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ORIGINAL ARTICLE

Comparing the distribution of common human papillomavirus genotypes among the population of Fars province in southwest Iran with the genotypes included in the available HPV vaccines

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Abstract

Background Given the strong association between high-risk HPV genotypes, such as HPV 16 and 18, and cervical cancer, this study aimed to compare the distribution of common HPV genotypes in the southwest Iranian population with those included in the available vaccines.

Methods Based on the sample quality, DNA was extracted from the biological samples of 8036 individuals included in the study using three different methods (automated instrument, column, and precipitation), and a total of 21 different HPV genotypes were detected using real-time PCR.

Results The majority of participants were women (> 99%), with a positive rate of HPV infection of 29.9%, in which high-risk genotypes were dominant in 84.9% cases. The highest rate of HPV infections was observed in the age ≤ 30 years (35.9%). HPV 6 and 16 were the most frequent low- and high-risk genotypes, respectively. Multi HPV infections were observed in 35% of positive samples and the highest cross infections were observed between HPV6 and 16. Co-infection with HPV 16 and 18 was observed in 21 positive samples (1%). Although vaccination is essential to reduce the outcome of HPV infections, such as cervical cancer, other frequently occurring high-risk genotypes are not included in the 9-valent vaccine.

Conclusion Since the association between cervical cancer and other high-risk HPV types rather than 16 and 18 has been less studied, investigating their pathogenicity in cervical cancer is recommended. Furthermore, the new generation of HPV vaccines should contain other frequently occurring high-risk genotypes beyond those currently covered in approved vaccines.

Keywords HPV · High-risk · Low-risk · Co-infection · Vaccine

Introduction

Human papillomavirus (HPV) is a DNA virus belonging to the *Papillomaviridae* family which is known as an agent for the most common sexually transmitted disease (STD). HPV is also responsible for about 4.5% of all human cancers including anal, cervical, oropharyngeal, penile, vaginal, and vulvar cancers, among them cervical cancer is predominant [1]. Analysis of virus structure revealed that HPVs typically contain eight expressed protein-coding open reading frames

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(ORFs) in their circular double-stranded DNA genomes with a size of approximately 8 kb [2]. Based on their expression timing, ORFs are named early (E) and late (L). HPV consists of 6E and 2 L ORFs [3]. Among them, E6 and E7 are considered oncoproteins that are responsible for degrading p53 and pRb, respectively [4], while L1 is a conserved region that reflects the diversity of HPV genotypes [5]. About 222 types of HPV have been reported until now [6]. However, only 14 types of them including HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV 66 and HPV68 have been reported to be associated with cervical cancer [7]. Therefore, most HPV infections are not carcinogenic and the immune system can clear nearly 80% of HPV infections from the body [8]. According to their roles in cancer development, HPV types can be classified into high- and low-risk genotypes. Of the high-risk genotypes, HPV16 and 18 are identified as the major inducers of HPV-related squamous cell carcinoma (HSCC) and cervical adenocarcinoma by changing the pathways associated with immune responses and cellular transformation [9]. To prevent HPV infections and consequently its attendant outcomes, the HPV vaccines were designed based on the virus-like particle (VLP) adsorbed to an aluminum adjuvant. There are currently different types of available vaccines such as bivalent, quadrivalent (4-valent), and nonavalent (9-valent). All the vaccines are highly effective in preventing infection with HPV types 16 and 18. The quadrivalent vaccine also prevents anogenital warts caused by HPV types 6 and 11. Population data analysis showed that the 4-valent HPV vaccine can significantly reduce the risk of invasive cervical cancer [10]. The 9-valent vaccine suggests extra protection against the other high-risk genotypes like 31, 33, 45, 52, and 58 [11].

Based on previous studies, the incidence rate of cervical cancer is rising among Iranian women, with 4.5 cases per 100,000 people, and an annual death rate of 9 cases per 100,000 women [12, 13]. This may be due to an increase in screening tests, higher HPV prevalence, and various risk factors such as marital status, age at marriage and first pregnancy, smoking, use of oral contraceptive pills, multiple sexual partners, family history, and multiple childbirths [14]. Thus, it seems that the frequency of HPV positivity rates, as well as alterations in the high-risk genotypes, especially HPV16 and HPV18, should be determined annually in each region. The present study aimed to evaluate the distribution of HPV common genotypes in a population from the Fars province in the southwest of Iran and compare the more frequent genotypes with those included in the HPV-approved vaccines.

Materials and methods

Study populations

As a cross-sectional study, from January 2021 to December 2023, 8,064 individuals were included, either referred for routine workups without any signs and symptoms, or presenting with signs and symptoms of HPV infection such as unusual vaginal discharge (thin, watery, and bloody discharge) and genital warts (vaginal, penile and anal). Unfortunately we did not have any information about the vaccination status in our study population. However, based on the HPV vaccine price, vaccination is not routine and its rate is very low in our country. Vaginal or tissue samples were collected by a gynecologist, and the collected sampling brushes in transport media were transferred to a referral diagnostic clinical lab (Farzanegan lab, Shiraz, Fars, Iran) for HPV genotyping. Given that Farzanegan lab is a referral center, in addition to Shiraz, the samples were also received from different cities within the Fars province in the southwest of Iran. A total of 8034 samples were collected from women and 30 from men. The samples were examined and analyzed as soon as they were received.

