Submitted: 08 April, 2023 Accepted: 09 May, 2023 Published: 15 August, 2023 DOI:10.22514/ejgo.2023.048

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Association of HPV and sexually transmitted infections among patients with genital warts and asymptomatic individuals: a cross-sectional study

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Abstract

STIs can impact HPV infection and persistence, potentially predisposing HPV-related cervical cancer development. This study examines HPV genotype prevalence and co-occurrence with other STIs to inform targeted prevention and treatment strategies for reducing cervical cancer incidence. 129 female patients aged 18–57 were enrolled based on the presence of anogenital warts, individuals with a history of risky sexual behaviors, having a partner with HPV infection, or voluntarily seeking HPV screening. Patients with a history of any STIs, prior HPV vaccination, systemic illnesses, or undergoing cancer treatment were excluded. Patients were divided into two groups: Genital warts group (31.8%) and asymptomatic group (68.2%). Among patients with genital warts, HPV types 6, 11, and 61 were prevalent, whereas in asymptomatic patients, HPV types 53, 31, and 16 were more common. The STI positivity rate among HPV-positive patients was 63.9%, significantly higher than HPV-negative cases. In the genital warts group at admission, Ureaplasma Parvum (UP) was the most common STI (40.0%), followed by Uraeplasma Urealyticum (UU) (28.5%), Mycoplasma Hominis (MH) (17.2%), and Chlamidia Trachomatis (CT) (11.4%). In the asymptomatic group, UP was also the most common STI (41.2%), followed by UU (17.6%), MH (15.8%), CT (9.7%), TV (6.2%), MG (5.3%), HSV-2 (2.6%), TP (0.8%), and NG (0.8%). The prevalence of UP was significantly higher (53.7%) in the HPV-positive group, suggesting a 6.96-fold greater risk of UP infection in individuals with HPV. This study demonstrates a high co-infection rate between HPV and UP, emphasizing the importance of genital infection screening for high-risk HPV-positive women. Further longitudinal research is needed to investigate the role of STIs as contributing factors in HPV-related cervical cancer development.

Keywords

HPV genotypes; STI co-infections; Ureaplasma parvum; Cervical infections; Cervical cancer risk

1. Introduction

Sexually transmitted infections (STIs) are very common in both men and women worldwide [1]. The most common STIs are Human papillomavirus (HPV) infections [2] and HPV is the most important factor in the etiology of cervical dysplasia and cervical cancer [3]. There are more than 300 identified types of HPV [4] and approximately 40 types of HPV show affinity to the genital area. HPV types are classified as low-risk, probably high-risk, and high-risk [5]. HPV Type 6 and HPV Type 11 belong to the low-risk HPV group and are responsible for 90% of anogenital warts. The main causes of cervical cancer development are HPV 16 and HPV 18 [6]. High-risk HPV types are detected in more than 95% of cervical cancer biopsies [6]. However, it is important to note that high-risk HPV types alone do not cause the development of cervical cancer, as genetic factors, smoking, and long-term use of oral contraceptives, among other factors, are also associated with cancer transformation [7].

Evidence of the predisposing role of other STIs in the development of cervical dysplasia and cancer in HPV-positive individuals is increasing day by day. The interaction between these pathogens, which share the same mucosal area as HPV, can accelerate cervical dysplasia and invasive cancer progression by increasing HPV persistence and replication. Tamim *et al.* [8] reported in their study that the combination of Chlamydia Trachomatis (CT) and HPV increases the risk of cervical cancer. CT can cause an inflammatory reaction that facilitates HPV entry into the cervical mucosa basal membrane. Similarly, pathogens such as Trichomonas Vaginalis (TV), Mycoplasma Hominis (MH), Ureaplasma Parvum (UP), and Uraeplasma Urealyticum (UU) can facilitate HPV entry into the cervix by triggering cervical inflammation [9].

Additionally, STIs may impact HPV infection and persis-

tence through various mechanisms, such as disruption of the cervical epithelium, increasing viral load and shedding, alterations in the cervical and vaginal microbiome, synergistic effects of co-infections, and hormonal changes [10–13]. Moreover, molecular mimicry exhibited by some STIs can compromise the host's defense against HPV infection, potentially leading to a higher likelihood of HPV persistence and progression to malignancy [14].

The study aims to assess the cross-sectional prevalence of various HPV genotypes and sexually transmitted infections (STIs), as well as their concurrent occurrence. Enhancing our understanding of the relationship between STIs and HPV infection can facilitate the development of targeted prevention and treatment methods, ultimately contributing to a reduction in the development of HPV-related cervical cancer.

2. Materials and methods

2.1 Study design and patient selection

A total of 129 women between 18 and 57 years of age who visited a private clinic in Istanbul, Turkey between 19 January 2023 and 01 April 2023 were included in this observational cross-sectional study. Patients were either diagnosed with anogenital warts, had a history of suspicious sexual activity, had a partner with HPV infection, or voluntarily wanted to undergo screening for HPV. Patients with a history of any STIs, prior HPV vaccination, systemic illnesses, or undergoing cancer treatment were excluded from the study. HPV and STI screening tests are applied to all patients.

