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Prevalence and genotype distribution of HPV infection among women in 2021–2023 in southern Iran: The rising trend of HPV infection among women

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Abstract

Background Human papillomavirus (HPV) infection is the leading cause of genital disease. More information on the prevalence and distribution of genital HPV subtypes in the female population is needed to inform preventive strategies. Geographical, social, economic, and ethnic barriers in developing countries challenge the development of preventive measures for cervical cancer. For the first time, we sought to determine the prevalence of HPV infection among women referred to gynecologists in southern Iran.

Methods This cross-sectional study was conducted in Hormozgan, Iran from July 2021 to July 2023 on various cervical specimens from all outpatients referred to the Virology Laboratory of the Infectious and Tropical Diseases Research Center. HPV DNA was extracted from genital swabs of the study participants using the QIAamp DNA Extraction Kit. Genotyping was performed on 3960 cases using multiplex PCR and a microarray chip (Master Diagnóstica, Granada, Spain) to identify 18 high-risk (HR) and 18 low-risk (LR) HPV genotypes. Demographic data were statistically analyzed in relation to viral data.

Results Of 3960 cases, 1303 (32.90%) were HPV positive. HPV positive patients were younger than negative patients. The positive rate was higher in the age group 20 to 40 years (72%). Single HPV infection was the most common pattern, accounting for 52.57% of cases while double infection accounted for 21.18% of the positive cases. The most common HR-HPV subtypes in southern Iran were HPV 16, 52, 31, and 53 (12.58, 8.36, 6.60, and 6.44%, respectively). Also, the most common LR-HPV subtypes were HPV6, 42, 62.81, and 44.55 (14.58, 12.58, 8.36, and 8.05, respectively). A comparison of the prevalence of HPV infection showed an increase in cases (in 2022–2023 (36.05%) compared with that in 2021–2022 (29.92%)), a decrease in the age of infected women (41.50% for 2022–2023 and 42.05% for 2021–2022) and an increase in multi-genotypic cases (42.44% for 2022–2023 and 31.24% for 2021–2022).

Conclusions These observations highlight the need for a program to educate the young population of the community as well as the implementation of infection control measures against HPV infection, especially in the case of mass HPV vaccination of the general population.

Keywords Human papillomavirus, Genotype, Prevalence, Low risk, High risk

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Background

Human papillomavirus (HPV) causes cervical cancer, the fourth most common cancer in women [1–3]. This virus plays an important role in morbidity and mortality in women and is therefore considered a public health priority. HPV infections are very common, affecting up to 90% of people at some point in their lives and leading to serious health problems, including cervical cancer in women. There are different types of HPV and most of these viruses do not cause complications and the symptoms go away on their own, but some HPV viruses are considered dangerous and the symptoms can worsen and eventually lead to cancer. In addition to cervical cancer, HPV can also cause other types of diseases in both men and women, including various types of cancer and genital warts [4–7]. Safe and effective vaccines are available to prevent HPV infection. The HPV vaccine is recommended for all adolescents (boys and girls) at 11 or 12 years of age, although we can administer the vaccine as early as 9 years of age [8–10]. Most people with HPV do not know they have the infection, and the virus causes no symptoms or health problems [4]. HPV has more than 200 genotypes [11]. To date, 231 HPV types (HPV-1 to HPV-231) have been identified, of which about 40 cause genital infections (https://www.hpvcenter.se/human_reference_clones/). A number of these viruses have been classified by the International Agency for Research on Cancer as high risk and carcinogenic to humans. In 2022, cervical cancer was the third most common cancer in women, with an estimated 662,301 cases, based on the data sources and methods used to produce global cancer and mortality statistics—GLOBOCAN estimates [12]. HPV 16 and 18 cause about 70% of all cases of cervix cancer. Types 31, 33, 45, 52, and 58 together account for about 15% of cervical cancers. HPV types 6 and 11 are less dangerous, do not cause cancer, and cause genital warts [13–18]. In 2017, the World Health Organization (WHO) estimated that the prevalence of HPV in women worldwide was 7.11%. The most common types of HPV are types 16 and 18, and women who are infected with one type of HPV may be infected with another type of HPV at the same time. The prevalence of HPV is also high in men in all regions of the world, so that the peak of infection in men occurs at a relatively older age than in women [19, 20]. Cytologic and molecular methods are currently used to diagnose HPV infection. Pap smear, as a cytologic test, is used as the main approach for screening cervical lesions. However, it can give significant false negative results. Therefore, molecular assays such as PCR hybridization, PCR, and real-time PCR have better and more accurate results for HPV detection and genotyping [4, 21–24]. Knowledge of the prevalence and distribution of HPV genotypes in each community is necessary for the development of new diagnostic methods and evaluation of vaccine efficacy in different geographic areas [25]. Today, many countries are seriously implementing screening and vaccination programs. These programs have been very effective in reducing cervical cancer [26]. In the present study, the health system of southern Iran aimed to investigate for the first time the prevalence and genotype diversity of HPV in the population of women referred to gynecologists and to identify the percentage of positive cases and the most common genotypes.