DNA extraction

Based on the quality of the specimens, different extraction methods were used to decrease false-negative results. Briefly, upon a centrifugation of the liquid base specimens at 15,000 rcf for 2 min, for specimens with adequate epithelial cell numbers, DNA was extracted using an automated DNA extraction instrument with the T200-32 kit (Zybio, China), specified for extracting viruses' genomes. For the highly mucoid and bloody samples or the samples with low cell numbers, column-based (Rastin Co., Iran) and precipitation-based (Amplisens, Russia) extraction methods were used, respectively. Briefly, upon centrifugation, 200 µl of the pelleted cells were used, and DNA extraction was performed according to the manufacturer's recommendations.

HPV genotyping

The types of HPV were determined using HPV genotyping kits (HPV QUANT-21, Russia) and performed on a Real-time PCR instrument (DTprime, Russia). This kit has been designed for in vitro diagnosis (IVD) of 21 HPV types including three low-risk (HPV 6, 11, 44) and eighteen high-risk (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) HPV types in human biological samples according to the manufacturer's instructions. Each well of 8-well strips contains an internal control to detect the amplification. Well number eight includes an internal control of

samples (SIC) to determine the quality and quantity of the extracted DNA. Based on the kit instruction manual, SIC < 4 is invalid and DNA was extracted from a new requested sample. To normalize the results, all extracted DNA samples with SIC ≥ 4 were included in the study.

Statistical analysis

To analyze the data, SPSS Statistics Version 27 (IBM), R programming software, and EPI Info were used. Using a non-parametric Chi-square test the association between age stratification and HPV frequencies was determined by EPI-info software (version 1.4.3, CDC). R programming package (version 4.3.3) was used to determine the frequencies of high- and low-risk HPV genotypes, co-, and cross-infection of HPV as well as to design the graphs [15]. *P* value < 0.05 was accepted as a significant level.

Results

Of 8,064 samples, 8,039 (99.7%) were from women (Mean ± SD; 35.59 ± 9.6 years old) and 24 (0.3%) were from men (Mean ± SD; 35.17 ± 12.8 years old). As shown in Fig. 1, 2,412 samples (29.9%) were positive and 5,652 (70.1%) samples were negative. Among the positive samples, 2,048 (84.9%) were positive for at least one high-risk HPV genotype, and 364 (15.1%) samples were positive for low-risk genotypes.

HPV genotypes distribution

HPV genotyping showed that genotype 6 was the most frequent, detected in 544 samples (22.6%). As shown in Fig. 2, among high-risk genotypes, genotype 16 was the most frequent, detected in 441 samples (18.3%), in contrast, genotypes 26 and 33 were each positive only in one sample (0.0004%), making them the least frequent. Among detected high-risk genotypes, seven HPV types (39%) were included in the approved 9-valent vaccine. Interestingly, following genotype 16, the frequencies of high-risk genotypes 53 (12.3%), 66 (9.99%), 51 (9.5%), and 39 (9.0%) were higher than those genotypes 52, 31, 18, 58, 45 and 33, which are included in the approved 9-valent vaccine.

The frequency of low-risk genotypes detected in the biological samples is shown in Fig. 3. Of the low-risk genotypes, genotypes 6 and 11 were the highest (22.6%) and the lowest (2.6%) frequent ones, respectively. In contrast to genotypes 6 and 11, which were included in the 4- and 9-valent vaccines, genotype 44, as the second low-risk frequent genotype (4.1%), is not included in the currently available vaccines.