2.2 HPV detection

The DNA of samples obtained from vaginal swabs was isolated using the Magnesia Viral Nucleic Acid Extraction kit, and HPV DNA Polymerase Chain Reaction (PCR) was performed using the Montana 4896 Real-Time PCR system. HPV typing was performed using the Single-Step PCR and Reverse Line Blot techniques with the Ampliquality HPV-Type Express v3.0 kit (02504-220607, AB Analytica, Padova, Italy). This test identified low-risk, probably high-risk, and high-risk HPV types (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68a, 68b, 69, 70, 71, 72, 73, 81, 82, 83, 84, 87, 89, 90) [15].

2.3 STI detection

Cervical swab samples collected from patients were examined using the Bosphore STIs Panel Kit v6 (BT3010, Anatolia Geneworks, Istanbul, Turkey) for the detection of STI. The kit includes an internal control for DNA isolation and PCR inhibition. Amplification data of the internal control added to the PCR reaction mixture can be visualized using specific filters. Different PCR master mixes were used to detect specific pathogens, including UU, UP, MH, Neisseria gonorrhea (NG), TV, Gardnerella vaginalis (GV), Mycoplasma genitalium (MG), Treponema pallidum (TP), Herpes Simplex Virus 1 (HSV-1), and Herpes Simplex Virus 2 (HSV-2). Detection of each pathogen was carried out using a specific filter in the respective PCR master mix tube [16].

This kit is can detect UU, UP, MH, NG, TV, GV, MG, TP, HSV-1 and HSV-2 viruses [16]. GV was not considered a STI in our study as it can be found in normal vaginal flora.

2.4 Statistical analysis

In the evaluation of the findings obtained in the study, the NCSS (Number Cruncher Statistical System) 2020 Statistical Software (NCSS LLC, Kaysville, UT, USA) program was used for statistical analysis. When evaluating the study data, quantitative variables were presented using descriptive statistical methods such as mean, standard deviation (Sd), median, minimum, and maximum values, while qualitative variables were presented using frequency and percentage. Shapiro Wilks test and Box Plot graphics were used to evaluate the normal distribution of the data.

The Student *t*-test was used for the evaluation of two quantitative groups showing normal distribution. The Mann Whitney-U test was used for the evaluation of variables that did not show normal distribution in two-group comparisons.

Chi-square test, Fisher's Exact test, and Fisher Freeman Halton test were used for the comparison of qualitative data. The results were evaluated at the 95% confidence interval, with a significance level of $p < 0.05$.

3. Results

Patients included in the study were divided into two groups. The first group was patients with genital warts (n = 41), and the second group was asymptomatic (n = 88). The demographic data are shown in Table 1.

3.1 HPV data

In the study, HPV was detected in 83.7% (n = 108) of the patients. Low-risk HPV was found in 72.2% (n = 78), probably high-risk HPV in 34.3% (n = 37), and high-risk HPV types in 63% (n = 68) of the patients. Among the patients, 13% (n = 14) had HPV Type 16, and 12% (n = 13) had HPV Type 18. HPV Type 6 was found in 18.5% (n = 20) and HPV Type 11 in 10.2% (n = 11). High-risk HPV types were detected in 16.7% (n = 18) of the patients with HPV Type 6 and/or HPV Type 11. Single-type HPV was present in 35.2% (n = 38) of the patients and multiple HPV types were found in 64.8% (n=70) of the patients. Low-risk and high-risk HPV types were detected together in 42.6% (n = 46) of the patients.

No significant difference was found between groups based on patients' admission symptoms ($p > 0.05$). Descriptive characteristics of the groups are provided in Table 1.

HPV Type 6 was detected in 48.5% (n = 16) of the cases with the complaint of genital warts, HPV type 11 was detected in 21.2% (n = 7), and HPV type 61 was detected in 15.2% (n = 5) (Fig. 1).

In asymptomatic patients, Type 53 was detected in 13 patients (25.3%), followed by Type 31 in 8 patients (20%) and Type 16 in 5patients (17.3%) (Fig. 2).

The comparison of HPV types according to the groups is presented in Table 2.

There was no statistically significant difference in the distribution of HPV presence among groups ($p > 0.05$). The

TABLE 1. The comparison of descriptive characteristics by groups.

	Genital warts at admission (n = 41)	Asymptomatic at admission (n = 88)	*p*
Age			
Mean ± Sd	29.61 ± 7.91	30.47 ± 7.57	[a]0.556
Median (Min-Max)	27 (20–48)	29 (18–57)	
BMI (kg/m^2)			
Mean ± Sd	25.74 ± 4.08	25.49 ± 3.94	[a]0.737
Median (Min-Max)	26.6 (17.6–33.9)	25.3 (17.6–34.6)	
Tobacco use			
No	30 (73.2%)	58 (65.9%)	[b]0.410
Yes	11 (26.8%)	30 (34.1%)	
Parity			
Null	13 (31.7%)	24 (27.3%)	[b]0.604
Multi	28 (68.3%)	64 (72.7%)	
Sexual partners in the last 2 years			
1	28 (68.3%)	63 (71.6%)	[c]0.890
2	11 (26.8%)	20 (22.7%)	
3–4	2 (4.9%)	5 (5.7%)	

[a] *Student-t Test.*
[b] *Pearson Chi-Square Test.*
[c] *Fisher Freeman Halton Test.*
Sd, Standard Deviation.

