Prevalence and Genotype Distribution of Human Papillomavirus Among Women Attending a Referral Papilloma Clinic in Tehran, Iran: Association with Risk Factors

Shaghayegh Saed[1], Mohammad Hadi Karbalaei Nia[1], Alireza Mohebbi[1], Seyed Mohammad Jazayeri[2], Shaghayegh Yazdani[3], Iman Rezaei[2], Masoumeh Norouzi[2], Parastoo Motamed Aria[1], Mehrnaz Hosseini Tehrani[1,2], Reza Aramideh Khouy[1,2], Atefeh Kachooei[1], Leila Mousavizadeh[1] and Angila Ataei-Pirkooh[1]*

[1] Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

[2] Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Microbiology-TeMS. C, Islamic Azad University, Tehran, Iran

*Corresponding Author

Angila Ataei-Pirkooh; E-mail: ataei.a@iums.ac.ir

Preprint

Abstract

Introduction: Human papillomavirus (HPV) is a significant public health concern because of its association with various anogenital diseases, including genital warts and cancers of the cervix. The prevalence of and risk factors for HPV infection vary across populations and are influenced by demographic, behavioral, and health factors. This study aims to investigate the prevalence of HPV genotypes and assess the influence of demographics, sexual behavior, and health factors on HPV infection among women attending a referral papilloma clinic in Tehran, Iran.

Methods: A cross-sectional study was conducted at Lolagar Papilloma Clinic, Tehran, between May 2023 and May 2024. A total of 311 women were enrolled, and 304 were included in the final analysis. HPV DNA was detected and genotyped using multiplex Real-Time PCR, while sociodemographic and behavioral data were collected via structured questionnaires. Statistical analyses were performed with significance set at $p < 0.05$.

Results: Of the 304 women included in the analysis, the mean age was 32.8 ± 8.7 years. Genital warts were present in 181 participants (58.2%, 95% CI: 52.7–63.7%). Overall, 57.8% (95% CI: 52.3–63.3) of the participants tested positive for HPV. The most common genotypes were HPV-6 (21.3%), HPV-16 (19.9%), and HPV-31 (13.1%). Genotype-specific analyses showed that HPV-33 ($p = 0.012$) and HPV-58 ($p = 0.0026$) were significantly more prevalent in the 25–40 years age group. HPV-53 was significantly associated with laser/hair removal use ($p = 0.042$) and with being unmarried but having a sexual partner ($p = 0.042$). Additionally, significant associations were observed between HPV-57 and number of sexual partners ($p = 0.005$), and between alcohol consumption and HPV-62 infection ($p = 0.0002$).

Conclusion: This study reveals high HPV burden in Tehran referral clinics, driven by specific genotypes and behavioral factors. It advocates integrated public health strategies—early vaccination, safe sexual practices, targeted interventions, and awareness campaigns—to curb transmission and long-term sequelae in Iran and similar settings.

Keywords: Cancer; Human papillomavirus; HPV genotypes; HPV prevalence; Risk factors

Preprint

1. Introduction

Human papillomavirus (HPV) is a significant public health challenge due to its well-established association with a wide spectrum of anogenital diseases, including genital warts and cancers of the cervix, vulva, vagina, penis, and anus [1, 2]. HPV is among the most common sexually transmitted infections worldwide. More than 200 genotypes have been identified, of which approximately 40 specifically infect the anogenital tract [3]. These genotypes are broadly classified into high-risk (HR-HPV) and low-risk (LR-HPV) types based on their oncogenic potential. HR-HPV types, particularly HPV-16 and HPV-18, are strongly associated with cervical and other anogenital cancers, whereas LR-HPV types, such as HPV-6 and HPV-11, are primarily responsible for benign conditions, including genital warts [4].

Globally, HPV prevalence differs between men and women. Studies have estimated the pooled prevalence of HPV infection to be approximately 11–12% in women and 15–20% in men, with higher rates often reported among men who have sex with men and individuals with multiple sexual partners [5-7]. Data on HPV prevalence in Iranian women and men remain limited and vary considerably depending on geographic region and study design. For example, a retrospective cross-sectional study of 2,969 outpatients between 2013 and 2016 reported an overall prevalence of 29.3% among Iranian women, with HPV-16 being the most common high-risk genotype (30.5%) and HPV-6 the most prevalent low-risk genotype (60.6%) [8]. Similarly, a study conducted in Mashhad between 2015 and 2020 identified HPV-31, HPV-16, HPV-51, HPV-18, and HPV-66 as the most frequent high-risk types, while HPV-6, HPV-53, and HPV-42 were the predominant low-risk types [9]. Other regional studies have consistently confirmed the dominance of HPV-16 as the leading high-risk genotype, particularly in cases of cervical cancer and abnormal cervical lesions [10, 11]. Together, these findings highlight significant heterogeneity in the HPV genotype distribution across different regions of Iran.