Methods

Clinical specimens

This cross-sectional study was conducted on various cervical specimens from outpatients referred to the Virology Laboratory of the Infectious and Tropical Diseases Research Center. Our sample size included all outpatients referred to this center. This laboratory is the main laboratory that collects specimens from various specialists, including gynecologists, throughout the province. In this study, we evaluated the prevalence of HPV infection and the frequency of genotypes between two consecutive years, 2021–2022 and 2022–2023. Cervical swabs were collected from 3960 participants aged 14 to 86 years between July 2021 and July 2023. Participants were divided into seven age groups: age group less than 20 years, age group $20 \leq \text{age} < 30$ years, age group $30 \leq \text{age} < 40$ years, age group $40 \leq \text{age} < 50$ years, age group $50 \leq \text{age} < 60$ years, age group ≤ 60 years < 70 years, and age group $70 \leq \text{age} < 86$ years. Patients were visited for various reasons, including physical examination, vaginitis, and female tumors. Written informed consent was obtained after explaining the study to each participant. Ethical approval was obtained from the University Ethics Committee.

For testing of outpatients seeking HPV identification, cervical and vaginal secretions were collected either by physicians or by trained laboratory personnel. These specimens were collected in a single cryo-tube. Upon delivery, the specimens were stored at -20°C until testing.

DNA extraction, PCR amplification, and HPV genotyping

HPV DNA was extracted using the QIAamp DNA Extract Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. HPV Direct Flow CHIP (Vitro, Master Diagnóstica, Granada, Spain) was used for virus identification and genotyping. 18 HR genotypes (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 18 LR HPV genotypes (HPV-6, 11, 40,42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84, and 89) were genotyped by PCR followed by reverse dot blot hybridization based on DNA flow technology. The manufacturer's instructions were followed for all experimental procedures.

Statistical analysis

Data were analyzed using Excel 2013 (Microsoft Corp., Redmond, WA, USA) and SPSS 20.0 (IBM Corp., Armonk, NY, USA). Two-way analysis of variance (ANOVA) was used to classify the age groups of those who tested positive and the genotypes of the virus based on high and low risk. Binary logistic regression was performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) to examine the relationship between year of study and positive test. The difference was considered statistically significant at the $p \leq 0.05$ level.

Results

Prevalence and type-specific distribution of HPV

Out of a total of 3960 outpatients referred to gynecologists and dermatologists, 1303 cases (32.90%) were positive and 2657 cases (67.10%) were negative. There was a significant increase in HPV infection in 2022–2023 (36.05%) compared to 2021–2022 (29.92%). HPV prevalence was significantly associated with age ($P < 00001$). In this study, we decided to compare the statistics of HPV infection in two consecutive years. The results of our survey showed that the infection statistics increased in 2023 compared with 2022. In both consecutive years, the highest prevalence of HPV was observed in the age group 30 to 40 years (42.05% for 2022 and 41.50 for 2023) (Table 1). The lowest prevalence of HPVs was observed in the age group 70 to 85 years (0.32% for 2022 and 0% for 2023), with the prevalence of infection decreasing with increasing age. More than 72% of the positive cases were in the age group 20 to 40 years (Table 1). The mean age was different between positive and negative cases (33.93 vs. 35.50 years, P value, < 0.001). Thus, HPV-positive patients were younger than negative individuals. The standard deviation of the total age was 8.631 and for the years 2021–2022 and 2022–2023 it was 9.037 and 8.252, respectively.