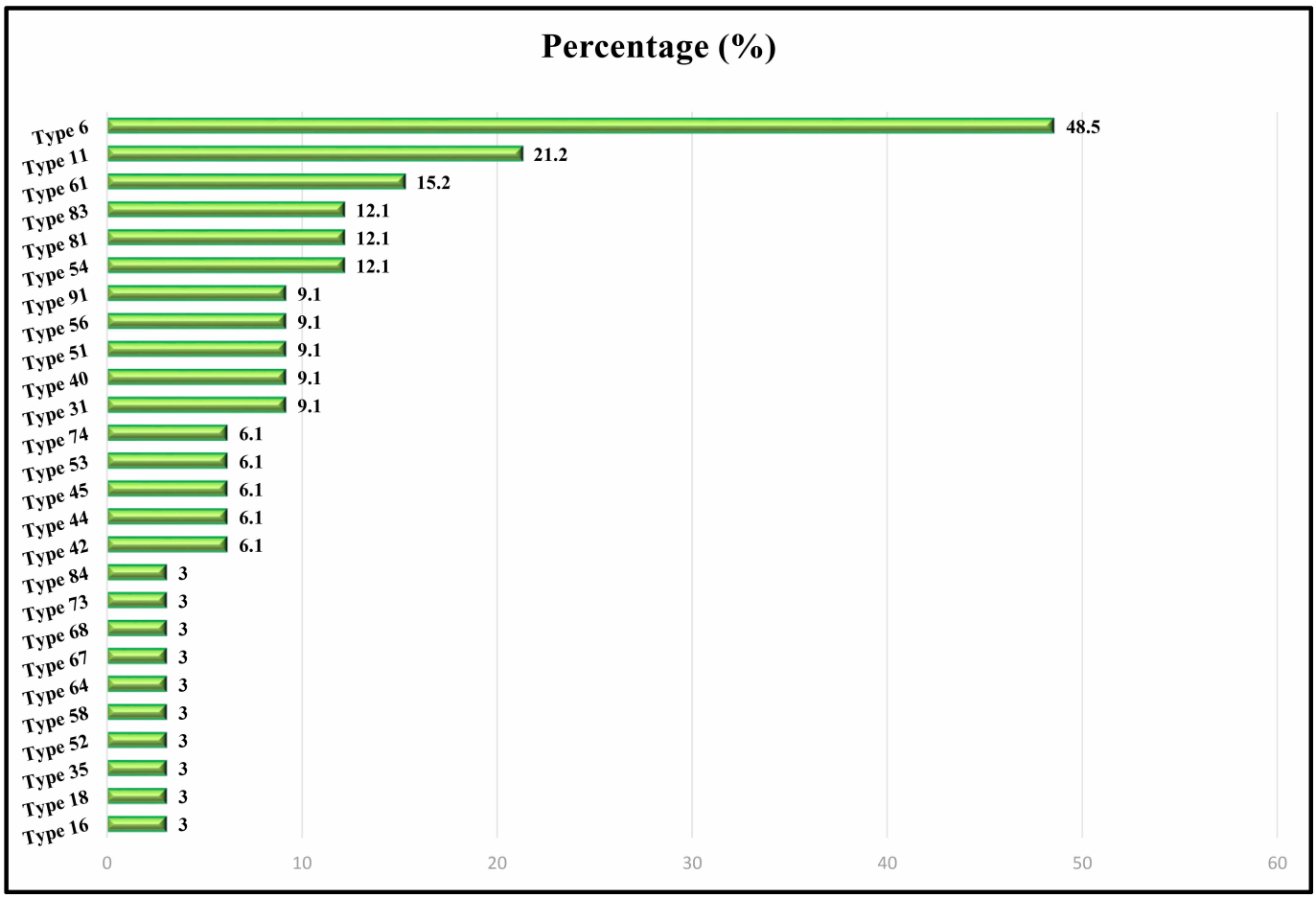

FIGURE 1. Distribution of HPV types among patients with genital warts at presentation.