The epidemiology of HPV is strongly influenced by demographic and behavioral factors. The evidence consistently indicates that early sexual debut, having multiple sexual partners, and engaging in high-risk sexual practices significantly increase susceptibility to infection [12, 13]. Additional correlates, including smoking, immunosuppression, and co-infection with other sexually transmitted infections (STIs), have also been implicated in HPV persistence and progression [14, 15]. In Iran, sociodemographic and cultural factors appear to play a critical role. For instance, a study in Zabol reported a slightly greater prevalence of HPV among polygamous women (37.9%) than among monogamous women (29%), suggesting that marital practices and sexual behaviors contribute to regional variation [16]. Age is another important determinant; younger women are generally at higher risk, although findings across Iranian studies remain inconsistent [17]. Moreover, lifestyle factors such as

Preprint

smoking, prolonged contraceptive use, and limited adoption of barrier contraceptives, together with the absence of a national HPV vaccination program, may further facilitate the transmission of high-risk genotypes [18].

Despite several regional investigations, comprehensive data that integrate HPV prevalence, genotype distribution, and associated risk factors in Iranian women remain limited. Addressing this gap is essential for informing evidence-based public health strategies. Therefore, this study aims to investigate the prevalence and genotype distribution of HPV and genital warts in a female population, with a particular focus on the influence of demographic, behavioral, and health-related factors. By clarifying these associations, our findings can contribute to the design of targeted interventions, including vaccination campaigns and awareness initiatives, to reduce the burden of HPV-related diseases.

2. Materials and methods

2.1. Study design and data collection

This cross-sectional study was conducted between May 2023 and May 2024 at the Lolagar Papilloma Clinic in Tehran, Iran. A total of 311 women were enrolled according to predefined eligibility criteria: (i) medical indication for HPV screening, (ii) willingness to participate, and (iii) provision of written informed consent. Participants were recruited through census sampling.

Data collection involved both biological sampling and a structured questionnaire. The questionnaire collected information on sociodemographic characteristics, sexual behavior, contraceptive use, smoking habits, and other relevant lifestyle factors. In addition, it assessed recognized risk factors for HPV transmission, including the number of sexual partners, age at first sexual intercourse, and history of sexually transmitted diseases (STDs).

2.2. Sample collection and DNA extraction

Genital specimens were collected from the cervix or vaginal secretions by trained clinicians using sterile swabs. The samples were immediately stored at −20°C and transported on dry ice to the laboratory, where they were preserved at −20°C until processing.

Viral DNA was extracted using the Viral DNA Isolation Kit (Aramesh Biogene, Tehran, Iran) according to the manufacturer's instructions, based on silica column purification. The extracted DNA was eluted in 50 μL of elution buffer and stored at −20°C for subsequent PCR amplification and genotyping analyses. DNA concentration and purity were assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), and the A260/A280 absorbance ratio was used to confirm sample purity.

Preprint

2.3. HPV genotyping

HPV genotyping was performed via the GeneMAP™ HPV HR & 6/11 Genotyping Kit (GENMARK Sağlık Ürünleri, Istanbul, Turkey). This CE-IVD–certified multiplex Real-Time PCR assay targets the HPV L1 region and allows detection of 16 genotypes, including 14 high-risk types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 2 low-risk types (HPV-6 and 11). An internal control supplied with the kit was included in each reaction to verify the quality of DNA extraction and to monitor potential PCR inhibition.

The reactions were prepared in a final volume of 20 µL using the supplied master mix with Uracil-DNA glycosylase (UDG) to prevent carry-over contamination. Amplification was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA) with the following cycling profile: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. The data were analyzed automatically via GeneMAP™ Viewer software (GENMARK, Turkey), which interprets probe-specific fluorescence signals (FAM, VIC/HEX, ROX, and Cy5) to determine the presence of specific HPV genotypes.

In addition, HPV genotyping results previously obtained from other accredited laboratories were incorporated for some participants. Although detailed information regarding the specific kits, target genes, and methodologies used in those laboratories was not available, these results were included to expand the detectable genotype spectrum beyond the 16 types covered by the geneMAP kit, thereby providing a more comprehensive estimation of HPV genotype distribution in the study population.

2.4. Statistical analysis

Data obtained from the questionnaires and HPV genotyping were coded and entered into a database, and each participant was assigned a unique identification code to ensure confidentiality. Descriptive statistics were calculated, with continuous variables (e.g., age, number of sexual partners) expressed as the mean ± standard deviation (SD) and categorical variables (e.g., smoking status, contraceptive use, HPV infection status) summarized as frequencies and percentages. Associations between categorical variables and HPV infection status were assessed using chi-square or Fisher's exact test, as appropriate. Statistical analyses were performed via SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All analyses were conducted with a 95% confidence level, and a p-value < 0.05 was considered statistically significant.