Table 1 The prevalence of HPV among women in different age groups

Age analysis of positive patients		
Age range	**2023–2022**	**2022–2021**
Not having age information	4.00%	1.30%
Age < 20	3.45%	1.62%
20 ≤ Age < 30	32.04%	30.36%
30 ≤ Age < 40	41.50%	42.05%
40 ≤ Age < 50	15.88%	19.32%
50 ≤ Age < 60	2.84%	3.41%
60 ≤ Age < 70	0.41%	1.62%
70 ≤ Age < 86	0	0.32%

Distribution of HPV genotypes

The HPV prevalence and the distribution of high-risk and low-risk HPV types in different age groups are shown in Table 2. All genotypes covered by the EUROArray HPV test were identified in the study population and the most prevalent HPV genotypes were HPV 6 (14.58%), HPV 16 and HPV 42 (12.58%), HPV 52 and 62/81 (8.36%), and HPV 44/55 (8.05%) (Table 2). Figure 1 illustrates the distribution of different high-risk and low-risk HPV genotypes in the women screened in 2021–2022 compared to 2022–2023. As shown in Table 2, among HPV-positive individuals, genotype 6 has the highest number of low-risk genotypes with 190 patients, and genotype 16 has the highest number of high-risk genotypes with 164 patients. No cases of the low-risk subtypes 71 and 69 have been reported. Among the high-risk genotypes, HPV 45 with 154.55%, HPV 39 with 95%, HPV 35 with 73.33%, and HPV 51 with 45.45% showed the highest increase in 2023 compared to 2022 (Fig. 1). Also, HPV 44.55 with 150%, HPV 61 with 100%, HPV 40 with 89.47%, HPV 6 with 75.36%, and HPV 42 with 73.33% showed the highest increase among low-risk types in 2023 (Fig. 1). In this study, single HPV infection was the most common pattern, accounting for 52.57% of cases. Double (21.18%), triple (7.98%), and multiple HPV infections were rare (Fig. 2). The two genotypes commonly found in the double infection were HPV52 + HPV58. The highest multiple infection in our study was 7 genotypes in the same patient. The genotypic diversity also increased in 2023 (Table 3) when comparing the phenotypic diversity between 2022 and 2023.

Analysis of the results showed that the odds of being HPV positive increased significantly in 2022–2023 (OR = 1.32, $p < 0.001$), with 36.05% of participants testing positive compared to 29.92% in the previous year (Table 4).

Discussion

In 2006, the European Medicines Agency (EMA) approved the first HPV vaccine to prevent HPV infection [27]. As a first step to end HPV-related cancers, the World Health Organization has published a strategy to reduce the global HPV prevalence rate from 13.3 per 100,000 women, adjusted for age, to 4 per 100,000 women by 2030 [28]. Knowledge of the prevalence rate and genotype type in the community can be useful in disease management and prevention efforts. To date, no study has been conducted in southern Iran to investigate common HPV genotypes. This study is one of the few to

Table 2 The results of the number of people who tested positive for HPV infection by high-risk and low-risk genotypes in 2021 to 2023