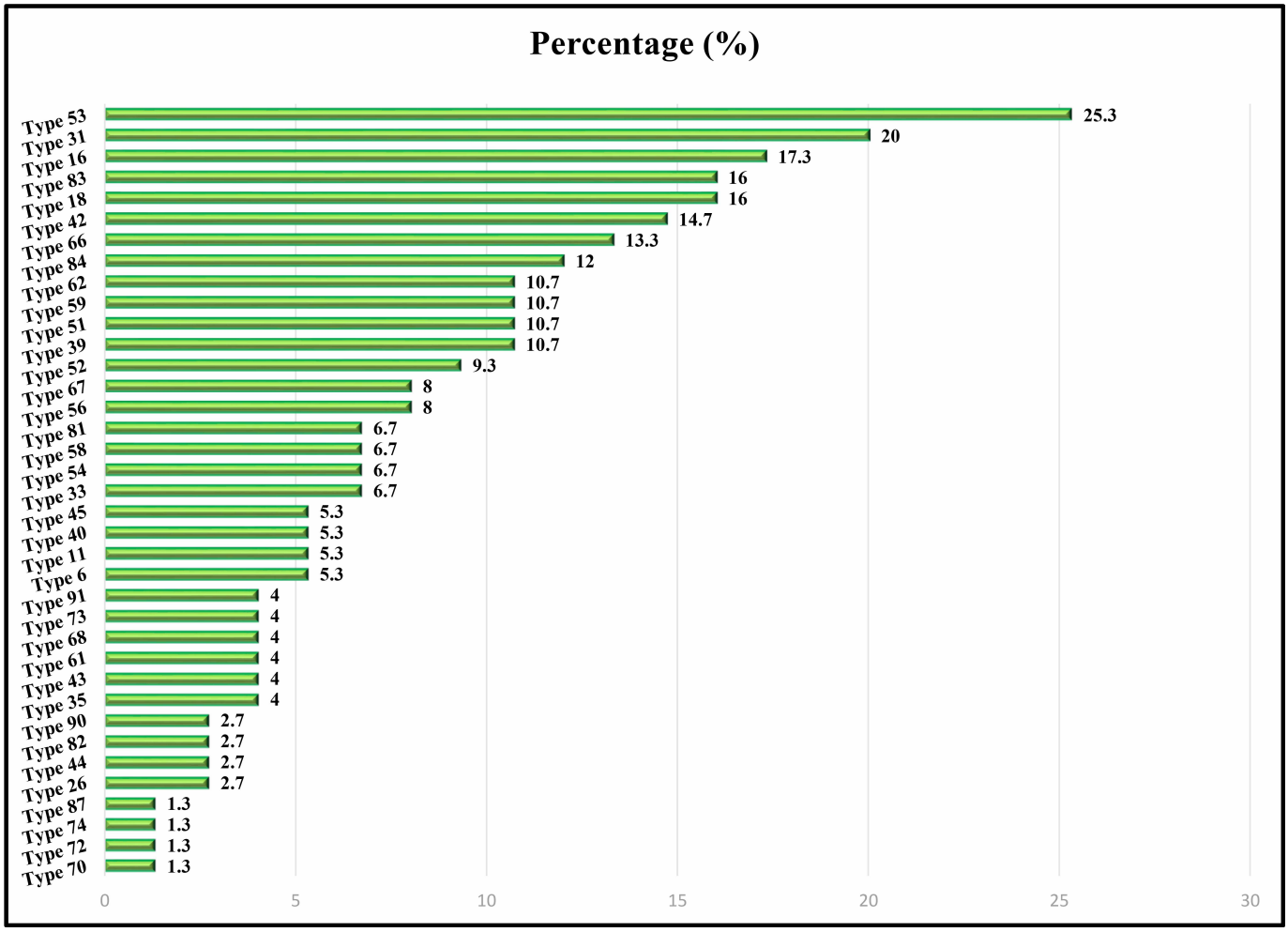

FIGURE 2. Distribution of HPV types among asymptomatic patients.

rate of low-risk HPV types in patients with genital warts at presentation was found to be significantly higher than that in asymptomatic patients ($p = 0.001$; $p < 0.01$). The likelihood of high-risk HPV types in asymptomatic patients at presentation was significantly higher than that in patients with genital warts ($p = 0.002$; $p < 0.01$). In women infected with HPV type 6 and/or 11, co-infection with high-risk type was significantly in patients with genital warts at presentation than in asymptomatic patients ($p = 0.001$; $p < 0.01$). There was no significant difference in the rate of infection with multiple HPV types or co-infection with low- and high-risk HPV types when comparing women with warts and asymptomatic women ($p > 0.05$).

3.2 STI data

All patients included in the study were tested with the STI 10 test, and at least one pathogen was detected positive in 58.1% of these patients.

STI test results of the patients who were positive for HPV were analyzed and the STI positivity rate was found to be 63.9%. This rate is higher than the STI positivity rate of HPV negative cases and statistically significant ($p = 0.003$) (Table 3).

STI test results of patients who were HPV positive were examined, the most notable difference is the higher prevalence of UP in the HPV positive group (53.7%), and this difference is statistically significant ($p < 0.01$). Although variations are observed among other diseases, no statistically significant relationship is found.

Based on these findings, we can conclude that individuals with HPV infection have a 6.96-fold higher risk of being positive for UP infection. (Table 4).

The distribution of STI types among the two groupscan be described as follows. For patients with genital warts at admission, UP was the most common STI, affecting 14 (40.0%) patients, followed by UU in 10 (28.5%) patients, MH in 6 (17.2%) patients, and CT in 4 (11.4%) patients. No cases of TV, MG, HSV-2, TP, NG, or HSV-1 were reported in this group.

For the asymptomatic group at admission, UP was also the most common STI, found in 47 (41.2%) patients. UU affected 20 (17.6%) patients, MH was present in 18 (15.8%) patients, and CT was found in 11 (9.7%) patients. The remaining STIs were observed as follows: TV in 7 (6.2%) patients, MG in 6 (5.3%) patients, HSV Type 2 in 3 (2.6%) patients, TP in 1 (0.8%) patient, and NG in 1 (0.8%) patient. No cases of HSV-1 were reported in the asymptomatic group.

