). The prevalence of multiple HR-HPV types (co-infection) was examined in a meta-analysis study and was found to be in second place (20.8%) after single HPV-16 (Gupta *et al*., 2022). In our study, the coinfection rate in HR-HPV positive samples was found to be 9.9%, which was lower than the rates reported in the literature. This may have been due to the fact that other types of hrHPV related to kit design were researched from a single pool. An increase in the incidence of co-infections has been reported in cervical cancer, especially those accompanied by HPV-16 (Li *et al*., 2019). It was found in our study that the most common type accompanying more than one infection was HPV-16 (141/158). Since there were no cases of cervical cancer in this study, the correlation with coinfections could not be evaluated. More studies are needed to determine the correlation between cervical cancer and single or multiple HR-HPV infections.

Indeed, in numerous studies, it has been reported that multiple HPV infections are an important risk factor for high-risk cervical lesions (LSIL; HSIL) and exhibit a synergistic effect for cervical cancer (Bello *et al.*, 2009; Pista *et al.* 2011; Spinillo *et al*., 2009).

In this study, it was also found that multiple HPV types of infections are associated with a higher risk for high-risk cervical lesions compared to infection with a single HPV type.

It has been reported in the literature that the detection rates of multiple HR-HPV infections are 1.5%-4.2% in women with normal cytology and 7.2%-20.8% in women with abnormal cytology lesions (Zhu *et al*., 2021; Altay-Kocak *et al*., 2022). In our study, the prevalence of multiple HR-HPV types was 0.2% in women with normal cytology results, while it was found to be significantly higher (6.2%) in women with abnormal cytological lesions, similar to previous studies.

Since HR-13HPV types were investigated in a single pool, a distinction between single or multiple infections could not be made, and they were presented as a different group.

In a broad-based screening program (ATHENA) in the United States in which the correlation of HR-HPV types associated with the risk of cervical precancerous lesions was investigated in women aged 25 and over, the overall prevalence was 10.3%, 9% in those with normal cytology findings, and higher in those with abnormal cytological findings. It was detected at a rate of 29.7% (Monsonego *et al*., 2015). In hospital-focused studies reported from Turkey, the overall HR-HPV prevalence was found to be from 14.2% to 34%, 17.4% to 20.9% in women with normal cytology, and 40.6% to 79.7% in women with abnormal cytological lesions (Kaleli *et al*., 2021; Seneldir *et al*., 2019; Altay-Kocak *et al*., 2022).

In our study, the prevalence of HR-HPV was found to be lower than in previous studies (5.7%) in those with normal cytology results, while it was found to be higher (55.4%) in those with abnormal cytological lesions, consistent with previous studies.

Long-term and persistent infection of HPV-infected epithelium is an important risk factor for cervical cancer. Therefore, it is not surprising that HR-HPV positivity was found to be higher in those with abnormal cytology.

In this study, HR-HPV positivity was found to be higher in women with abnormal cytology (55.4% versus 5.7%) than in women with normal cytology, and the pool of HR-13HPVs was higher in both abnormal cytology (39.7%) and women with CINI and CINIII histopathology. It was detected most frequently in the samples (9.6%).

In our study, HR-13HPV(s) were found to be significantly higher in ASCUS (79.3%) and LSIL (16.7%) cases with abnormal cytology diagnoses.

Altay-Koçak and colleagues from Turkey investigated HR-HPV genotypes (HPV-16, HPV-18, and other pooled HR-HPV types) with clinical data from a large number of female cases and found the frequency of HR-HPV positivity in samples with abnormal cervical cytology. They found that the rate was higher (41%), consistent with our study. In the same study, it was reported that other (non-16 and non-18 HPV types) HR-HPV(s) were in first place (22.6%), while HPV-16 was in second place (9.5%) (Altay-Kocak *et al*., 2022).

In a university hospital in Turkey, the HR-HPV positive detection rates in women with abnormal cytological test results (ASCUS, LSIL, and HSIL) were 94%, 89%, and 100%, respectively (Beyazit *et al*., 2018).

Weizhi Sen and colleagues from China found the HR-HPV detection rate to be 62.80% (50.2%, 77.1%, and 89% for ASCUS, LSIL, and HSIL, respectively) in a large number of women aged 21-65 with an abnormal cytological diagnosis (You *et al*., 2018).

In our study, we likewise observed higher rates of

HPV among patients with abnormal cytology. As cytological atypia increased, the rate of HPV infection was also observed to increase (ASCUS: 52.5%, LSIL: 64.1%, HSIL: 100%) (p<0.001).

The data we obtained show that it is useful to detect HR-HPV at the early stages of cytological dysplasia (at the ASCUS level).

These findings are compatible with national and the World Health Organisation's HPV screening and elimination programmes, which include the early diagnosis and effective management of cervical cancer (Gultekin *et. al*., 2020; WHO, 2023).

The main limitation of this study could be attributed to its retrospective nature. Transmission routes and risk groups could not be evaluated in patients. Subtype definitions were limited due to the design of the kit used to identify the genotypes.

CONCLUSION

In this study, overall HR-HPV prevalence was found to be 12.6%. The most commonly detected HR-HPV type is HPV-16. HR-13HPV types were detected at a higher frequency in samples with abnormal cytology and in samples with CINI and CINIII histopathology. By determining the separate frequency of HR-13 HPVs other than HPV-16 and HPV-18, specific HPV-targeted vaccination can be encouraged in countries such as Turkey, where a routine HPV vaccination program is not yet implemented.

Since migrations may for various reasons cause changes in ethnic structure as well as differences in HPV prevalence and genotypes, we think that the updated data obtained as a result of frequent regional follow-up will contribute to epidemiological studies.

Our findings of a correlation between HPV DNA PCR tests and cytological evaluation results showed that early diagnosis is beneficial.

Ethics Committee Approval

Ethical approval was obtained for this study from the Mersin Toros University Ethics Committee (Reference Number: 142), September 23, 2022.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Concept - M.Y., E.A.O., A.B.; Design - M.Y., E.A.O.; Data Collection or Processing - M.Y., E.A.O., A.B.; Analysis or Interpretation - A.B.; Literature Search - M.Y., E.A.O.; Writing- M.Y., E.A.O., A.B.; Critical Review - M.Y., A.B.

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Conflicts of Interest

No conflicts of interest were declared by the authors.

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