Risk assessment							
LOW RISK	Genotypes	Number	Percent	**HIGH RISK**	Genotypes	Number	Percent
	6	190	14.58		16	164	12.59
	11	23	1.77		18	65	4.99
	40	55	4.22		26	9	0.69
	42	164	12.59		31	86	6.6
	43	46	3.53		35	41	3.15
	54	84	6.45		39	59	4.53
	61	24	1.84		45	39	2.99
	67	47	3.61		51	81	6.22
	69	0	0		52	109	8.37
	70	6	0.46		53	84	6.45
	71	0	0		56	66	5.07
	72	5	0.38		58	48	3.68
	84	34	2.61		59	55	4.22
	89	2	0.15		66	71	5.45
	44.55	105	8.06		68	68	5.22
	62.81	109	8.37		73	59	4.53
	*	*	*		82	51	3.91

provide detailed information on the current prevalence of HPV subtypes in a general population of women living in southern Iran. The present study was conducted on fresh specimens collected from samples referred to the virology laboratory of the Infectious and Tropical Diseases Research Center. Recent studies from Iran show that genital HPV infection has increased rapidly in the sexually active Iranian population over the past decade. One of the main reasons for this increase in the number of HPV-positive cases is the limited access to vaccination and the lack of knowledge among women about this infection [29–33].

In this cross-sectional study of women with outpatient specialist referrals between 2021 and 2023, the prevalence of HPV infection was 32.90%. We observed a significant association between the age of the women and the prevalence of HPV infection. This prevalence is about three times higher in women between 20 and 40 years of age than in women over 45 years of age. This means that younger women are more likely to be infected with HPV, which is consistent with previous findings. Studies conducted in Iran show that between 5.5 and 57.4% of women's vaginal secretions are herpes-positive in different populations and different study methods, and the highest amount of HPV-positive and high-risk genotypes are usually found between the ages of 20 and 40 [29, 34–36]. An increase in HPV prevalence in middle age and a decrease in cases with increasing age have been reported in internationally published data on women [34, 37]. One of the most important implications of the above Iranian research could be the need for rapid targeted vaccination against HPV in the 20s to 40s or even earlier. Several studies have demonstrated the effectiveness of HPV vaccination at younger ages. Based on cost-effectiveness analyses, even vaccinating women up to age 25 is generally cost-effective [38]. In addition, the FDA has approved the expanded use of Gardasil 9 for people between the ages of 27 and 45 [39].

Our results showed that single infections were more common than double or multiple infections. In addition, this diversity was greater in 2022–2023 compared with 2021–2022. Patients with multiple HPV infections have longer and more persistent HPV infections than patients with single HPV infections [40].

HPV genotypic association with cervical cancer has been reported in different racial and geographic groups [41]. Therefore, epidemiological data on HPV genotypes in each country can be very useful for implementing appropriate cervical cancer prevention strategies [32]. Using a precise molecular method, this study presents a recent report on HPV genotype distribution among women in southern Iran. The results of our investigations showed that the most common HR genotypes are HPV 16, HPV 52, HPV 31, HPV 53, and HPV 66 and the most common LR genotypes are HPV 6, HPV 42, HPV 62.81, and HPV 44.55. In the study by Rezaei Azhar and colleagues to investigate the prevalence of HPV genotypes in women in Iran, HR genotypes HPV 16, HPV 66, HPV 18,