Preprint

2.5. Ethical statement

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Iran University of Medical Sciences (IUMS), Tehran, Iran (IR.IUMS.FMD.REC.1402.536). Written informed consent was obtained from all participants prior to enrollment. Confidentiality and privacy were ensured by anonymizing data and samples, and participants were informed of their right to withdraw at any stage without any consequences.

3. Results

3.1. Sociodemographic characteristics

The demographic data of the 311 enrolled participants were incomplete for 7 individuals, leaving 304 participants for analysis. The mean ± SD age of the participants was 32.8 ± 8.7 years (range: 14–73). Marital status information was available for all 304 participants. They were categorized as married (n = 146, 48.0%), unmarried without a sexual partner (n = 28, 9.2%), and unmarried with a sexual partner (n = 130, 42.8%). Educational level was classified into four categories: illiterate–below high school diploma, high school diploma–associate degree, bachelor's degree, and master's degree–PhD. The most common educational level was a bachelor's degree (n = 143, 47.0%) (Table 1).

Table 1. Sociodemographic characteristics of study participants (n = 304)

Variable	Category	n	%
Age (years)	<18	5	1.6
	18–25	60	19.2
	25–40	190	61.1
	40–55	50	16.1
	>55	6	1.9
Marital status	Married	146	48.0
	Unmarried	28	9.2
	Unmarried with partner	130	42.8
Education level	Illiterate–Below diploma	22	7.2
	High school–Associate degree	77	25.3
	Bachelor's degree	143	47.0
	Master's–PhD	62	20.4

Preprint

3.2. Behavioral and vaccination characteristics

3.2.1. Sexual behavior

As shown in Table 2, different categories for the age at first sexual intercourse were established. The majority of females reported initiating sexual activity after the age of 18 years (70.4%). Regarding the number of sexual partners within the last two years, most participants reported having one partner (47.7%), followed by having no partner (23.0%). In addition, the lifetime number of sexual partners was recorded as part of the questionnaire, with the majority reporting 2–5 partners (74.6%).

Table 2. Behavioral characteristics, contraceptive methods, and HPV vaccination status of participants (N = 311)

Variable	Category	n	%
Age at first sexual intercourse	>18 years	219	70.4
	15–18 years	24	7.7
	<15 years	10	3.2
	Missing/Unspecified	58	18.6
Number of sexual partners in the past two years	None	66	23.0
	1 partner	137	47.7
	2 partners	49	17.1
	3 partners	21	7.3
	4–9 partners	11	3.8
	>10 partners	3	1.0
	Missing	24	7.7
Total number of sexual partners	1 partner	24	5.3
	2–5 partners	226	74.6
	6–9 partners	17	5.6
	>10 partners	9	2.9
	Missing/Unspecified	35	11.5
Hair removal or laser use	Yes	215	69.1
	No	96	30.9
Substance use	Tobacco (cigarette/hookah)	100	32.1
	Alcohol consumption	76	24.4
	Drug use	5	1.6
Contraceptive methods	Withdrawal method	140	54.3

Preprint

	Oral contraceptive pills	38	14.8
	Condom use	122	47.3
	IUD	15	5.8
	Other methods	14	5.4
	Missing	54	17.4
HPV vaccination status	Gardasil 4	160	51.4
	Gardasil 9	1	0.3
	Papilloguard	5	1.6
	Not vaccinated	113	36.3

IUD: Intrauterine device

3.2.2. Personal behaviors, habits, and contraceptive methods

Among the 311 participants, 215 (69.1%) reported undergoing hair removal or laser treatments, whereas 96 (30.9%) did not. In terms of tobacco use, 211 participants (67.8%) reported no use of cigarettes or hookah, while 100 (32.2%) reported current use. Alcohol consumption was reported by 76 women (24.4%), and only 5 individuals (1.6%) reported drug use.

With respect to contraceptive practices, the withdrawal method was the most frequently reported (140 participants, 54.3%), whereas 118 participants (45.7%) indicated non-use. Oral contraceptive pills were used by 38 participants (14.8%). Condom use was relatively balanced, with 122 participants (47.3%) reporting use and 136 (52.7%) reporting non-use. The use of intrauterine devices (IUDs) was reported by 15 participants (5.8%), and 14 participants (5.4%) reported using other contraceptive methods listed in the questionnaire.