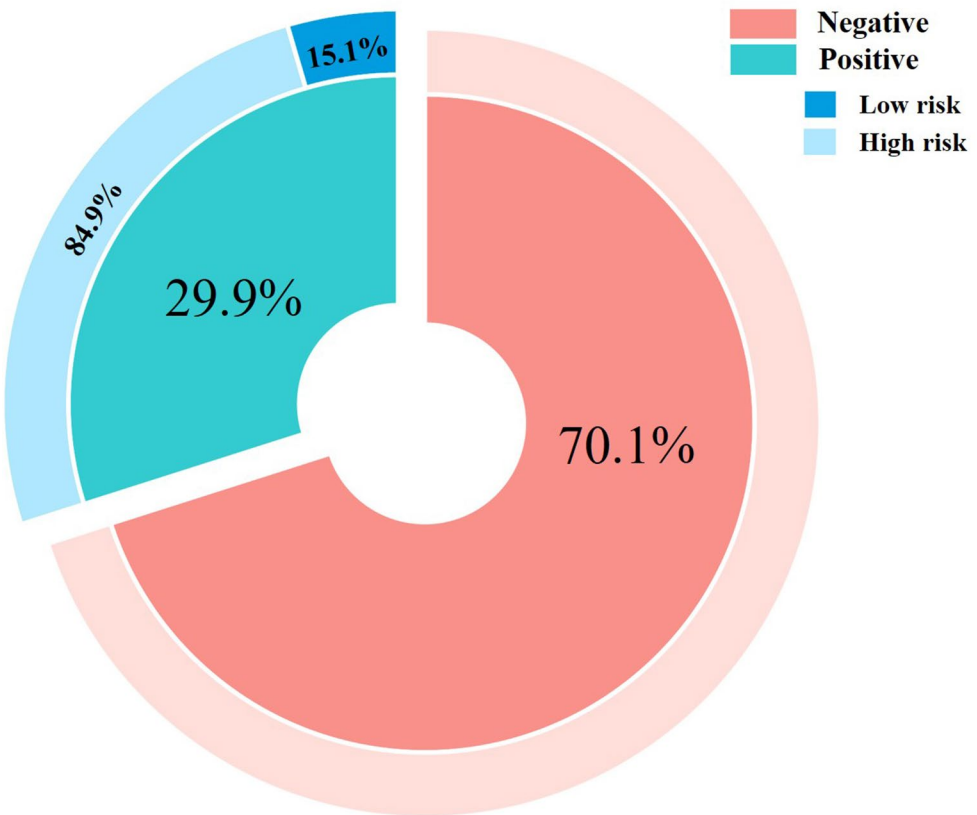

Fig. 1 The donut graph represents the frequency of positive samples as well as the distribution of samples with low- and high-risk genotypes

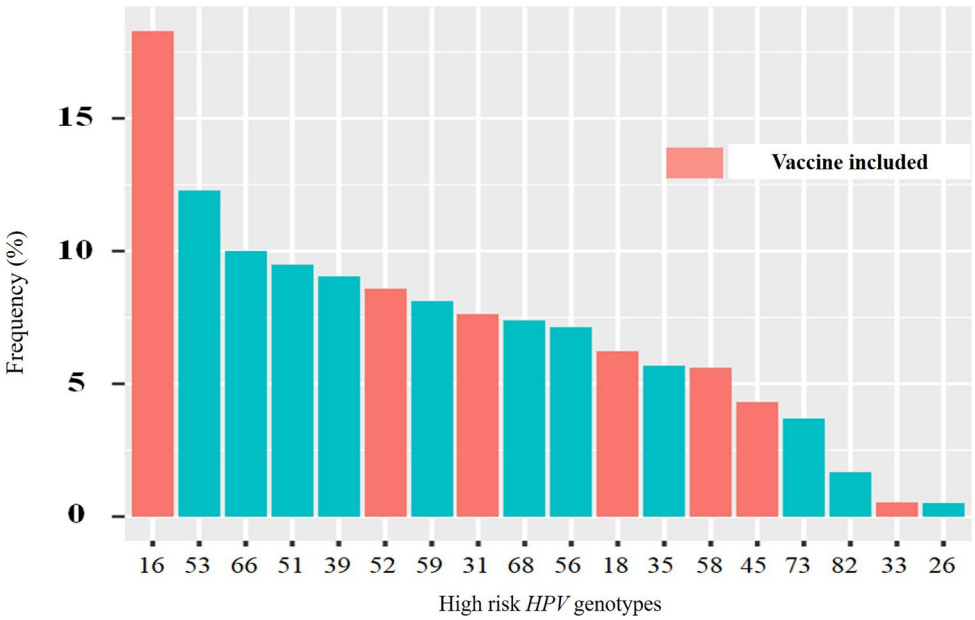

Fig. 2 The bars represent the frequency of high-risk genotypes in the biological samples. The red bars indicate HPV genotypes that are included in the approved HPV 9-valent vaccine