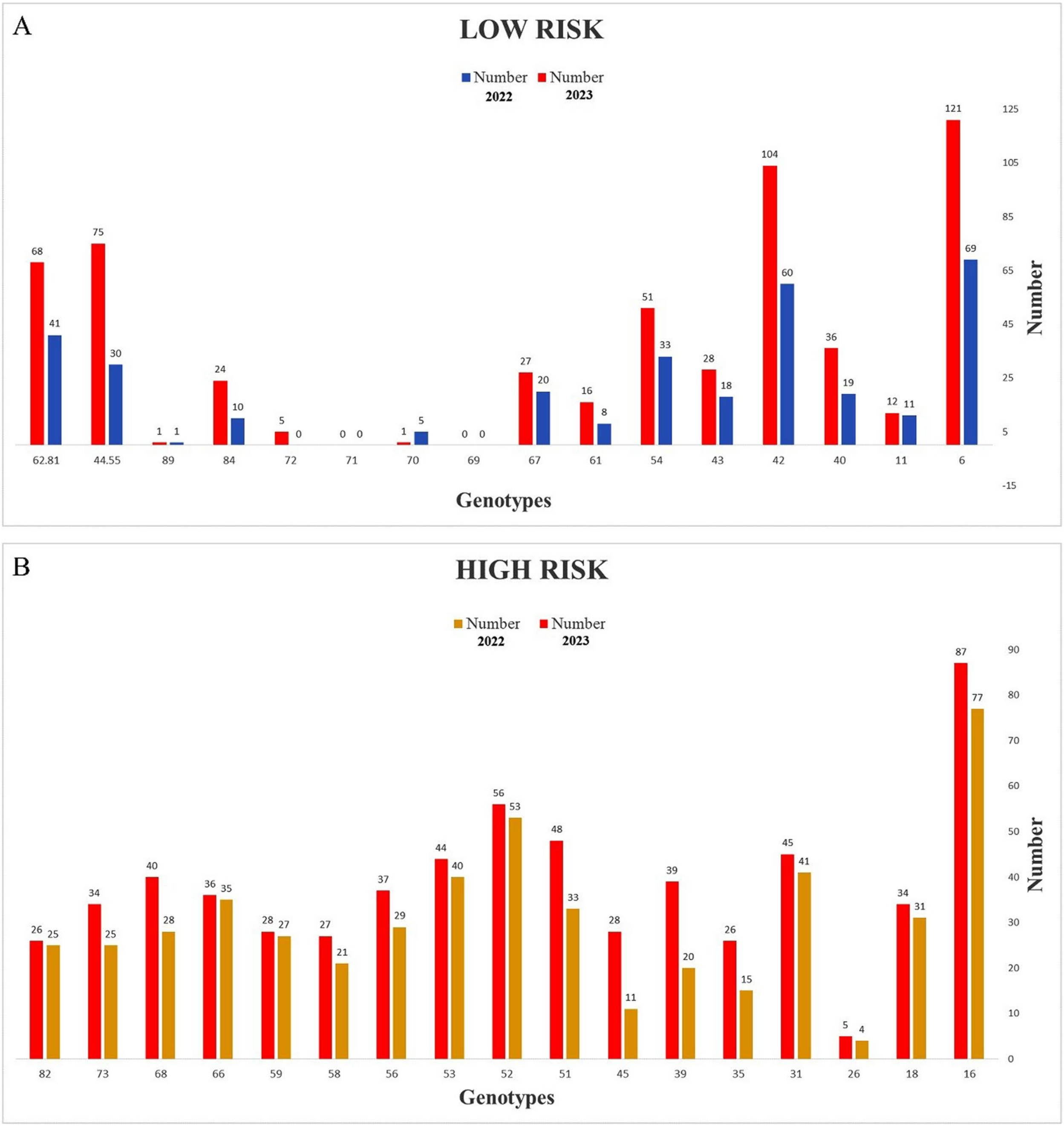

Fig. 1 Total infection rate of different genotypes of LR-HPV (**A**) and HR-HPV (**B**) in the years 2021–2022 compared to the years 2022–2023

HPV 31, HPV 52, and HPV 39 were the most common genotypes. In both communities, HR genotypes HPV 16, HPV 52, and HPV-31 were common [34]. In the largest study conducted in Iran, Mobini Keshe et al. performed herpes typing on 10266 samples from 31 provinces of Iran and the results showed that 49.5% ($n=5085$) of the samples were HPV positive among which the most common genotypes were HPV 6 (43.3%), HPV 11 (11.4%), HPV 16 (16.6%) and HPV 52 (9.6%) [29]. Sabet et al. also investigated the prevalence of HPV genotypes in both sexes in three northeastern provinces of Iran. They found that the prevalence of HPV positivity was 35.3% of the population, and the most common genotypes were HPV 6 (50%), HPV 11 (10%), and HPV 16 (15%) [36]. Two genotypes, HPV-6 and HPV-16, were consistent with our study. In another study from Iran, Shalchimanesh et al.,

