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# Prevalence and genotype distribution of human papillomavirus in individuals referred to a laboratory in Urmia, Iran



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# Abstract

**Background and aim** Human papillomavirus (HPV) is a major contributor to sexually transmitted infections, especially common in sexually active populations. Although the majority of HPV infections resolve naturally, certain cases can develop into different types of cancer. This study focused on evaluating the prevalence and distribution of HPV genotypes across males and females of different age groups who visited a laboratory in Urmia, Iran.

**Materials and methods** Samples from the genital area were obtained from participants in the study. DNA extraction was performed using the Favorgen extraction kit (Favorgen, Taiwan), followed by genotyping through Real-Time PCR. Genotypes were determined using the MehrViru HPV genotyping kit (MehrViru, Iran). Additionally, demographic details, including age, were analyzed in conjunction with the statistical virological data.

**Results** Between 2022 and 2023, a total of 447 individuals, including both referred and routine visitors, attended the laboratory, comprising 431 females and 16 males. Of these, 195 tested positive for HPV, resulting in an overall prevalence rate of 43.6%. Among the positive cases, 90 individuals (46.2%) were infected with a single HPV genotype, while 105 cases (53.8%) had multiple genotype infections. The most common genotypes identified were HPV-6 (41.0%), HPV-16 (15.4%), HPV-56 (10.8%), and HPV-90 (10.8%). The least genotype identified was HPV-43, which was detected in 5 cases (2.6%). Additionally, our analysis revealed that women under 30 who tested positive were predominantly infected with the LR genotype, a pattern also seen in the four men in the same age group, all of whom were infected with the LR genotype.

**Conclusion** Our findings underscore the significant presence of HPV among both females and males visiting the laboratory in Urmia, particularly in individuals under 30 years old. The identification of HPV-6 and HPV-16 as the most prevalent genotypes highlights the importance of age-specific intervention strategies. Although vaccination programs cover HPV-6 and HPV-16, HPV-56 is not included, which underscores the need for comprehensive screening and preventive measures to address the potential long-term impacts of HPV-related diseases.

Keywords Human papillomavirus (HPV), Genotype distribution, HPV prevalence, Sexually transmitted infection

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# Introduction

Human papillomavirus (HPV) is a circular, doublestranded DNA virus that lacks an envelope and is classified within the *Papillomaviridae* family [1]. HPV ranks among the most common sexually transmitted infections and typically spread through sexual contact, such as vaginal, anal, or oral intercourse, with an infected individual. Nearly all sexually active individuals are expected to contract the virus at some point in their lifetime [2, 3]. Although most HPV infections are temporary and benign, with about 70% clearing within a year and over 90% resolving within two years, some infections can persist [4]. These persistent infections greatly increase the likelihood of developing several types of cancer. These include cervical cancer, cancers of the head and neck, oropharyngeal squamous cell carcinoma, penile cancer, and anal carcinoma [5]. Worldwide, HPV is linked to 4.5% of all cancer diagnoses, equating to about 630,000 new cases each year. Among these, 8.6% are found in women, while 0.8% are in men [6]. To date, over 200 HPV types have been discovered, with more than 40 specifically affecting the genital region. HPV genotypes are classified according to their cancer-causing potential into high-risk (HR) and low-risk (LR) categories [7]. The most frequent HR-HPV genotypes include HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV59. These genotypes are strongly associated with the development of various cancers, with cervical cancer being the most prominent [8, 9]. Notably, HPV-16 and HPV-18 are responsible for over 70% of cervical cancer cases worldwide, and cervical cancer stands as the fourth most commonly diagnosed cancer among women globally [10, 11]. LR-HPV genotypes, such as HPV 6, 11, 40, 42, 43, 44, 61, 70, 72, and 81, are closely associated with non-cancerous conditions. These strains are usually identified in cases of benign or slightly abnormal cervical tissue and are frequently linked to the development of genital warts [12].