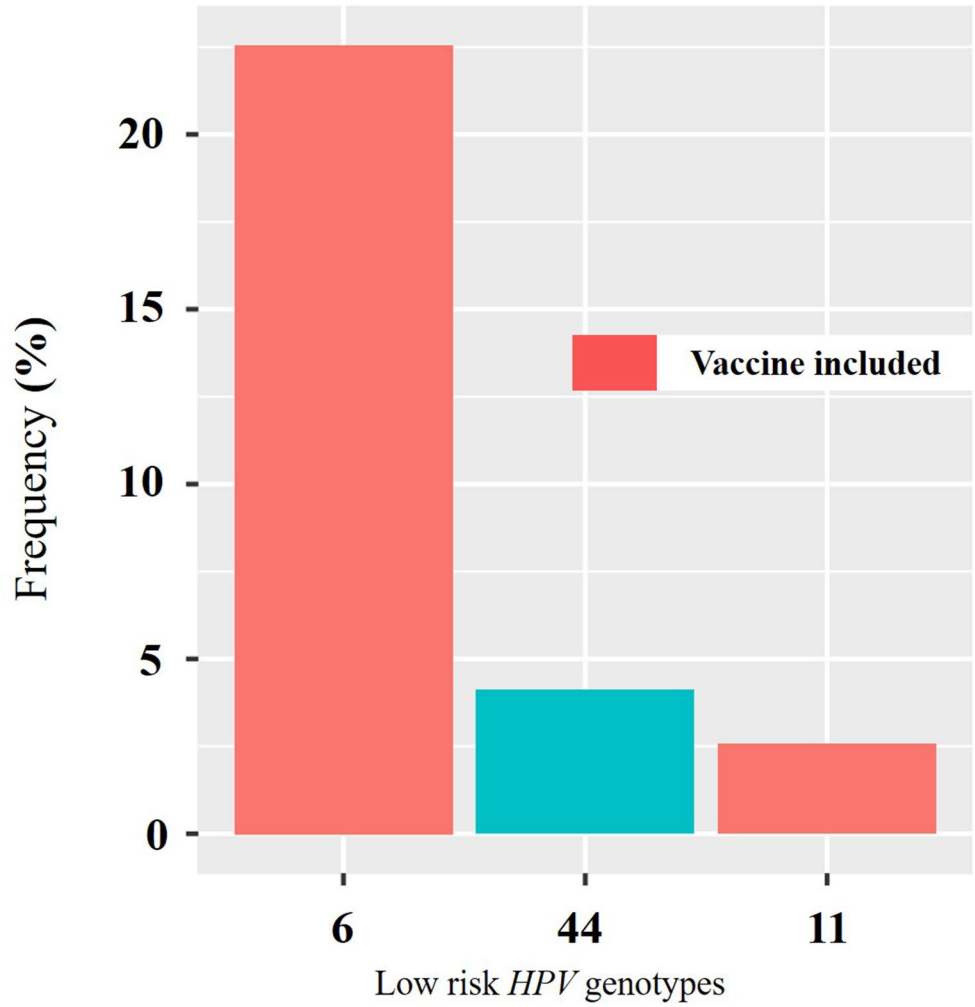

Fig. 3 The bars show the frequency of low-risk genotypes in the biological samples. The red bars show the genotypes included in the 4- and 9-valent vaccines

Table 1 The frequency of HPV positivity and co-infection rates in different age groups of the participants. P1 and P2 values were calculated based on the comparison of the positive rate and co-infection rates of each row with the sum of other rows. Bold numbers represent significant values

Group	Age (y)	Sample size	Positive (%)	Co-infection (%)	P1 value	P2 value
1	≤20	294	113 (38.4)	44 (15)	**0.001**	**0.047**
2	21–30	2407	858 (35.65)	332 (13.8)	**0.00001**	**0.00001**
3	31–40	3175	918 (28.9)	304 (9.5)	0.11	**0.0008**
4	41–50	1626	383 (23.56)	115 (7)	**0.0001**	**0.00009**
5	51–60	422	107 (25.36)	43 (10.2)	**0.035**	0.5
6	≥60	139	33 (22.02)	15 (10.8)	0.1	0.77

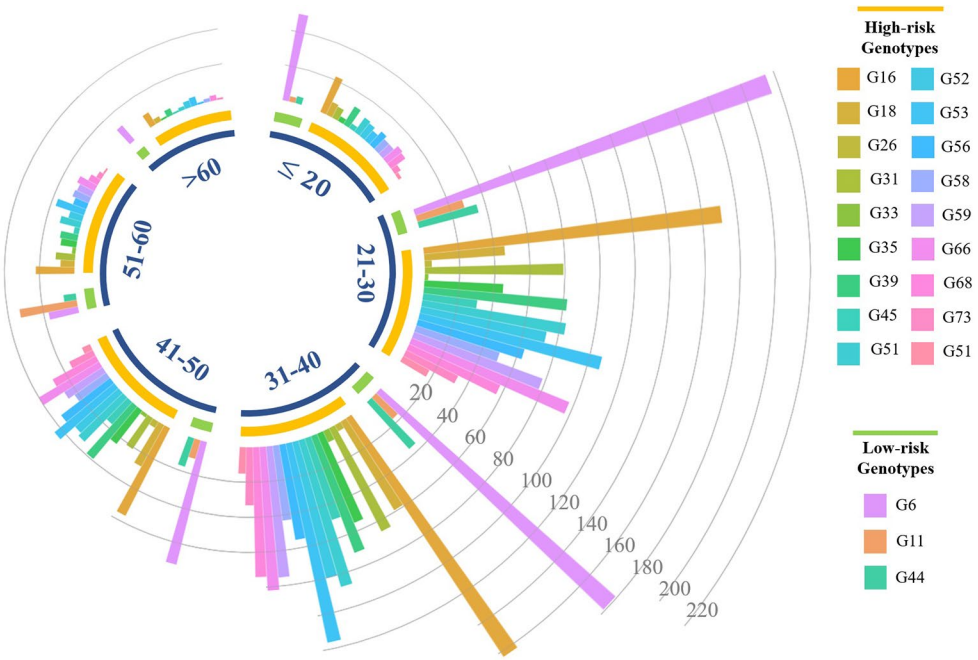

Fig. 4 Circular bar charts represent the frequency of high- and low-risk genotypes of HPV across different age groups. The numbers in the circle elucidate the age ranges (in years), while the numbers on the circles represent the absolute frequency of each genotype

Distribution of HPV in different age ranges

To determine the frequency of HPV in different age groups, we stratified individuals into six groups (G 1–6) based on their age: ≤20, 21–30, 31–40, 41–50, 51–60, and ≥60 years old.