The distribution of STI pathogens according to HPV types is given in Table 5.

TABLE 2. Distribution of HPV characteristics by groups.

	Genital warts at admission (n = 41)	Asymptomatic at admission (n = 88)	*p*
HPV			
Negative	8 (19.5%)	13 (14.8%)	[b]0.497
Positive	33 (80.5%)	75 (85.2%)	
Low risk			
No	2 (6.1%)	28 (37.3%)	[b]0.001**
Yes	31 (93.9%)	47 (62.7%)	
Probably high-risk			
No	29 (87.9%)	42 (56.0%)	[b]0.002**
Yes	4 (12.1%)	33 (44.0%)	
High risk			
No	20 (60.6%)	20 (26.7%)	[b]0.001**
Yes	13 (39.4%)	55 (73.3%)	
Type 6 and/or Type 11			
No high risk	21 (63.6%)	69 (92.0%)	[b]0.001**
With high risk	12 (36.4%)	6 (8.0%)	
HPV Types			
Single Type	13 (39.4%)	25 (33.3%)	[b]0.662
Multiple Type	20 (60.6%)	50 (66.7%)	
Low risk and high risk			
Not together	21 (63.6%)	41 (54.7%)	[b]0.385
Together	12 (36.4%)	34 (45.3%)	

[b]*Pearson Chi-Square Test.*
***p < 0.01.*
HPV, Human Papilloma Virus.

TABLE 3. The association between HPV positivity and STI positivity.

	HPV		[b]*p*
	Negative	Positive	
STI			
Negative	15 (71.4%)	39 (36.1%)	0.003**
Positive	6 (28.6%)	69 (63.9%)	

[b]*Pearson Chi-Square Test.*
***p < 0.01.*
HPV, Human Papilloma Virus; STI, Sexually transmitted infections.

TABLE 4. Comparison of STI pathogenes by HPV positivity.

	HPV (−)	HPV (+)	[e]*p*	OR	95% Confidence Interval
Mycoplasma Genitalum	1 (4.8%)	6 (5.6%)	1.000	1.176	0.134–10.310
Mycoplasma Hominis	1 (4.8%)	23 (21.3%)	0.122	5.412	0.689–42.483
Ureraplasma Urealyticum	3 (14.3%)	27 (25.0%)	0.401	2.000	0.546–7.321
Trichomonas Vaginalis	3 (14.3%)	4 (3.7%)	0.085	0.231	0.045–1.119
Chlamydia Trachomatis	2 (9.5%)	13 (12.0%)	1.000	1.300	0.271–6.237
Ureaplasma Parvum	3 (14.3%)	58 (53.7%)	0.001**	6.960	1.936–25.019
Neisseria Gonorrhoeae	0 (0)	1 (0.9%)	1.000	0.836	0.774–0.903
Herpes Simplex Type 1	0 (0)	0 (0)	-	-	-
Herpes Simplex Type 2	1 (4.8%)	2 (1.9%)	0.416	0.377	0.033–4.362
Treponema Pallidum	0 (0)	1 (0.9%)	1.000	0.836	0.774–0.903

[e]*Fisher's Exact Test.*
***$p < 0.01$.*
HPV, Human Papilloma Virus.
OR, Odds Ratio.

4. Discussion

The study findings revealed that the co-occurrence of low-risk and high-risk HPV types was observed in patients with anogenital warts, where high-risk HPV types could be present along with low-risk HPV types. Additionally, we can conclude that individuals with HPV infection have a significantly higher risk of being positive for UP infection.

In our study, the most common HPV types in cases with genital warts were HPV Type 6, HPV Type 11 and HPV Type 61. Consistent with the literature, studies by Patel *et al*. [17] and Wang *et al*. [18] also reported HPV Type 6 and HPV Type 11 as the predominant types in genital warts cases. Although specific prevalence rates may vary by population and region, the findings of our study align with global trends.

In our study, when examining HPV types in asymptomatic cases during admission, HPV Type 53 was found to be the most common type in 25.3% of cases, followed by HPV Type 31 and HPV Type 16. When compared to other publications in the literature, these findings appear to be generally consistent. In a study conducted by Račić *et al*. [19], the prevalence of any high-risk HPV was estimated at 13.1%, with HPV Type 16 being the most common type, followed by HPV Type 31 and HPV Type 51. In contrast, our study found HPV Type 53 to be the most common type in 25.3% of cases, followed by HPV Type 31 and HPV Type 16. The difference in the prevalence of Type 53 between the two studies may be attributed to differences in the HPV types analyzed. Račić *et al*. [19] only examined high-risk HPV types, while our study included both probably high-risk and high-risk types.

In the nationwide study conducted by Gültekin *et al*. [20], which enrolled 1 million women from Turkey, the most common HPV genotypes detected among 37,515 HPV positive women were 16, followed by 51, 31, 52 and 18. This comprehensive research provided valuable insights into the prevalence of various HPV genotypes in the Turkish population.