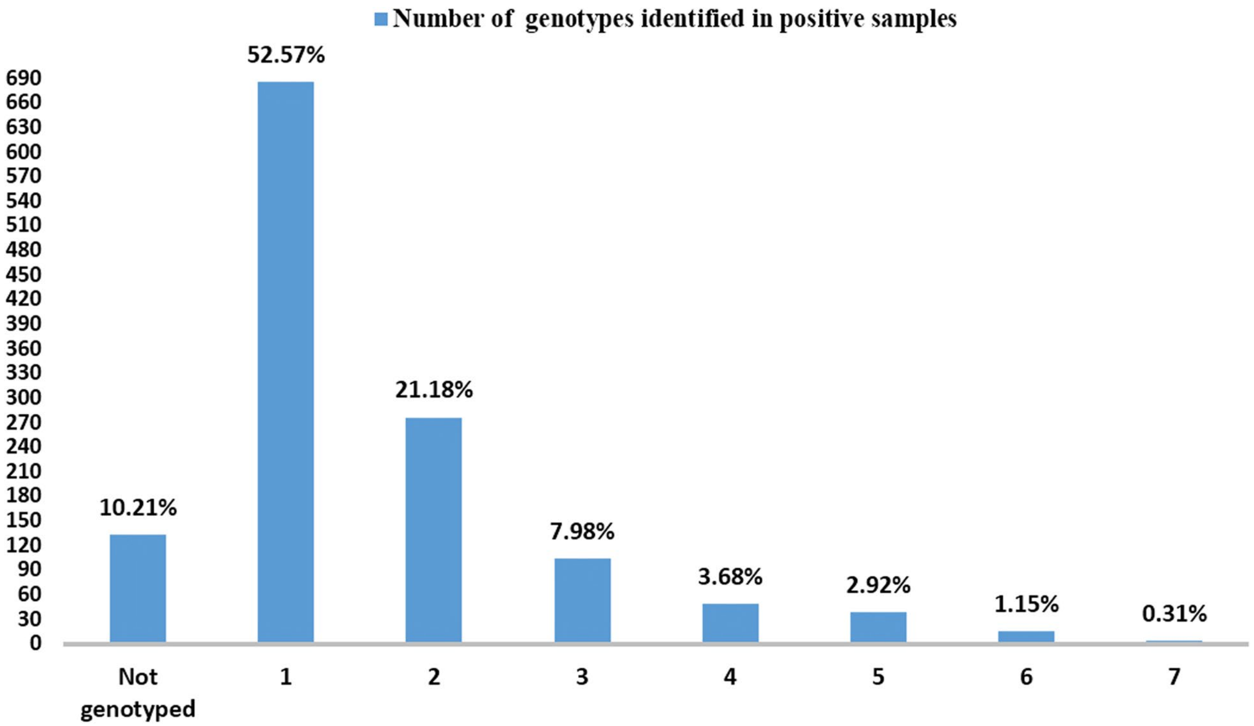

Fig. 2 The overall distribution of single and multiple genotypes of HPV infection

Table 3 Genotypic variation in positive HPV cases in 2022 compared to 2023

	Genotypes identified in positive samples in 2022–2023		Genotypes identified in positive samples in 2021–2022	
Number of genotypes	**Number**	**Percent**	**Number**	**Percent**
Not genotyped	43	6.19	90	14.8
1	357	51.37	328	53.95
2	160	23.02	116	19.08
3	61	8.78	43	7.07
4	36	5.18	12	1.97
5	26	3.74	12	1.97
6	9	1.29	6	0.99
7	3	0.43	1	0.16

Table 4 Test variation positive HPV cases in 2022 compared with 2023

HPV	HPV Positive *n* (%)	HPV Negative *n* (%)	OR (95%)	*p*-value
Total	3960 (100)			
	1303 (32.90)	2657 (67.10)		> 0.001*
2021–2022	608 (29.92)	1424 (70.08)		
2022–2023	695 (36.05)	1233 (63.95)	1.32	