In a study conducted by Mobini Kesheh et al. to evaluate the prevalence of HPV genotypes in the Iranian population, it was found, 49.5% of the total participants (n=10,266) tested positive for HPV [13]. Among the most frequently identified low-risk HPV genotypes, HPV-6 was detected in 77.7% of males and 43.3% of females, followed by HPV-11 in 13.7% of males and 11.4% of females. In terms of high-risk HPV types, HPV-16 was observed in 5.5% of males and 16.6% of females, while HPV-52 appeared in 3.2% of males and 9.6% of females [13]. A cross-sectional study conducted in Urmia from December 2015 to December 2016 analyzed 80 samples and found that 30 individuals (37.5%) tested positive for HPV infection. Among the positive cases, 16 participants (53.4%) were infected with low-risk HPV genotypes (6, 11, 26, 53, 67), while 7 individuals (23.3%) were infected solely with high-risk genotypes (16, 31, 58, 18). Additionally, 7 participants (23.3%) were found to have co-infections, carrying both low-risk and high-risk HPV genotypes [14].

Altogether, HPV epidemiology differs based on factors such as race/ethnicity, the age at which an individual first engages in sexual intercourse, urban or city residence, and sexual behaviors [15, 16]. Moreover, several risk factors associated with HPV infection have been identified, including HIV positivity, the number of sexual partners, and smoking [17]. One of the most effective measures to prevent HPV infection is vaccination. Currently, three FDA-approved vaccines offer protection against varying numbers of HPV types. The bivalent vaccine (Cervarix) targets HPV types 16 and 18. The quadrivalent vaccine (Gardasil) shields against types 6, 11, 16, and 18, while the 9-valent vaccine (Gardasil-9) covers types 6, 11, 16, 18, 31, 33, 45, 52, and 58 [18]. The latest guidelines for HPV vaccination recommend it for individuals starting at age 9 up to 26, as well as for adults aged 27 to 45 who are at risk of new HPV infections and may benefit from vaccination [19]. The WHO primarily targets girls aged 9 to 14 years for the vaccine, advising a two-dose regimen before they become sexually active. For girls aged 15 and older, a three-dose series is recommended [19]. However, in some developing countries, the HPV vaccination program has not been well established or widely implemented, which can limit access and coverage for those who could benefit from it [20]. As well, while the vaccine offers protection against many HPV infections, vaccinated individuals are still at risk for HPV-related diseases from strains not covered by the vaccine [21].

Therefore, in areas where HPV vaccination efforts are insufficiently established, placing a greater emphasis on screening and HPV genotyping can greatly improve public health outcomes by empowering prompt identification and prevention of cancers associated with HPV. Understanding the distribution of various HPV types across countries is essential for creating targeted vaccines and formulating effective national immunization plans. Moreover, having detailed regional HPV genotype statistics is crucial for refining public health strategies, assessing the effectiveness of existing vaccines, and determining whether customized vaccine solutions are needed in specific areas. Considering the significance of HPV prevalence and the existence of HR strains, this investigation aims to evaluate the frequency of HPV infections and determine the related genotypes across various age groups in both males and females who visited a laboratory in Urmia, Iran.

## **Material and method**

#### Study group and sample collection

The study population consisted of 447 individuals (431 women and 16 men) who visited a laboratory in Urmia, Iran, over the period from October 2022 to October 2023. The participants in our study were people who visited the laboratory for HPV screening, driven by a range of factors. These included personal requests for testing, diagnostic recommendations from their doctors, recent engagement in high-risk sexual activities, abnormal results from Pap smears, the existence of genital warts, or being in a relationship with someone diagnosed with HPV. These factors motivated them to pursue testing for their own peace of mind regarding their health.

The samples were collected by experienced specialists. For female participants, samples were taken from the vaginal and cervical areas using brushes. In males, samples were collected using brush or swab methods from the urethra penis to detect HPV. All collected samples were placed in liquid-based cytology (LBC) medium and stored at 4 °C. Extraction was completed on the same day, and genotyping conducted the following day. The Ethics Committee of Urmia University of Medical Sciences granted approval for this study under the reference number IR.UMSU.REC.1403.043. As the data analysis was conducted anonymously, no informed consent or further actions were required from the patients.

## **DNA extraction and PCR**

After collecting samples, we proceeded with DNA extraction and HPV genotyping. HPV DNA was extracted using the Favorgen extraction kit (Favorgen, Taiwan), according to the manufacturer's protocol. According to the kit instructions, 20 µL of Proteinase K was initially added to a microtube. Then, 200 µL of the patient sample, previously vortexed, was added to the microtube containing Proteinase K and vortexed again. Subsequently, 200 µL of FABG was added, and the mixture was incubated in a heater block for 15 min. Afterward, 200 µL of absolute alcohol was added and vortexed, and the entire content was transferred to the extraction column, followed by centrifugation at 6000 RPM for 1 min. The extraction column was then transferred to a new collection tube. Next, 400 µL of Wash Buffer 1 was added to the column and centrifuged at 18,000 RPM for 30 s. Subsequently, 750 µL of Wash Buffer 2 was added to the column and centrifuged at 18,000 RPM for 30 s. After replacing the collection tube, this step was repeated for 3 min. Finally, the column was transferred to a new microtube, and 70 µL of Elution Buffer was added to the filter. After 3 min at room temperature, it was centrifuged at 18,000 RPM for 1 min, completing the genetic material extraction process.