3.2.3. Vaccination status

Among all participants, 196 (63.0%) reported receiving the HPV vaccine, while two individuals (0.6%) did not provide information regarding their vaccination status. Among the vaccinated participants, Gardasil 4 was the most commonly administered vaccine (167 participants, 53.7%), followed by Papilloguard (9.3%) and Gardasil 9 (0.6%). Notably, 36.3% of participants reported not receiving any HPV vaccine. The mean age of unvaccinated individuals was 39 years, compared with 32 years among those vaccinated, suggesting that younger participants were more likely to have received the vaccine. Most vaccinations occurred in participants' early thirties.

3.3. Clinical and virological findings

3.3.1. Prevalence of HPV and other STIs

Preprint

Among the 311 participants, 182 (57.8%, 95% CI: 52.3–63.3) tested positive for HPV, whereas 129 (42.2%) were HPV negative. Genotyping revealed that the most prevalent HPV genotypes were HPV-6 (21.3%), HPV-16 (19.9%), HPV-31 (13.1%), and HPV-68 (9.7%). High-risk HPV types were dominated by HPV-16 (19.9%), followed by HPV-31 (13.1%) and HPV-68 (9.7%), while HPV-33 represented the least frequent subtype (1.9%). Low-risk genotypes were led by HPV-6 (21.3%) and HPV-11 (6.3%). Possibly high-risk types were also identified, with HPV-53 being the most frequent (6.8%) (Table 3).

Table 3. Distribution of HPV genotypes among HPV-positive participants

Risk Category	HPV Genotype	n	%
High-Risk	HPV-16	41	19.90
	HPV-18	18	8.74
	HPV-31	27	13.11
	HPV-33	4	1.94
	HPV-35	11	5.34
	HPV-39	16	7.77
	HPV-45	11	5.34
	HPV-51	13	6.31
	HPV-52	18	8.74
	HPV-56	16	7.77
	HPV-58	11	5.34
	HPV-59	11	5.34
	HPV-66	11	5.34
	HPV-68	20	9.71
Low-Risk	HPV-6	44	21.36
	HPV-11	13	6.31
	HPV-40	3	1.46
	HPV-42	5	2.43
	HPV-43	3	1.46
	HPV-44	1	0.49
	HPV-54	4	1.94
	HPV-61	4	1.94
	HPV-62	4	1.94

Preprint

	HPV-81	2	0.97
	HPV-83	2	0.97
	HPV-84	2	0.97
	HPV-89	1	0.49
	HPV-90	4	1.94
Possibly High-Risk	HPV-53	14	6.80
	HPV-67	8	3.88
	HPV-73	6	2.91

With respect to other sexually transmitted infections (STIs), most participants (93.9%) tested negative for concurrent infections. However, 11 individuals (3.5%) were diagnosed with genital herpes, 1 (0.3%) with HCV, 3 (0.9%) with HIV, and 4 (1.3%) with other STIs. No significant association was observed between HPV infection and the presence of other STIs ($p = 0.62$).

3.3.2. Clinical presentation and management of genital warts

Genital warts were reported in 181 participants (58.2%, 95% CI: 52.7–63.7%), while 130 participants (41.8%) showed no clinical evidence of warts. The pubic triangle was the most commonly affected site (13.7%), followed by the external genitalia/penile shaft (9.0%), anus (6.2%), groin (4.0%), and the perineal region between the anus and vagina (2.0%). Less common sites included the oral cavity and cases with multiple affected regions, highlighting the diverse anatomical distribution of HPV-associated lesions (Table 4).

Table 4. Clinical findings of the study population

Category	Finding	Frequency (%)
Genital warts	With warts	181 (58.2)
	Without warts	130 (41.8)
Wart location	Pubic triangle	35 (13.7)
	External genitalia/penile shaft	23 (9.0)
	Anus	16 (6.2)
	Groin	10 (4.0)
	Perineum	5 (2.0)
Treatment method	Cryotherapy	83 (44.3)
	Topical medications	23 (28.2)
	Laser	17 (17.7)

Preprint

	Electrocautery	15 (15.3)
	Surgery	12 (12.8)
	Plasma therapy	1 (1.2)
Wart recurrence	No recurrence	45 (54.2)
	Recurrence	38 (45.8)
Pap smear results	Normal	211 (67.9)
	Inflammation	54 (17.3)
	ASC-US	39 (12.5)
	ASC-H	6 (1.8)
	Cervicitis	2 (0.6)
Colposcopy findings	Normal	13 (34.2)
	CIN-1	15 (39.5)
	CIN-2	3 (7.9)
	Cervicitis	7 (18.4)

ASC-US: Atypical squamous cells of undetermined significance

CIN: Cervical intraepithelial neoplasia

Among the 83 individuals who received treatment for genital warts, cryotherapy was the most frequently employed method (44.3%), followed by topical medications (28.2%), laser therapy (17.7%), electrocautery (15.3%), surgery (12.8%), and plasma therapy (1.2%). Wart recurrence was reported in 38 participants (45.8%), whereas 45 (54.2%) did not experience recurrence. The highest recurrence rates were observed following cryotherapy (55.6%) and topical medication (25%), whereas lower recurrence were reported for laser therapy (19.4%), electrocautery (16.7%), and surgery (13.9%). Notably, no recurrence occurred among individuals treated with plasma therapy.