In contrast, our study investigated both low-risk and high-risk HPV genotypes in a smaller sample of 129 patients. Interestingly, we found the highest prevalence for HPV 53, which is classified as a probable high-risk genotype but not included in the established high-risk group. Among the high-risk HPV genotypes, our study discovered a different sequence of prevalence: 31, 16, 18 and 51. Despite the differences in ranking, the same HPV genotypes were observed in both studies, with the addition of HPV 53 in our study.

It is also crucial to note the differences in the HPV test systems used in each study. The Cobas test system, employed in Gültekin *et al*.'s [20] study, is designed to detect only high-risk HPV genotypes. In contrast, our study utilized a test capable of detecting both low-risk and high-risk HPV genotypes, offering a more comprehensive view of HPV prevalence in the population.

The discrepancies in ranking could be attributed to factors such as geographic variations, differences in the target populations tested, and the smaller sample size in our study might have also contributed to the differences in the ranking of high-risk HPV genotypes.

Martinelli *et al*. [21]. evaluated Italian women with abnormal smear results and found at least one HPV type positive in 132 out of 177 patients, with HPV Type 16 being the most common high-risk type followed by HPV Type 53, HPV Type 42 and HPV Type 31. Despite these differences, both studies demonstrate the high prevalence of HPV Type 53 in HPV-positive individuals and highlight the importance of its detection in clinical practice. Therefore, our findings are consistent with the broader literature on the prevalence and distribution of high-risk HPV types.

TABLE 5. The co-infection of HPV types and STI types.

HPV TYPE	MG	MH	UU	TV	CT	UP	NG	HSV-1	HSV-2	TP
TYPE 6	1	4	7	-	5	8	-	-	-	1
TYPE 11	-	6	7	-	1	6	-	-	-	-
TYPE 16	3	4	6	-	5	7	-	-	1	1
TYPE 18	1	6	5	2	1	11	1	-	-	-
TYPE 26	-	1	-	-	-	1	-	-	-	-
TYPE 31	5	3	5	2	6	10	-	-	1	-
TYPE 33	-	1	1	-	1	4	-	-	-	-
TYPE 35	1	3	3	1	1	1	-	-	-	-
TYPE 39	-	1	2	1	1	5	-	-	-	-
TYPE 40	-	2	2	-	1	4	-	-	1	1
TYPE 42	2	2	5	-	2	8	-	-	1	-
TYPE 43	-	1	-	-	-	2	-	-	-	-
TYPE 44	-	1	1	-	-	2	-	-	-	-
TYPE 45	-	1	-	-	-	4	-	-	1	-
TYPE 51	1	3	5	-	2	4	-	-	-	1
TYPE 52	-	3	3	-	-	5	-	-	-	-
TYPE 53	2	6	4	-	-	11	-	-	-	-
TYPE 54	1	3	4	2	3	5	1	-	-	-
TYPE 55	-	-	-	-	-	-	-	-	-	-
TYPE 56	1	3	1	-	1	6	-	-	-	-
TYPE 58	-	1	-	1	-	5	-	-	-	-
TYPE 59	1	1	3	-	1	-	-	-	-	1
TYPE 61	-	1	3	-	1	3	-	-	1	-
TYPE 62	-	4	3	2	2	6	1	-	-	-
TYPE 64	-	-	-	-	-	-	-	-	-	-
TYPE 66	3	4	6	1	3	3	1	-	-	-
TYPE 67	-	2	4	-	3	6	-	-	-	-
TYPE 68	1	2	3	-	-	2	-	-	-	-
TYPE 69	-	-	-	-	-	-	-	-	-	-
TYPE 70	-	-	-	-	-	1	-	-	-	-
TYPE 71	-	-	-	-	-	-	-	-	-	-
TYPE 72	-	1	1	1	-	1	-	-	-	-
TYPE 73	-	1	2	1	-	2	-	-	-	-
TYPE 74	-	2	1	-	-	2	-	-	-	-
TYPE 81	-	3	3	-	-	6	-	-	-	-
TYPE 82	-	1	1	-	1	1	-	-	-	-
TYPE 83	1	2	1	-	1	11	-	-	-	-
TYPE 84	2	3	4	2	3	5	-	-	-	-
TYPE 87	-	-	-	-	-	-	-	-	-	-
TYPE 89	-	-	-	-	-	-	-	-	-	-
TYPE 90	-	-	-	-	-	2	-	-	-	-
TYPE 91	1	1	2	-	2	4	-	-	-	-

HPV, Human Papilloma Virus; MG, Mycoplasma Genitalium; MH, Mycoplasma Hominis; UU, Uraeplasma Urealyticum; TV, Trichomonas Vaginalis; CT, Chlamidia Trachomatis; UP, Ureaplasma Parvum; NG, Neisseria Gonorrhea; HSV, Herpes Simplex Virus; TP, Treponema Pallidum.

Zhu *et al*. [22] reported a 37.3% prevalence of high-risk HPV types in patients with anogenital warts. In comparison, our study found that 13 out of 41 patients (approximately 31.7%) had a high-risk HPV type along with anogenital warts, which is similar in prevalence.