After extraction, HPV genotyping was conducted using the MehrViru HPV genotyping kit (MehrViru, Iran). This

kit can detect 12 high-risk HPV genotypes, namely 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, and it identifies an additional 7 genotypes (26, 53, 66, 67, 68, 73, and 82) as potentially high risk. It also covers 11 lowrisk HPV genotypes, such as 6, 11, 40, 42, 43, 44, 54, 61, 62, 89, and 90. Overall, the kit is capable of detecting 30 HPV genotypes and consists of 8 distinct HPV Reaction Mixes. Each mix is carefully prepared with specific primers and fluorescent probes tailored to identify particular HPV genotypes. For each strip, 20 µL of the MehrViru kit's master mix and 5  $\mu$ L of the patient's sample were added. Following centrifugation, the strips were placed in a real-time PCR machine. According to the kit's protocol, the following thermal cycling program was set: an initial denaturation cycle at 90 °C for 10 min, followed by 40 cycles consisting of three steps (95 °C for 10 s, 58 °C for 45 s, and 70 °C for 15 s), and a final extension at 35 °C for 30 s. After the completion of the run, the amplification curves were analyzed based on the kit's protocol to determine the results.

# Statistical analysis

The statistical analysis was done using SPSS software, version 27. Data description and the assessment of HPV prevalence and genotype distribution were carried out using frequency counts. To compare prevalence rates and analyze genotype distribution across various subgroups, Fisher's exact test and chi-square test were applied.

# Results

#### Demographic data and HPV prevalence

Between 2022 and 2023, a total of 447 individuals, including both referred and routine visitors, attended the laboratory, comprising 431 females and 16 males. The age range of the participants spanned from 17 to 67 years. Of these, 195 tested positive for HPV, resulting in an overall prevalence rate of 43.6% (42.5% among females and 75.0% among males) (Table 1). As the exact ages were recorded only for HPV-positive cases, the mean and standard deviation were calculated exclusively for this group. The average age of the HPV-positive population was  $33.73 \pm 8.59$ years, with a mean age of 33.76±8.77 years for females and 33.33±5.44 years for males (Table 2). The participants were divided into four distinct age categories for analysis. Table 1 outlines the frequency and percentage of infections across these groups. Persons under 30 years old had the largest proportion of positive test results at 47.5%, followed by those aged 31 to 40, with a positivity rate of 42.7%. However, the 31-40 age group had the highest absolute number of HPV-positive cases. As well, 447 cases were categorized into two groups: those who had a Pap smear test (189 individuals) and those who did not (258 individuals). Then the prevalence of HPV within each group was assessed. Among the 189 individuals who

Table 1	Demogr	aphic data	and HPV	prevalence
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	Total	HPV	HPV	P-
		positive	negative	value
Characteristics				
Female	431 (96.4)	183 (42.5)	248 (57.5)	0.01*
Male	16 (3.6)	12 (75.0)	4 (25.0)	
Age category				
<=30	160 (35.8)	76 (47.5)	84 (52.5)	0.58*
31–40	192 (43.0)	82 (42.7)	110 (57.3)	
41–50	76 (17.0)	29 (38.2)	47 (61.8)	
>=51	19 (4.3)	8 (42.1)	11 (57.9)	
Pap Smear test				
No	258 (57.7)	108 (41.9)	150 (58.1)	0.38*
Yes	189 (42.3)	87 (46.0)	102 (54.0)	
Sign/symptom				
Discharge	24 (5.6)	6 (25.0)	18 (75.0)	0.19**
Burning	16 (3.7)	5 (31.3)	11 (68.8)	
Discharge and burning	4 (0.9)	2 (50.0)	2 (50.0)	
Itching	17 (3.9)	11 (64.7)	6 (35.3)	
Discharge and itching	7 (1.6)	4 (57.1)	3 (42.9)	
Burning and itching	28 (6.5)	12 (42.9)	16 (57.1)	
All three (Discharge, burning, and itching)	26 (6.5)	13 (50.0)	13 (50.0)	