3.3.3. Cytological and colposcopic findings

Cytological evaluation revealed that 67.9% of Pap smear samples were normal. Inflammatory changes were identified in 17.3% of samples, while 12.5% demonstrated atypical squamous cells of undetermined significance (ASC-US). Additionally, 1.8% of the patients exhibited atypical squamous cells, HSIL (ASC-H) could not be excluded, and 0.6% were diagnosed with cervicitis.

Colposcopic examination was performed on 38 individuals. The most common finding was cervical intraepithelial neoplasia grade 1 (CIN-1) in 39.5% of the patients, followed by cervicitis in 18.4% and CIN-2 in 7.9%. Normal colposcopy results were reported in 34.2% of the patients (Table 4).

Preprint

3.4. Demographic, Behavioral, and Clinical Associations

We examined demographic, behavioral, and clinical characteristics in relation to HPV infection, genotype-specific distribution, and genital wart status to identify potential associations. The mean age of HPV-positive participants was 32.9 ± 8.5 years compared with 34.2 ± 10.3 years in HPV-negative women ($p = 0.17$). No overall association was observed between age and HPV infection; however, genotype-specific analyses revealed significant associations for HPV-33 ($p = 0.012$) and HPV-58 ($p = 0.0026$), both of which were predominantly detected in the 25–40 year age group. Marital status was not significantly related to HPV infection ($p = 0.69$), although HPV-53 was more frequent among unmarried women with a sexual partner ($p = 0.042$). Educational level showed no significant associations with HPV positivity or genital wart status.

Regarding behavioral factors, age at first sexual intercourse was not linked to overall HPV infection, but HPV-52 and HPV-68 were more frequently detected among women whose first intercourse occurred after age 18 ($p < 0.05$). The number of sexual partners was not associated with HPV infection overall ($p = 0.45$), although HPV-57 showed a significant distribution difference across categories ($p = 0.005$). The use of hair removal/laser treatments was not associated with overall HPV positivity ($p = 0.36$) but was significantly related to HPV-53 detection ($p = 0.042$). Smoking and hookah use were not correlated with overall HPV infection ($p = 0.35$) but were associated with HPV-35 ($p = 0.049$), HPV-40 ($p = 0.012$), and HPV-83 ($p = 0.041$). Alcohol consumption was not linked to HPV infection overall ($p = 0.21$) but was strongly associated with HPV-62 ($p = 0.0002$). Drug use was not significantly related to HPV status.

In clinical analyses, the mean age of women with genital warts (32.9 ± 9.6 years) did not differ significantly from that of those without warts (32.8 ± 8.1 years). Genital wart status was not associated with marital status, education, or behavioral factors. Pap smear findings were also not significantly associated with multiple HPV infections ($p = 0.33$), although multiple HPV genotypes were more frequently observed in women with normal Pap results.

In summary, significant genotype-specific associations were identified for age (HPV-33, HPV-58), marital status (HPV-53), age at first intercourse (HPV-52, HPV-68), number of sexual partners (HPV-57), hair removal/laser use (HPV-53), smoking/hookah (HPV-35, HPV-40, HPV-83), and alcohol consumption (HPV-62) (Table 5).

Table 5. Significant associations between demographic, behavioral, and clinical factors and HPV genotypes

Risk factor	Associated HPV genotype	p value
Age group (25–40 years)	HPV-33	0.012

Preprint

	HPV-58	0.0026
Marital status	HPV-53	0.042
Age at first intercourse >18 years	HPV-52	<0.05
	HPV-68	<0.05
Number of sexual partners	HPV-57	0.005
Hair removal/laser	HPV-53	0.042
Smoking/hookah	HPV-35	0.049
	HPV-40	0.012
	HPV-83	0.041
Alcohol consumption	HPV-62	0.0002

4. Discussion

HPV remains the most common sexually transmitted infection worldwide and a major cause of cervical cancer and other anogenital malignancies. This study provides comprehensive data on HPV prevalence, genotype distribution, and associated demographic and behavioral factors among women attending a referral clinic in Tehran, contributing to the limited evidence available from Iran.