Another study conducted by Bruni *et al*. [23] found that the prevalence of high-risk HPV types among patients with anogenital warts ranged from 4.4% to 36.7%. Our study found a 31.7% prevalence of high-risk HPV types in patients with anogenital warts, which is in line with the prevailing literature. Therefore, our results are consistent with previous research in this area.

Our findings highlight the potential association between anogenital warts and higher than expected risk of cervical carcinogenesis due to increased rates of cervicovaginal high-risk HPV infection. We suggest routine assessment of cervical high-risk HPV status for women with anogenital warts, followed by appropriate changes in follow-up recommendations if positive results are obtained. However, potential drawbacks such as increased costs and psychological impact on patients should be considered.

Mortaki *et al*. [24] found that the group of anogenital warts had a significantly higher number of smokers compared to both the asymptomatic cervical HPV group and the control group. This is not unexpected since smoking has been shown to increase the incidence of anogenital warts. However, the significant difference in smoking rates between the group of anogenital warts and the asymptomatic cervical HPV group highlights the need for further research to explore the potentially more detrimental role of smoking in the development of anogenital warts. In our study, we compared patients with anogenital warts and asymptomatic HPV-positive patients and did not find a significant difference in smoking status between the two groups. The difference in results between the two studies may be attributed to differences in study design, sample size, and patient population. Mortaki *et al*. [24] focused especially on patients with anogenital warts, while our study compared patients with anogenital warts and HPV-positive asymptomatic patients. Additionally, other factors such as age, gender, and socioeconomic status may have contributed to the observed differences in smoking rates between the two studies.

In a study conducted by Kataja *et al*. [25], the number of sexual partners was identified as a known risk factor for HPV, particularly within the prior two years. The study involved 691 HPV positive and 706 HPV negative patients. However, in our study, we did not find a significant relationship between the number of sexual partners and HPV infection. Specifically, our study had a negative HPV percentage of 16.3% of the total sample size, which may have influenced the observed relationship between the number of sexual partners and HPV infection. Additionally, since our study had only 7 patients with 3–4 sexual partners, with this small sample size we may not have been able to fully capture the relationship between the number of sexual partners and HPV infection.

We found a strong association between HPV positivity and STI positivity. Our findings are consistent with previous studies that have evaluated the co-occurrence of HPV and other STIs.

In Martinelli *et al*.'s [21] study, HPV and STI coinfection was observed in 36.7% of Italian women with abnormal cervical cytology. In contrast, our study found a higher coinfection rate of 63.9%. Martinelli *et al*.'s [21] study included asymptomatic patients with abnormal cervical cytology, whereas our study included both patients with anogenital warts and HPV-positive asymptomatic patients. The difference in coinfection rates may be attributed to differences in the inclusion criteria of the two studies. In the same study, UP was the most common STI detected in high-risk HPV positive patients, followed by CT, MG, and TV. In our study, UP was also the most common STI detected in HPV-positive patients, followed by UU and MH.

Several studies suggest that STIs such as CT and TV may influence the persistence and progression of HPV infection, subsequently increasing the risk of cervical cancer [26, 27]. Research indicates that CT infection may promote HPV-associated persistence and progression, while bacterial vaginosis, associated with STIs like TV, may contribute to HPV persistence [27].

STIs trigger an inflammation process associated with facilitating HPV entry and persistence of infection [28]. Immunological defects resulting from HPV or STI infections can increase an individual's susceptibility to other pathogens and reduce HPV clearance. This phenomenon is particularly observed in the presence of high-risk HPV genotypes [26].

Wang and colleagues [29] hypothesize that co-infection of HPV and CT in cervical cells contributes to carcinogenesis through a "hit-and-run" model, whereby HPV-infected cells are subsequently infected by CT, resulting in augmented centrosomal defects. This sequence of events is supported by their cell culture model and previous research showing cervical cancer cells lack active Chlamydia infection. Wang *et al*.'s [29] findings suggest that past CT infections could increase cervical cancer risk in HPV-infected women, indicating a need for enhanced screening and potential vaccine development. Apart from CT, further investigation is needed to identify other pathogens that may potentially contribute to increased centrosomal defects when co-infected with HPV. These potential pathogens may include Ureaplasma, Mycoplasma, and other STIs. Nevertheless, the nature and mechanisms of these relationships remain unclear.

According to the study conducted by Horner *et al*. [30], asymptomatic carriage of bacteria such as MH, UP, and UU is common, and most individuals do not develop any disease. Routine testing and treatment of these bacteria can result in the selection of microbial resistance, substantial economic costs for society and individuals, and unnecessary treatment. On the other hand, studies on the co-infection of HPV and UP are still limited. However, existing data suggest that the co-presence of these two infections may increase the risk of cervical cancer. There is some evidence that UP may play a role in conjunction with HPV in cervical cancer. In a study, UP was detected in cervical lesions along with HPV16 and HPV18 [28]. This is because UP infection can affect the immune system, creating an environment that can facilitate the chronicity and progression of HPV infection to cancer.

It has been proposed that genital infection with GV and UU, contribute to HPV persistence through the impairment of immune response pathways. Vaginal secretions from in-

dividuals infected with GV presented increased expression of IL10, which is associated with a reduced cytotoxic Th1 T-cell response [31].