\* Chi square test, \*\* Fisher's exact test

 Table 2
 Mean age of HPV positive cases

Gender	Mean	Frequency	Std. Deviation
Female	33.76	183	8.77
Male	33.33	12	5.44
Total	33.73	195	8.59

underwent a Pap smear, 87 (46.0%) were HPV-positive, and 102 (54.0%) were negative. Among the 258 individuals who did not undergo a Pap smear, 108 (41.9%) were HPV-positive, and 150 (58.1%) were negative. Statistical analysis yielded a p-value of 0.38, indicating no significant difference in HPV prevalence between the two groups. In addition to our broader analysis, we assessed HPV prevalence among individuals presenting with specific symptoms. Symptom data were available for a subset of cases, and the results are summarized in Table 1.

In our research, a significant proportion of HPVpositive cases exhibited infections with multiple HPV genotypes. Figure 1 illustrates that out of the total positive cases, 90 individuals (46.2%) had a single HPV genotype infection, whereas 105 cases (53.8%) presented with infections involving two or more genotypes. Among the cases with mixed infections (n = 105), 66 (62.9%) had dual genotype infections, 18 (17.1%) had triple infections, 14 (13.3%) had quadruple infections, and 7 (6.7%) had infections with more than four genotypes. One subject exhibited a maximum of seven distinct genotypes (Table 3).

# HPV genotype distribution

We identified a total of 32 unique HPV genotypes among the HPV-positive patients (n = 195). This includes 14 high-risk (HR-HPV) genotypes, 11 low-risk (LR-HPV) genotypes, and 8 potential high-risk (pHR-HPV) genotypes. Table 4; Fig. 2 clearly shows that the predominant types of infections in this study were LR-HPV6, HR-HPV16, HR-HPV56, and HR-HPV90. The most prevalent high-risk HPV (HR-HPV) genotypes, ranked from highest to lowest, are as follows: HPV-16 (15.4%), HPV-56 (10.8%), HPV-52 and HPV-59 (7.2%), HPV-51 (6.7%), HPV-33 (5.1%), HPV-18 (4.1%), and HPV-35 (3.1%). The most common pHR-HPV genotypes were HPV-53 (9.7%), HPV-67 (5.1%), and HPV-82 (4.6%). In terms of LR genotypes, the most commonly observed genotypes were HPV-6 (41.0%), HPV-90 (10.8%), HPV-11 (7.7%), HPV-44 and HPV-61 (5.6%), HPV-62 (4.6%), and HPV-54 (4.1%). Regarding co-infections with multiple HPV genotypes, 105 individuals (52.8%) were found to have such infections. The most frequently identified genotypes among the 105 mixed cases were HPV-6 (43 cases), followed by HPV-16 (24), HPV-56 (20), HPV-53 (18), and HPV-90 (16). Table 5 illustrates the distribution of HPV genotype groups across various age categories. Among HPV-positive females (n = 183), the majority were infected with low-risk genotypes, which were found in 124 cases (67.7%). High-risk genotypes were identified in 98 females (53.6%). Similarly, among HPV-positive males (n = 12), the low-risk genotypes were the most common, found in 11 cases (91.7%). In comparison, high-risk genotypes were identified in 3 males (25%). Our findings also indicate that women under 30 years old who tested positive predominantly had the LR genotype. This trend was consistent among the four men in the same age group, all of whom were also infected with the LR genotype. Similarly, for women aged 31–40 who tested positive, the LR genotype was the most common. The same genotype predominated among men in this age bracket as well. Additional details for other age groups are available in Table 5.

# Discussion

This study offers an up-to-date analysis of HPV genotype distribution among men and women in Urmia. Between 2022 and 2023, a total of 447 individuals, comprising 431 females and 16 males, were referred to the laboratory. Among them, 195 were diagnosed with HPV, indicating an overall infection rate of 43.6% (42.5% in females and 75.0% in males). The highest rate of positive cases occurred in individuals under 30 (47.5%) and those aged 31–40 (42.7%). As well, the cases were categorized into two groups: individuals who underwent a Pap smear and