As shown in Table 1, G3 and G6 had the largest and the smallest sample sizes, comprising 3175 (39%) and 139 (1.7%) of the total samples (8,064), respectively. The highest and lowest positivity rates were observed in groups 1 and 6, respectively. The results revealed that the positivity rate in groups 1, 2, 4, and 5 were significantly different from those in the other groups (Table 1).

According to age classification, the most frequent low-risk genotype in all age groups was HPV6, except for Group 5, where HPV11 was more prevalent than the other types. Among high-risk genotypes, HPV16 was the most frequent in all age groups (Fig. 4).

HPV co-infection

To determine the frequency of samples infected with one or more genotypes, results analysis revealed that most samples were infected just with one HPV type, even though infection with 2 to 10 different HPV types was also observed. Based on the results, 1,561 samples (65%) were infected with one HPV type and each co-infection with 8 and 9 HPV types was observed in only one sample. In addition, 2 samples were positive with 10 different HPV genotypes (Fig. 5). Detection of co-infection based on the different age ranges revealed the highest and the lowest co-infection in the age groups 1 (15%) and 4 (7%), respectively (Table 1; Fig. 6).

HPV genotypes cross-infection

We defined cross-infection based on the co-infection of each genotype with the others which is determined according to the cross tabulation of the frequency of each genotypes placed in the vertical and horizontal columns. To assess the cross-infection between different genotypes, data analysis revealed that the highest cross-infection rate was observed

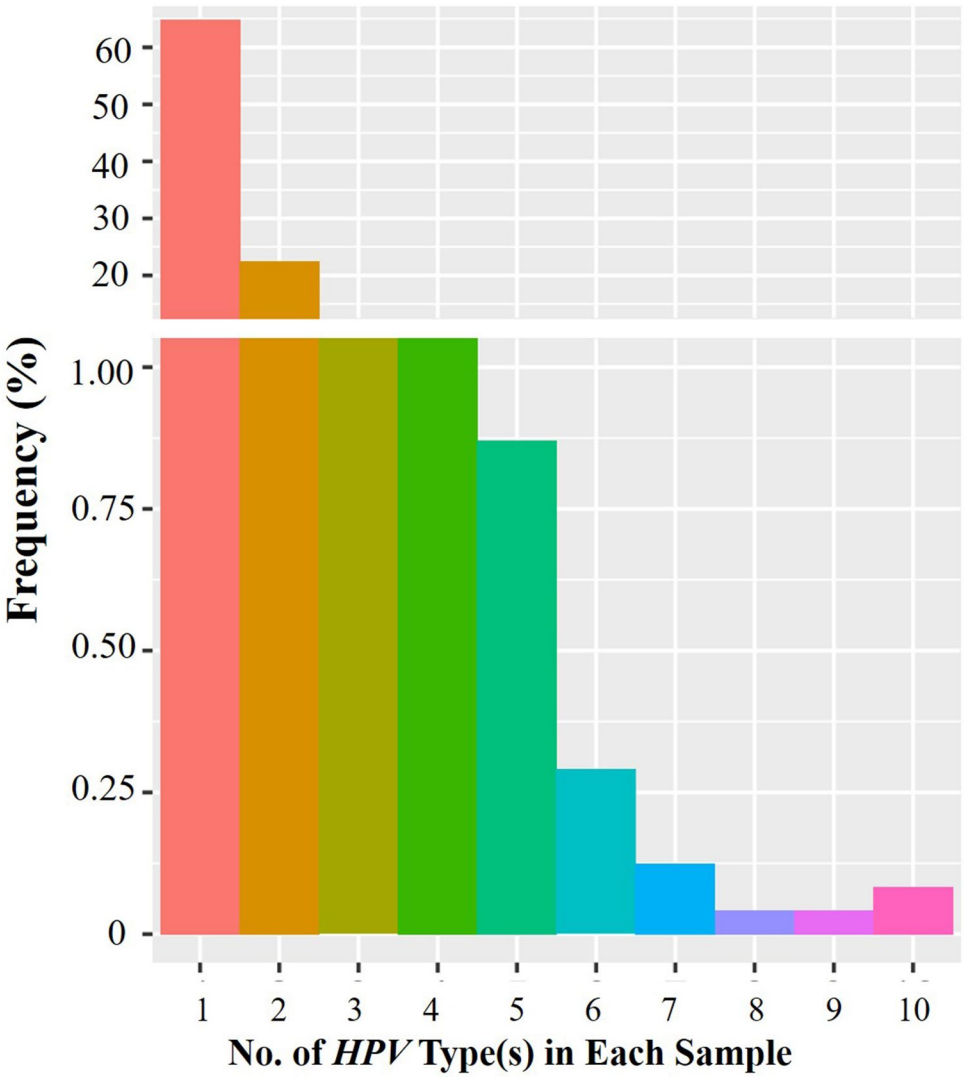

Fig. 5 The bars represent the frequency of samples infected with one to 10 different HPV genotypes

between genotypes 16 and 6 with a frequency of 52 (2.2%) of positive samples. Following genotype 16, the highest cross-infection of genotype 6 was observed with genotypes 66 and 53, respectively. Given its importance in the development of cervical cancer, cross-infection between HPV genotypes 16 and 18 was observed in 22 positive samples (1%). Because eight genotypes had a frequency less than 5% and their cross-infection with other genotypes caused the heatmap to enlarge and they could not be distinguished from each other due to color interference, therefore genotypes with frequency > 5% were included to draw heatmap in order to be more informative (Fig. 7).