The HPV prevalence observed in this study was considerably higher than that reported in community-based studies in Iran, where infection rates of approximately 9–10% have been described among asymptomatic women [18]. This difference is likely explained by the referral-based nature of our study population, consisting of women undergoing HPV testing for clinical indications. Similar discrepancies between community- and clinic-based populations have been consistently reported, emphasizing the influence of study setting on HPV prevalence estimates [19]. Internationally, HPV prevalence among sexually active women is substantially higher, with a weighted prevalence of 42.9% reported among women aged 18–59 years in the United States [20]. Therefore, our findings are consistent with those reported in other high-risk clinical populations.

The most frequently detected genotypes were HPV-6, HPV-16, and HPV-31, consistent with previous studies identifying HPV-6, HPV-11, and HPV-16 as the predominant circulating types [21]. Likewise, studies from Tehran have demonstrated the prominent role of HPV-16 and HPV-18 in cervical disease, although Khodakarami et al. also reported associations between HPV positivity and certain marital characteristics that were not observed in our study [22].

Preprint

No significant associations were identified between HPV infection and marital status, age at first sexual intercourse, or number of sexual partners. However, HPV-33 and HPV-58 were significantly associated with women aged 25–40 years, supporting previous evidence that younger age remains an important determinant of HPV acquisition [23]. Although the highest HPV prevalence in our cohort was observed among women whose sexual debut occurred after 18 years of age, sexual activity itself remains the principal risk factor for HPV infection, particularly among adolescents and young adults [24].

Vaccination remains one of the most effective strategies for HPV prevention. In our cohort, 63% of participants had received HPV vaccination, predominantly Gardasil 4. Vaccinated women did not report higher levels of risky sexual behavior, consistent with previous studies demonstrating that HPV vaccination is not associated with increased sexual risk-taking, earlier sexual debut, or a greater number of sexual partners [25,26]. These findings further support strengthening public confidence in HPV vaccination programs and expanding vaccine coverage

Behavioral and lifestyle factors also warrant attention. While hair removal and laser treatments were not linked to overall HPV positivity, HPV-53 showed a notable association. Smoking and hookah use were significantly associated with certain genotypes, including HPV-35, HPV-40, and HPV-83. Similar findings have been reported elsewhere, with Liang et al. identifying smoking and abortion history as key risk factors in rural populations [27]. These data highlight the importance of considering both biomedical and lifestyle determinants when designing prevention strategies.

Clinical and virological outcomes observed in this study, including the prevalence of genital warts and their association with Pap smear results, provide further insight into the morbidity linked to HPV infection. The pubic triangle was identified as the most common site for genital warts, and recurrence rates varied according to treatment modality, with cryotherapy and topical agents showing higher recurrence. These findings are valuable for informing clinical management strategies and optimizing treatment approaches for HPV-related conditions.

Despite the strengths of this study, several limitations should be acknowledged. The cross-sectional design restricts the ability to infer causal relationships between identified risk factors and HPV infection. In addition, reliance on self-reported data regarding sexual behavior and lifestyle may have introduced recall or social desirability bias, potentially affecting data accuracy. The study population was recruited from a single referral papilloma clinic in Tehran. However, data regarding participants' specific place of residence, such as districts within Tehran or other provinces, were not collected. Consequently, it is unclear whether the study population was limited to residents of Tehran or included individuals referred from other regions of Iran. This lack of

Preprint

information may limit the generalizability of the findings to the broader female population in Iran and should be considered when interpreting the results. Another limitation is that HPV genotyping results from other accredited laboratories were used for a subset of participants. As detailed information regarding the specific assays and kits employed in those laboratories was not available, potential heterogeneity in genotyping methods cannot be ruled out. Moreover, the absence of male participants and other potential sources of HPV transmission, along with the lack of longitudinal follow-up, prevented assessment of the natural history of infection or progression to malignancy.

Future research should adopt longitudinal and multicenter designs to provide a more comprehensive understanding of the interplay between demographic, behavioral, and virological factors in HPV infection. Expanding the study population to include diverse regions and both sexes would enhance the applicability of findings and improve insights into transmission dynamics. Furthermore, investigations into the long-term impact of vaccination programs, the effectiveness of behavioral interventions, and the psychosocial determinants of HPV prevention and health-seeking behaviors are warranted to guide more targeted and sustainable public health strategies.

5. Conclusion

This study demonstrated a high prevalence of HPV infection among women attending a referral papilloma clinic in Tehran, with high-risk genotypes, particularly HPV-16, HPV-31, and HPV-68, being the most common. Certain modifiable behaviors, including smoking, hookah use, and laser hair removal, were associated with specific HPV genotypes, whereas demographic factors such as marital status, age at first sexual intercourse, and number of sexual partners showed no significant association. These findings emphasize the population-specific nature of HPV epidemiology and the importance of identifying context-dependent risk factors. Although vaccination coverage was relatively high, many women remained vulnerable to HPV infection, highlighting the need for strengthened prevention strategies. Public health efforts should prioritize HPV vaccination, education on safe sexual behaviors, smoking cessation, and regular cervical screening to facilitate early detection of precancerous lesions. Future multicenter longitudinal studies are needed to clarify causal relationships and support the development of evidence-based, targeted HPV prevention and control programs.