**Fig. 1** Prevalence of mixed infection according to the type of infection. Among the cases with mixed infections (n = 105), the majority, 66 individuals (62.9%), had dual genotype infections. Triple infections were observed in 18 cases (17.1%), quadruple infections in 14 cases (13.3%), and 7 cases (6.7%) had five or more genotypes involved (other infections)

Number of genotypes	Frequency	Percentage among total positive cases (n = 195)	Percent- age among mixed infec- tion (n = 105)
2	66	33.8	62.9
3	18	9.2	17.1
4	14	7.2	13.3
5	4	2.1	3.8
6	2	1.0	1.9
7	1	0.5	1.0
Total	105	53.8	100.0

Table 3 Prevalence of mixed infection

those who did not. As shown in our results, the prevalence of HPV had no statistically significant difference between two each group. Additionally, a notable percentage of HPV-positive individuals had infections with multiple genotypes. Of the total positive cases, 90 (46.2%) had a single HPV genotype, while 105 (53.8%) were infected with two or more genotypes. The most common types identified were LR-HPV6, HR-HPV16, HR-HPV56, and HR-HPV90.

A systematic review and meta-analysis that evaluated 26 eligible studies with a total sample size of 5,560 women revealed an overall HPV prevalence of 23% among Iranian women. The highest prevalence was observed in Tehran, while the lowest prevalence was found in Isfahan [22]. In alignment with our results, a study conducted in Tehran on 219 HPV-positive cases (160 females and 59 males) found that HPV-16 (17.4%) was the most prevalent HR type, and HPV-6 was the most common LR type [23]. However, contrary to our findings, this study indicated that the prevalence of HR-HPV was greater than that of LR-HPV in their population [23]. Another study conducted in Tehran aimed at analyzing the molecular characteristics of HPV genotypes among 5,176 male and female patients revealed that 2,727 (53%) tested positive for HPV [24]. Of these, 2,372 (87%) were women, and 355 (13%) were men. In line with our findings, the survey recognized HPV-6 as the most predominant LR type (32% in females and 31% in males) and HPV-16 as the most common HR type (12% in females and 11% in males). The highest frequency of HPV was observed in the 31–40 and 21–30 age groups. Additionally, a significant proportion of the study population, 1,306 patients (52%), were found to have infections with multiple genotypes, similar to what we observed in this research [24]. In the largest survey conducted in Iran, which analyzed 10,266 samples from 31 provinces, Mobini Kesheh et al. reported that 49.5% (n = 5085) tested positive for HPV DNA [13]. Similar to our findings, the most frequent

Table 4 Distribution of HPV genotypes in 195 HPV positive cases						
Geno-	HPV	Female (n=	= 183)	Male (n = 12)		
type	Genotypes	Frequency	Percent	Frequency	Percent	
group						
LR	6	72	39.3	8	66.7	
HPV	11	15	8.2	0	0.0	
	40	3	1.6	0	0.0	
	42	2	1.1	0	0.0	
	43	4	2.2	1	8.3	
	44	11	6.0	0	0.0	
	54	8	4.4	0	0.0	
	61	11	6.0	0	0.0	
	62	9	4.9	0	0.0	
	89	5	2.7	1	8.3	
	90	19	10.4	2	16.7	
pHR	26	6	3.3	0	0.0	
HPV	53	19	10.4	0	0.0	
	66	6	3.3	1	8.3	
	67	9	4.9	1	8.3	
	68	7	3.8	1	8.3	
	73	4	2.2	0	0.0	
	82	9	4.9	0	0.0	
HR	16	28	15.3	2	16.7	
HPV	18	8	4.4	0	0.0	
	31	6	3.3	0	0.0	
	33	10	5.5	0	0.0	
	35	6	3.3	0	0.0	
	39	3	1.6	0	0.0	
	45	2	1.1	0	0.0	
	51	13	7.1	1	8.3	
	52	14	7.7	0	0.0	
	56	21	11.5	0	0.0	
	58	6	3.3	0	0.0	
	59	14	7.7	0	0.0	