Discussion

In the present cross-sectional study, the prevalence of HPV genotypes in the biological samples of a population from the southwest of Iran (Fars province) was assessed. The results showed a positivity rate of 29.9% in which the high-risk genotypes comprised the most genotypes in the studied population. To determine the prevalence of HPV genotypes, several studies were performed in different parts of Iran. Of them, a previous study from the northeast of Iran (2013 to 2018) on 567 biological samples including vaginal and urethral discharges, paraffin-embedded tissue, and genital wart specimen (fresh or fixed) reported a positivity rate of 33.6% for HPV infection [16]. Another study reported HPV infection prevalence of 49.5% in 10,266 samples (penile and anal biopsies from males and Dacron swabs or brush from the vagina and cervix of females) collected from different provinces of Iran between April 2011 and April 2016 [17]. In addition, a recent study from the capital city of Iran, Tehran, reported an HPV positivity rate of 53% in 5176 genital samples collected from 2017 to 2021 [18]. Also, another study from Iran, Tehran, was carried out from 2016 to 2018 on 12,076 biological samples and reported an HPV infection rate of 38.68% [19]. In our neighboring country, Iraq,

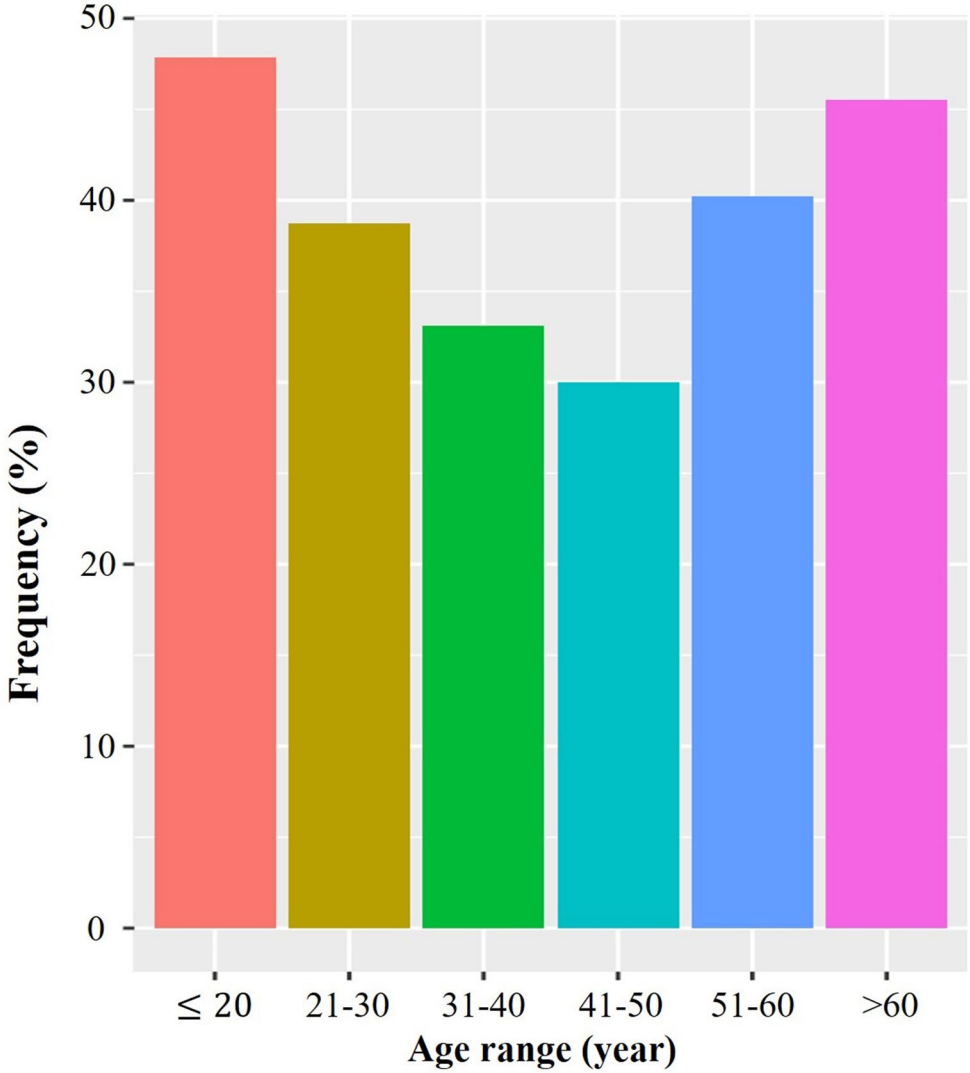

Fig. 6 The relative frequency of HPV co-infection based on the different age ranges