[*] *p*-value was calculated with binary logistic regression

by examining the prevalence of HPV genotypes in 219 HPV-positive patients, they introduced that the most common types of HR-HPV are HPV 16, HPV 68, and HPV 51 and the most common types of LR HPVs include HPV 6, HPV 81, and HPV 62 [32]. As it is clear from the mentioned studies, the most common HR genotypes in Iran include HPV 16, HPV 66, HPV 18, HPV 31, HPV 52, HPV 39, HPV 53, and HPV 68. The highest frequency is associated with HPV 16 and HPV 52 which is consistent with our studies. By studying HPV prevalence and genotyping and knowing common genotypes in the community, it is possible to introduce multivalent vaccines that cover the common genotypes in the community. Today, HPV vaccination is to some extent part of the national immunization program in Iran, and the quadrivalent vaccine that prevents HPV 16 and 18 infection is now available, which can help reduce the health and economic burden of this disease [35, 42]. The most common non-vaccine HPV variants with significant geographic prevalence are HPV 39, HPV 52, HPV 68, and HPV 81. HPV 39 and HPV 51 also have significant prevalence in the analysis of unvaccinated populations in Finland [43].

In our study, the prevalence of HR-HPV was high, and this prevalence for HPV 35, HPV 39, HPV 45, and HPV 51 showed a significant increase in 2023 compared to 2022. It is important to note that HPV 35 and HPV 39 are not among the types targeted by commercial HPV vaccines, but are commonly identified in cases of cervical cancer in women [44]. This increase was 95% for HPV 39 and 73.33% for HPV 35, which may be a warning of increasing prevalence of these variants. Several studies have reported HPV 35 as the fourth most common HPV type in South African women with cervical cancer [45]. Current HPV vaccines do not target HPV 35. The addition of HPV 35 to the Gardasil®9 variants increases protection against HPV-associated disease in African American women and other community-shared genotypes [45].

The limited sample size and provincial level survey are important limitations of this study. This HPV prevalence rate may not reflect the distribution of HPV genotypes in the country. Another limitation is the lack of statistics on HPV prevalence in men. In this study, we only examined HPV prevalence in women. Also, demographic information of the participants such as marital status, occupation, and number of sexual partners were not available. Despite the study limitations, this study provides a general overview of the current status of HPV genotype distribution in southern Iran for the first time, which is useful for policy making.

Conclusion

In general, HPV 16, HPV 52, HPV 31, HPV 53, and HPV 66 genotypes are the most dominant. HR-HPV genotypes, and the highest prevalence is associated with the age group of 20 to 40 years. Cases in the younger age group increased in 2023 compared to 2022. The results also showed that the prevalence and incidence of multivariate is increasing in this province. The increase in cases of genotypes HPV 35, HPV 39 and HPV 51, which are not included in the formulation of common vaccines against HPV, leads to the conclusion that it is necessary to add these genotypes to the formulation of vaccines. This report is valuable for cervical cancer screening and provides local governments with valuable guidance for future targeted next-generation HPV vaccination. In particular, high-risk HPV infections should be the subject of close surveillance.

Abbreviations

WHO	World Health Organization
HPV	Human papillomavirus
HR-HPV	High-risk human papillomavirus
LR-HPV	Low-risk human papillomavirus

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Not applicable.

Authors' contributions

RA. AA. Data Collection; KA. BSH. HG. ES. ZGH. Manuscript writing; ZGH. KHA. Data analysis; KHA. HG. Supervision and Manuscript review. All authors reviewed the manuscript.

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Data availability

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

Declarations

Ethics approval and consent to participate

This research study was conducted retrospectively from data obtained for clinical purposes. The ethics approval was obtained from the Ethics Committee of Hormozgan University of Medical Sciences, Bandarabbas, Iran (approval number: HUMS.REC.1396.013). The Ethics Committee of Hormozgan University of Medical Sciences concluded that informed consent was not required because the data were appropriately anonymized and the study was retrospective.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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