LR-HPV type identified was HPV-6 (77.7% in males and 43.3% in females), while the most common HR type was HPV-16 (5.5% in males and 16.6% in females). Additionally, they observed mixed HPV infections in 14.0% of HPV-positive males (n = 174) and 42.2% of HPV-positive females (n = 1620). In line with our findings, HPV-6 was the most frequently detected genotype in mixed infections across both genders. However, unlike our results where HPV-16 was the second most common genotype, their analysis found HPV-11 to be the second most common in cases of mixed infections [13]. Also, the highest HPV burden was found in individuals aged between 30 and 44, accounting for 51.8% of cases [13]. A study conducted in Sari included 90 participants, with 83 women and 7 men. Among the women, 65% (54 out of 83) tested positive for HPV, while all 7 men were positive [25]. Unlike our findings, the most common LR-HPV type was HPV-11 (23%), and the most frequent HR types were HPV-56 (18%) and HPV-39 (16%). In line with our findings, the highest HPV prevalence of 75% (36 cases) was found in individuals under the age of 31. However, unlike our results, most of the positive cases in this age group were associated with HR genotypes [25]. Among individuals with HPV infections, 39 cases (around 61.3%) exhibited co-infections with multiple genotypes. In contrast to our findings, where HPV-6 was the most common genotype in mixed infections, HPV-11 was the most prevalent genotype, appearing in 14 out of 61 cases [25].

In a study by Sabet et al., out of 567 participants in northeast Iran, 35.3% (n = 200) tested positive for HPV [26]. The distribution of HPV genotypes in this population included HPV-6 (50%), HPV-11 (10%), HPV-67 (5%), HPV-16 (15%), HPV-31 (10%), HPV-54 (5%), and HPV-89 (5%). They also observed multiple HPV genotypes in Razavi and North Khorasan, where 11.2% (28/108) had co-infections with two genotypes, 4.8% (12/108) with three, and 0.4% (1/108) with four or more genotypes. In South Khorasan, 3% (6/37) were co-infected with another genotype, while within a more diverse group, 8.6% (10/37) had two-genotype co-infections, 0.9% (1/37) had three, and 1.7% (2/37) had four or more genotypes [26]. In a separate study conducted in Sanandaj, out of 950 participants, 933 (98%) were female, while 17 were male [27]. Within the female group, 236 (25%) tested positive for HPV, whereas among the male participants, 13 out of 17 (76%) were HPV positive. Similar to our findings, the most prevalent HR genotype was HPV-16, and the most common LR genotype was HPV-6. Additionally, the highest percentage of positive HPV cases was seen in individuals under 20 years old (40%) and those aged 21-30 (35%). However, in terms of the total number of HPV-positive individuals, the 31-40 age group had the highest count of cases [27]. Furthermore, few studies have been carried out in Urmia to examine the prevalence of HPV genotypes. In the initial research conducted by Olia et al., focused on determining the HPV genotypes linked to vaginal infections among women, results indicated that 30 out of 80 samples (37.5%), were positive for HPV [14]. Similar to our findings, the majority of cases, 16 people (53.4%), were infected with LR genotypes. In addition, seven patients (23.3%) had infections with HR HPV genotypes, and another seven individuals (23.3%) had co-infections involving both LR and HR genotypes [14]. However, it is important to note that this investigation predominantly focused on female participants and did not include any males in the sample.

Overall, our study emphasizes a significant observation regarding the notably high prevalence of HPV-6 and HPV-16. Although current available vaccines offer good protection against HPV-6 and HPV-16, these remain among the most frequently detected strains in our research. Multiple factors may have contributed to this occurrence. In Iran, the provision of HPV vaccines is managed by private companies, and are not covered by



**Fig. 2** Prevalence of HPV genotypes. HPV-6 was the most common genotype, accounting for 80 cases (41.0%). This was followed by HPV-16 with 30 cases (15.4%), HPV-56 and HPV-90 each with 21 cases (10.8%), HPV-53 with 19 cases (9.7%), HPV-11 with 15 cases (7.7%), HPV-59 and HPV-52 both with 14 cases (7.2%), HPV-51 with 13 cases (6.7%), and HPV-44 and HPV-61 both with 11 cases (5.6%). Additionally, HPV-67 and HPV-33 were identified in 10 cases (5.1%), HPV-62 and HPV-82 in 9 cases (4.6%), HPV-54, HPV-68, and HPV-18 in 8 cases (4.1%), and HPV-66 in 7 cases (3.6%). Lastly, HPV-26, HPV-31, HPV-35, HPV-58, and HPV-89 each appeared in 6 cases (3.1%), while HPV-43 was found in 5 cases (2.6%). Genotypes with lower frequencies are not displayed in this figure