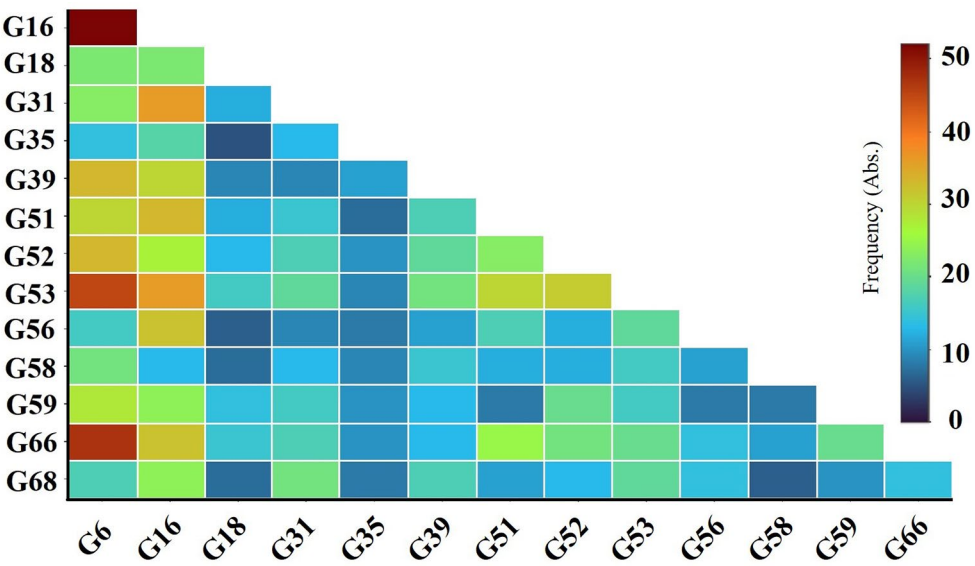

Fig. 7 The heatmap graph represents the cross-infection between different HPV genotypes with frequency > 5%

with which we share many religious ties, the positivity rate of HPV infection was reported as 20.9% for 320 samples collected from recruited patients with recurrent genital infections between January 2018 and September 2020 [20]. Another study reported an HPV-positive rate of 17.96% in 362 Iraqi women aged 15 to 50 years [21]. The discrepancy in results may also be attributed to differences in DNA preparation, sampling origins and preservation (e.g., FFPE, fresh biopsies, smears), and extraction methods (commercial kits versus in-house methods). In addition, increased community awareness of HPV infections and their possible complications, as well as vaccine availability and more intensive preventive behaviors, may also be reasons for the observed decline in HPV transmission.

The distribution of HPV genotypes revealed that samples with positive high-risk genotypes were more frequent than those with positive low-risk genotypes. Of the positive samples, the most frequent genotypes were HPV6 and HPV16, respectively. In concordance with our study, genotypes 6 and 16 were also reported as the most low- and high-risk genotypes, respectively, in other studies from Iran [16, 22, 23].

Geographically, comparing the distributions of HPV in our results with those from different continents and countries, the highest prevalence has been reported in the Asian, African, and European regions, respectively. Also, the prevalence of HPV infection in European areas revealed a lower rate of positivity in developed countries in comparison with developing countries [24–26]. Like our findings, a global study reported that high-risk genotypes are more frequent than low-risk ones [27–29]. Furthermore, in agreement with our results, a study from China, where we have a lot of travel and business dealings, reported a higher frequency of high-risk genotypes (19.2%) compared to low-risk ones (6.4%) [30].

Remarkably, we observed that genotypes 53, 66, 51, and 39 were the most frequent high-risk genotypes after HPV16. However, other Iranian studies reported different high-risk genotypes following HPV16, such as genotypes 52 [22], 66 [31], 51 [16], and 68 [32]. Given that the available 9-valent HPV vaccine is protective against high-risk genotypes 16, 52, 31, 18, 58, 45, and 33, as well as low-risk genotypes 6 and 11, and based on both our study and the other Iranian studies [15, 21, 30, 31], it seems that some more frequent high-risk HPV genotypes among Iranian population, such as 53, 66, 51 and 39, are not covered by the currently available 9-valent vaccine. In addition, in the present study, we observed that genotype 44 is the second most frequent low-risk genotype which is not included in the 9-valent vaccine. The exploration of the distribution of HPV genotypes in other countries revealed that in China, HPV genotypes 52, 58, 16, 51, and 56 were more frequent [33]. Another study from Beijing, China, reported that genotypes HPV 52, 58, 16, 39, and 51 were the most common high-risk genotypes [34]. Additionally, a study from Weifang, China, reported that HPV genotypes 16, 52, 58, 53, and 68 were more frequent. A report from Maputo city, Mozambique, showed that high-risk HPV genotypes 52, 35, 16, 53, 58, and 51 were more frequent [35]. Moreover, researchers from Shanghai, China, reported that high-risk HPV genotypes 52, 58, 16, 53, 51, and 81 were more prevalent [36] and a study from the Metropolitan Area of Naples, Italy, reported that genotypes 16, 31, 18, and 51 were the most frequent high-risk HPV types [36], suggesting incorporating other more frequent high-risk genotypes with worldwide distribution into new vaccine formulations which could significantly reduce the incidence of HPV infections globally, particularly in our country, Iran.