In a meta-analysis by Liang *et al*. [32], varying outcomes were found among three separate studies examining the correlation between UU and HPV infection. Nonetheless, when the findings of all studies were combined, a statistically significant relationship was identified (Odds Ratio (OR) 1.35, 95% CI: 1.20–1.51, $p < 0.05$). Similarly, Lu, H [33] reported a significantly higher detection rate of mycoplasma in the HPV-positive group (6.5%) compared to the HPV-negative group (1.2%). In our study, although not statistically significant, we found a 2-fold increase in UU prevalence and a 5.4-fold increase in MH prevalence among HPV-positive patients.

Liang *et al*. [32] presented findings from five studies that investigated the relationship between CT and HPV infection. The detection rate of CT was 5.9% (225/3821) in the HPV-positive group and 2.8% (196/6982) in the HPV-negative group. A statistically significant correlation was discovered when the outcomes of all studies were analyzed together (OR 3.16, 95% CI: 2.55–3.90, $p < 0.05$). In our research, we observed a 1.3-fold increase in CT positivity among HPV-positive patients, but it was not statistically significant.

Liang *et al*. [32] and our study suggest a potential association between UU prevalence and HPV infection. While Liang *et al*.'s [32] meta-analysis demonstrated a statistically significant association ($p = 0.00001$), our study showed a similar trend, albeit not statistically significant. Additionally, our study revealed an increased prevalence of MH in HPV-positive patients.

It is important to note that the co-occurrence of STIs is a natural possibility due to shared risk factors and modes of transmission [34]. However, the presence of these pathogens together is not sufficient to evaluate their potential facilitative and potentiating effects on one another. Indeed, recent studies have reported that certain STIs can influence the course of other infections, potentially exacerbating disease progression or altering immune responses [35]. For example, CT and NG coinfection has been associated with increased rates of treatment failure and antimicrobial resistance [36].

In vitro research is required to determine STIs function as cofactors, taking advantage of the immune tolerance and irregular cell cycle control induced by HPV. Additionally, clinical studies involving women with abnormal cervical cytology results will help deepen our understanding of the relationship between genital infections and HPV infections [21].

Our study presents both strengths and weaknesses in its comprehensive investigation of HPV infection and the coexistence of other STIs among a selected population. The strengths of the study lie in the utilization of reliable and sensitive diagnostic methods, such as the Montana 4896 Real-Time PCR system for detecting various HPV types. This system can identify low-risk, probably high-risk, and high-risk HPV types, thus providing a thorough analysis of the patient's HPV status. Additionally, the study employs the Anatolia Geneworks Bosphore STIs Panel Kit v6 to screen for multiple STIs, thereby enriching the collected data.

However, the study has some limitations. The sample size of 129 female patients is relatively small, which may not provide sufficient power for drawing broad generalizations from the results. The study population is also limited to individuals who visited a private clinic in Istanbul, Turkey, potentially restricting the generalizability of the findings to other populations or regions. Moreover, the exclusion criteria may have removed patients with complex medical histories or those who have been vaccinated against HPV, which could have offered valuable insights into the relationship between these factors and HPV infection.

Furthermore, if the study had incorporated long-term follow-up of the patient's cervical cytology, it would have been possible to compare the rate of cancer transformation attributed to HPV infection alone and in conjunction with other STIs.

5. Conclusions

This study reveals a high occurrence of co-infection between HPV and UP. The elevated prevalence of STIs in high-risk HPV-positive women, who face a greater risk of cervical disease development, highlights the necessity for genital infection screening in this group. By identifying and treating STIs through this screening, the potential adverse impact of concurrent microorganisms on HPV infection can be reduced. Further longitudinal research is needed to explore the role of STIs as contributing factors in HPV-related cervical cancer development.

ABBREVIATIONS

HPV, human papilloma virus; STIs, sexually transmitted infections; CT, Chlamydia Trachomatis; TV, Trichomonas Vaginalis; MH, Mycoplasma Hominis; MG, Mycoplasma Genitalium; UP, Ureaplasma Parvum; UU, Uraeplasma Urealyticum; YP, Treponema Pallidum; NG, Neisseria gonorrhea; GV, Gardnerella Vaginalis; HSV-1, Herpes Simplex Virus Type 1; HSV-2, Herpes Simplex Virus Type 2.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during the current study are not publicly available due to personal data protection regulations but are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

EA and SŞA—conceived and designed the research. EA—collected data and conducted research, analyzed and interpreted data, and wrote the initial paper. SŞA—edited and revised the paper. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Istanbul Gelisim University Ethics Committee on 18 January 2023 with decision

number 2023-02-40. The study was conducted in accordance with the principles outlined in the Helsinki Declaration. Informed consent was obtained from all patients for this study.

ACKNOWLEDGMENT

We would like to thank Empiar Statistics Center for their valuable assistance with statistical analysis in this study.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Eser Ağar, Seda Şahin Aker. Association of HPV and sexually transmitted infections among patients with genital warts and asymptomatic individuals: a cross-sectional study. European Journal of Gynaecological Oncology. 2023; 44(4): 145-155. doi: 10.22514/ejgo.2023.048.