Table 5	Distribution of	of HPV g	genotype	groups	across	various	age
categorie	es						

HPV	Gender	<=30	31–40	41–50	>=51
genotypes					
LR HPV	Female ( <i>n</i> = 124)	50	49	21	4
		(40.3%)	(39.5%)	(16.9%)	(3.2)
	Male (n = 11)	4 (36.4)	7 (63.6)		
pHR HPV	Female ( <i>n</i> = 49)	19	17	12	1
		(38.8)	(34.7)	(24.5)	(2.0)
	Male $(n=2)$	0 (0)	2 (100)		
HR HPV	Female ( <i>n</i> = 98)	46	37	12	3
		(46.9)	(37.8)	(12.2)	(3.1)
	Male $(n=3)$	0 (0)	3 (100)		

insurance, making them unaffordable for many [28, 29]. Additionally, the vaccine has yet to be integrated into the national immunization schedule, and evidence supporting its cost-effectiveness in Iran is lacking [30, 31, 32]. Other barriers, such as high vaccine prices, financial difficulties, and a lack of public awareness, significantly impede vaccination initiatives [33]. This highlights the critical necessity for comprehensive national initiatives and enhanced regulation to enhance the availability and adoption of the HPV vaccine in Iran. Moreover, although existing vaccines offer strong protection against HPV-6 and HPV-16, they do not include HPV-56, which has become one of the most frequently identified strains in this study. This limitation inn vaccine coverage highlights

the urgent need for expanded preventive strategies and enhanced screening initiatives.

It's essential to recognize certain limitations in our research. Firstly, the majority of contributors were women, with a much smaller sample of men, which may not accurately represent the true epidemiology of HPV. Consequently, these findings are not applicable to men, highlighting the necessity for additional focused studies that incorporate a larger group of male participants in this area. Larger studies with broader sample sizes, especially focused on men, are necessary to better understand the prevalence and impact of HPV infections across different demographics. Secondly, it is essential to acknowledge that these observations may not be applicable to the broader population. This study's participants were individuals who came to the laboratory expressly for HPV testing, likely due to noticeable symptoms or engagement in high-risk activities. Consequently, the heightened infection rates seen in specific subgroups mirror the characteristics of those actively seeking testing, rather than representing the general population. Another limitation of this study is the lack of DNA genotyping using specific primers (like GP5+/GP6+) followed by sequencing, which could have provided valuable data for phylogenetic analysis. Such an analysis would have offered a more detailed understanding of the HPV strains circulating in the region. This limitation highlights the potential for future research to incorporate such analyses to

further enhance the understanding of HPV strain distribution. Additionally, the relationship between co-infections and the symptoms and prognosis of patients was not explored in this study. Further investigation into this matter would be valuable in understanding the clinical implications of co-infections. This limitation should be considered, and future studies could focus on the impact of mixed infections on patient outcomes.

# Conclusion

This survey found that 43.6% of both male and female participants tested positive for HPV in our population. The individuals who participated in the study were prompted to undergo testing due to current high-risk sexual activity, abnormal Pap smear findings, genital warts, or concerns related to an infected sexual partner. Among those who tested positive, 46.2% had single HPV genotype infections, while 53.8% were co-infected with multiple genotypes. Our results showed that low-risk genotypes, particularly HPV-6, were the most common, followed by high-risk types like HPV-16 and HPV-56. The prominence of these high-risk strains underscores the urgent need for expanded HPV vaccination programs in Iran, especially for younger individuals. Moreover, the absence of coverage for genotypes like HPV-56 in existing vaccines calls for more comprehensive preventive measures and screening programs. Implementing vaccination alongside increased awareness and education, particularly targeted toward individuals under 30, could extensively diminish the incidence of HPV-associated diseases, such as cancer, ultimately improving public health outcomes in the area. Along with the vaccine recommendation, due to the high prevalence of high-risk HPV in individuals under 30 and the potential for infection to lead to cancer after at least 10 years, it is advised that these individuals undergo regular follow-up.

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#### Author contributions

S.M and M.N: Supervision, Review and editing, Validation, Methodology, Z.KJ and O.SA: Methodology, Investigation, Writing original draft. H.RS: Statistical analysis. M.J and F.AS: Investigation, Review and editing, Validation.

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## Data availability

No datasets were generated or analysed during the current study.

### Declarations

#### **Consent for publication** None.

#### **Competing interests**

The authors declare no competing interests.

#### Ethics approval and consent to participate

The study received approval from the Ethics Committee of Urmia University of Medical Sciences, assigned the reference number IR.UMSU.REC.1403.043.

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