Investigating the association of HPV genotypes with age range showed that a higher frequency of HPV infection was observed in age groups younger than 30 years old, while in individuals older than 60 years, the prevalence rate of HPV infection was statistically reduced. In agreement with our study, another study [22] from Iran and studies from other countries [37–39] reported the same association between HPV prevalence and age ranges. In almost all of these studies, the highest frequency of HPV infection was observed in groups younger than 30 years old [22, 37–39]. The Centers for Disease Control and Prevention (CDC) recommends two schedules for HPV vaccination based on the age of vaccinated individuals. A two-dose series (0, 6–12 months) is recommended for most persons who initiate vaccination at ages 9 through 14 years, while a three-dose series (0, 1–2, 6 months) is recommended for those who initiate vaccination at ages 15 through 45 years, as well as for immunocompromised patients [40]. So, based on the CDC recommendations and the prevalence rate of HPV infection, HPV vaccination for individuals younger than 30 years old could be most effective in preventing high-risk age groups from high-risk HPV infections and associated cancers in the future.

A previous study [41] reported that co-infection with more than one HPV type is common in 20–50% of HPV-infected women, especially among young women. Infection with multiple HPV types is often considered a risk factor leading to the development of cervical cancer. In agreement with previous studies, we also observed a 35% co-infection rate in our study groups. Among them, co-infection with 10 genotypes was also detected in two samples, and co-infection with the very high-risk genotypes 16 and 18 was determined in 1% of the positive samples. Given the frequency of co-infection in our study and previous studies where more co-infection was observed between genotype 6, the most frequent genotype, and the high-risk genotypes 16, 66,

and 53, it seems that vaccination with a vaccine containing more than 2 (Cervarix) and 4 (Gardasil, 4vHPV) genotypes, such as the 9-valent vaccine, is essential for future vaccination strategies. In a large Chinese study, co-infection of genotypes 16 and 18 was similar to our findings, and authors also reported that co-infection of HPV16 with other high-risk genotypes occurred in 23.54% of cases [42]. Among multi-genotype HPV infections and their disease outcomes, it has been reported that the risk of high-grade squamous epithelial lesions increases with the number of HPV types (odds ratio for single, 2 to 3, and 4 to 6 HPV types were 41.5, 91.7, and 424, respectively), compared with HPV negative samples [43]. A recent study reported that TLR4 rs10759931 is protective against multiple high-risk HPV infections. In contrast, the haplotype ACAC (at the loci rs7873784, rs4986791, rs4986790 and rs4986791) in TLR4, which functions as a pathogen recognition receptor (PRR) on innate immune cells, increases the risk of infection with multiple high-risk genotypes [44]. This suggests that gene variants associated with immune responses can act as predisposing factors for multiple HPV genotype infections. Our study limitations include the fact that we selected the study population from individuals with and without symptoms who were referred to a subspecialty clinic for routine checkups. This suggests that, our sample may represent a higher-risk group instead of the general population, which could affect the observed prevalence of HPV in the community. In addition, there was no information available about Pap smear results and clinical manifestations in the HPV-infected individuals to compare with those who were HPV-negative. Furthermore, the status of cervical cancer in the patients was not clear. As the positive rate of HPV infection was higher (29.9%) in our study compared with the results obtained from other population-based studies (9.73% in Ethiopia [45], 13.22% in China [46], and 13.6% in the Canary Islands [47], future studies with larger sample sizes collected from healthy individuals in different regions of Iran are warranted to accurately reflect HPV prevalence and distribution.

Conclusion

In conclusion, we observed that HPV 6 and 16 were the most frequent low- and high-risk genotypes, respectively, and are providentially included in the available 4- and 9-valent HPV vaccines. However, other prevalent high-risk genotypes such as 53, 66, 51, and 39 in the Fars province population, located in the southwest of Iran, are not included in the current vaccine formulations. Therefore, it is recommended to incorporate these more frequent high-risk genotypes in future HPV vaccine formulations. Additionally, further studies are required to assess the impact of other high-risk HPV genotypes, beyond 16 and 18, on the development of cervical cancer.

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Author contributions MeK and AM conceived and designed the experiment, FM, HK, AR and GHK performed the experiments and collected the data, MeK and MaK collected the data and performed the analysis, and wrote the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate The authors have no conflicts of interest to declare. The authors declare that they have conducted the project ethically according to the World Medical Association Declaration of Helsinki. The present study was approved by the Ethics Committee of Fasa University of Medical Sciences with approval number IR.FUMS.REC.1403.050. No informed consent or other action from the patients was required because of the anonymity of the data analyses.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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