Acknowledgments

Preprint

The authors would like to thank the students and colleagues of the Research Center for Clinical Virology at Tehran University of Medical Sciences, as well as the Department of Virology at Iran University of Medical Sciences in Tehran, Iran, for their valuable cooperation and support.

Author contributions

S.S. Writing- original draft, Data curation, Investigation; **M. H.K.N.** Methodology, Validation; **A.M**. Writing-original draft, Visualization, Formal analysis; **S.M. J**. Methodology, Project administration; **S.Y.** Resources, methodology; **I.R.** Resources, Investigation; **M.N.** Resources, Investigation; **P.M.A.** Investigation; **M.H.T.** Formal analysis, writing review and editing. **R.A.K.** Validation, Writing-review and editing; **A.K.** Investigation; **L.M.** Supervision, Writing-review and editing; **A.A.P.** Conceptualization, Project administration, Funding acquisition, Supervision, Writing- original draft. All authors have read and agreed to the submitted version of the manuscript.

Conflict of interest

The authors declare no competing interests**.**

Ethics approval

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Iran University of Medical Sciences (IUMS), Tehran, Iran (Approval ID: IR.IUMS.FMD.REC.1402.536).

Funding

This research was funded and supported by Iran University of Medical Sciences (grant no. 25546).

Data availability

All data supporting the findings of this study are presented within the article**.**

References

1. Williamson AL. Recent Developments in Human Papillomavirus (HPV) Vaccinology. Viruses. 2023;15(7).10.3390/v15071440
2. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020;40(5):602-8.10.1080/01443615.2019.1634030
3. Seyoum A, Seyoum B, Gure T, Alemu A, Alemayehu DH, Alemu A, et al. High rate of non-vaccine targeted high-risk HPV genotypes circulate among women in Eastern Ethiopia. Sci Rep. 2024;14(1):958.10.1038/s41598-024-51594-7

Preprint

4. Pešut E, Đukić A, Lulić L, Skelin J, Šimić I, Milutin Gašperov N, et al. Human Papillomaviruses-Associated Cancers: An Update of Current Knowledge. Viruses. 2021;13(11).10.3390/v13112234
5. Kombe Kombe AJ, Li B, Zahid A, Mengist HM, Bounda GA, Zhou Y, et al. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. Front Public Health. 2020;8:552028.10.3389/fpubh.2020.552028
6. - Laprise JF DM, Boily MC, et al. . Prevalence of human papillomaviruses in men: a systematic review and meta-analysis. The Lancet Global Health. 2010;2023;11(9):e1345-e1357. .doi: 10.1016/S2214-109X(23)00305-4
7. - Hörner L, Nkurunziza T, Rank S, Fiengo Tanaka L, Klug SJ. Global prevalence of cervical human papillomavirus in women aged 50 years and older with normal cytology: a systematic review and meta-analysis. Lancet Microbe. 2025;6(1):100955. doi:10.1016/j.lanmic.2024.100955
8. Chalabiani S, Nazari MK, Shabani M, Davoodi NR, Sarafnejad A, Amirzargar AA. Retrospective analysis of prevalence of high-risk and low-risk Human Papillomavirus (HPV) genotypes in iranian women during 2013-2016. Asian Pacific Journal of Cancer Biology. 2017;2(4):85-90.10.31557/apjcb.2017.2.4.85-90
9. Bakhshani A, Ganjali R, Tabatabaeizadeh SE. Prevalence of Human Papillomavirus (HPV) Genotypes among Women During 2015-2020 in Mashhad, Iran. Arch Iran Med. 2023;26(8):419-26.10.34172/aim.2023.64
10. Salavatiha Z, Farahmand M, Shoja Z, Jalilvand S. A meta-analysis of human papillomavirus prevalence and types among Iranian women with normal cervical cytology, premalignant lesions, and cervical cancer. J Med Virol. 2021;93(8):4647-58.10.1002/jmv.26928
11. Ahmadi S, Goudarzi H, Jalilvand A, Esmaeilzadeh A. Human Papilloma Virus Genotype Distribution in Cervical lesions in Zanjan, Iran. Asian Pac J Cancer Prev. 2017;18(12):3373-7.doi:10.22034/apjcp.2017.18.12.3373
12. Bobadilla ML, Villagra V, Castro H, von Horoch M, Araya S, Deluca G, et al. Human Papillomavirus (HPV) Infection and Risk Behavior in Vaccinated and Non-Vaccinated Paraguayan Young Women. Pathogens. 2024;13(3).10.3390/pathogens13030209
13. George C, Roberts R, Brennen D, Deveaux L, Read SE. Knowledge and awareness of Human Papillomavirus (HPV) and HPV vaccines among Caribbean youth: the case of the Bahamas. Hum Vaccin Immunother. 2020;16(3):573-80.10.1080/21645515.2019.1661205
14. Onwuamah CK, Feng N, Momoh AE, Uwandu M, Ahmed RA, Idigbe I, et al. Prevalence and risk factors for high-risk human papillomavirus infection among women from three southern geopolitical zones of Nigeria. Front Oncol. 2023;13:1254304.10.3389/fonc.2023.1254304
15. Del Pino M, Vorsters A, Joura EA, Doorbar J, Haniszewski M, Gudina IA, et al. Risk factors for human papillomavirus infection and disease: A targeted literature summary. J Med Virol. 2024;96(2):e29420.10.1002/jmv.29420
16. Shahramian I, Heidari Z, Mahmoudzadeh-Sagheb H, Moradi A, Forghani F. Prevalence of HPV Infection and High Risk HPV Genotypes (16, 18), among Monogamous and Polygamous Women, In Zabol, Iran. Iran J Public Health. 2011;40(3):113-21. https://pmc.ncbi.nlm.nih.gov/articles/PMC3481649/
17. Mobini Kesheh M, Keyvani H. The Prevalence of HPV Genotypes in Iranian Population: An Update. Iran J Pathol. 2019;14(3):197-205.10.30699/ijp.2019.90356.1861
18. Hamkar R, Shoja Z, Ghavami N, Heydari N, Farahmand M, Jalilvand S. Type-Specific Human Papillomavirus Prevalence in Iranian Women with Normal Cervical Cytology: The Impact of Current HPV Vaccines. Intervirology. 2018;60(4):125-30.10.1159/000485898
19. Bruni L, Albero G, Rowley J, Alemany L, Arbyn M, Giuliano AR, et al. Global and regional estimates of genital human papillomavirus prevalence among men: a systematic review and meta-analysis. Lancet Glob Health. 2023;11(9):e1345-e62.10.1016/s2214-109x(23)00305-4
20. Devarakonda SS, Neppalli A, Liu L, Friday E, Shi R. Abstract 278: Risk factors and prevalence of genital HPV infection among adult females in US between 2003-2010: Data from NHANES study. Cancer Research. 2014;74(19_Supplement):278-.10.1158/1538-7445.Am2014-278
21. Xiang J, Han L, Fan Y, Feng B, Wu H, Hu C, et al. Prevalence and Genotype Distribution of Human Papillomavirus Among Attendees at a Sexually Transmitted Diseases Clinic in Urban Tianjin, China. Int J Gen Med. 2021;14:1983-90.10.2147/ijgm.S308215
22. Khodakarami N, Clifford GM, Yavari P, Farzaneh F, Salehpour S, Broutet N, et al. Human papillomavirus infection in women with and without cervical cancer in Tehran, Iran. Int J Cancer. 2012;131(2):E156-61.10.1002/ijc.26488
23. Burk RD, Kelly P, Feldman J, Bromberg J, Vermund SH, DeHovitz JA, et al. Declining prevalence of cervicovaginal human papillomavirus infection with age is independent of other risk factors. Sex Transm Dis. 1996;23(4):333-41.10.1097/00007435-199607000-00013
24. Wiley D, Masongsong E. Human papillomavirus: the burden of infection. Obstet Gynecol Surv. 2006;61(6 Suppl 1):S3-14.10.1097/01.ogx.0000221010.82943.8c

Preprint

25. Madhivanan P, Pierre-Victor D, Mukherjee S, Bhoite P, Powell B, Jean-Baptiste N, et al. Human Papillomavirus Vaccination and Sexual Disinhibition in Females: A Systematic Review. Am J Prev Med. 2016;51(3):373-83.10.1016/j.amepre.2016.03.015
26. Hansen BT, Kjær SK, Arnheim-Dahlström L, Liaw KL, Jensen KE, Thomsen LT, et al. Human papillomavirus (HPV) vaccination and subsequent sexual behaviour: evidence from a large survey of Nordic women. Vaccine. 2014;32(39):4945-53.10.1016/j.vaccine.2014.07.025
27. Liang LY, Du H, Zhang W, Chen Y, Wu B, Qu XF, et al. [Relevant factors to female human papillomavirus infection in city and rural areas of Shenzhen]. Zhonghua Liu Xing Bing Xue Za Zhi. 2013;34(8):796-9. https://pubmed.ncbi.nlm.nih.gov/24423766/

Preprint