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Distribution of HPV genotypes in Mashhad, Iran: insights from a 2022–2023 study

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Abstract

Background Human papillomavirus (HPV), is one of the main causes of cervical cancer and also one of the most common sexually transmitted infections (STIs). HPV is responsible for almost all cases of cervical cancer and plays a principal role in causing other cancers including oropharynx, penis, larynx, oral cavity, anus, vulva, and vagina. The study aims to investigate the prevalence and distribution of HPV genotypes among patients referred to private laboratories in Mashhad, located in the northeast of Iran.

Methods and materials 428 samples including 382 females (89.3%) and 46 males (10.7%) between January 10, 2022, and February 11, 2023, in Mashhad, Iran were evaluated to detect HPV and determine its genotypes. Cervical swabs and urine samples were collected from females and males, respectively. Viral DNA was extracted by using a CedExtra purification kit (cedbio, Iran) and viral genotypes were identified with a High + Low Papillomastrip kit (Operon, Spain). Mann Whitney U test and Chi-square test were accomplished for statistical analysis.

Result From the total of 428 participants analyzed, the HPV test result was positive for 129 patients (30.1%) and negative for 299 people (69.9%). Among the participants, 115 female (30.1%) and 14 male (30.4%) were positive for HPV infection. The prevalence of HPV infection among the referring people was about 30%. The most common genotype identified was HPV-6 (10.3%), followed by HPV-16 (8.7%) and HPV-51 (7.7%), the second and third most common genotypes, respectively. Additionally, HPV-39 was detected at a frequency of 6.70%. HPV-11, HPV-61, HPV-91, and HPV-44 with a frequency of 1% were among the least genotypes identified among the patients.

Conclusion In line with the results of this study, the prevalence of HPV genotypes in both genders is 30%. The results likely reflect differences in the prevalence of high-risk HPV genotypes, that are less common. Also, HPV-6 and HPV-16 genotypes that are covered by the vaccine had a significant prevalence. On the other hand, with the prevalence of HPV-51 and HPV-39 genotypes in infected people who are not covered by the Gardasil (quadrivalent) vaccine, there is a risk of related cancers in the future.

Keywords Human papillomavirus (HPV), HPV prevalence, Viral genotyping, Sexually transmitted infections

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Introduction

Human papillomavirus (HPV) is a small circular double-stranded DNA genome, non-enveloped, and identified as the most common sexually transmitted infection (STIs) [1, 2]. HPV infection, which is the leading cause of cervical cancer, is estimated to exist in about 300 million females in the whole world, which 604,127 and 341,831 new cases and deaths were reported in 2020, respectively [3]. So far, 444 HPV genotypes have been detected, which are divided into 5 genera: alpha, beta, gamma, mu, and Nu papillomaviruses, with the oncogenic genotypes of HPV belonging to the alphapapillomavirus genus [4, 5]. In general, HPVs are divided into 2 main groups, which are: low-risk HPV (LR-HPV such as types 6, 11, 42, 43, 81, and 83) and high-risk HPV (HR-HPV such as types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) [6]. Consistent with a meta-analysis study, the global prevalence of HPV is 31% and high-risk HPV is 21%. Genotype 16 is known as the most rampant HR-HPV, followed by HPV-6 as the most common LR-HPV. The prevalence of HPV reaches its maximum in young people between the ages of 25 and 29 [7]. According to previous studies to evaluate and estimate the amount of HPV infections among Iranian females, the prevalence of HPV in this population was reported to be 23% [8].

HPV is a substantial contributor to cancers of the anus, vagina, oropharynx, vulva, and almost all cervical cancers [9]. Cervical cancer holds the position of the fourth most prevalent cancer globally, which is caused by HPV, causing 604,000 new cases and 342,000 deaths per year in females, and almost 100% of this cancer is attributed to HPV [10]. 60% of penile cancers, 70% of vulvar and vaginal cancers, 70% of oropharyngeal cancers, and 90% of cervical and anal cancers are occurred because of continuous infection with high-risk genotypes of HPV [2]. In Iran, the rate of cervical cancer is reported to be 1.90 per 100,000 in a research conducted in 2024 [11]. From 2001 to 2019, 72,421 new cases in females and 37,147 new cases in males of HPV-related squamous cell carcinoma were diagnosed in the United States [12]. Also, in 2020, 50,865 new cases of anal cancer have been diagnosed worldwide [13]. In a cross-sectional research that performed in Iran, anal HPV test were positive for 23.1% of females [14]. HPV is also known as a main cause of oropharyngeal cancers with a prevalence of 72.8% [15]. A study in Shiraz, Iran reported that 14% of 100 cases of oral cancer were infected with HPV [16]. Malignancies caused by high-risk HPVs and Genital warts by low-risk types can be easily prevented with effective FDA-approved vaccines (bivalent, quadrivalent, and 9-valent vaccines) [17]. Due to cervical screening and effective preventive vaccines against it, most cervical cancers are preventable [18]. The injection of at least

one dose of HPV vaccine was approved by the World Health Organization (WHO) in 2022, especially in low-income and middle-income countries for whom the consequences of cervical cancer are irreparable [19]. In order to develop an effective vaccination program and perform HPV-related malignancy screening, it is important to know the distribution of HPV genotypes in different regions [20, 21].

Latest research indicates that the human papilloma virus appreciably increases the risk of various types of cancer, especially cervical cancer. Vaccination and screening are among the main approaches to prevent and control this virus. Nevertheless, complete information about the frequency of HPV genotypes in different population groups, is not available. Consequently, this investigation was carried out to explore the frequency and spread of HPV genotypes based on genotype groups (high-risk, low-risk, and potentially high-risk) in individuals referring to the private laboratories of Mashhad in northeastern Iran in 2022–2023.

Methods and materials

Study population

In this cross-sectional study, 428 samples, comprising 382 (89.3%) females and 46 (10.7%) males, referred to private laboratories in Mashhad, Iran between January 10, 2022, and February 11, 2023, were assessed for HPV detection and determination of its genotypes distribution. Samples were collected by gynecologists and experienced laboratory personnel, which included tissues or cells in the cervix (Cervical) samples of cervical secretions for females and urine samples for males. Contributors who consented to participate in the study completed the informed consent form (IR.TUMS.SPH.REC.1402.317). Inclusion criteria included females who had abnormal cytological results and whose gynecologists had referred them to the laboratory for diagnostic tests and HPV genotyping or routine checkup. Also, males who had signs suspected of HPV infection or had recently had high-risk sex with an HPV-infected partner were included in this study. Among participants, the age range spanned from 17 to 70 years old. Exclusion criteria were those without consent of participation in the study.

Sample collection

Swab samples collected from females were transferred into a vial pre-filled with a liquid-based (Pap-Preps) preservative solution and kept at 2–8 °C until ready for analysis. Also, two urine samples were collected from males, including one first morning urine sample and another midstream urine sample. Finally, all samples collected from participants were stored at 4 °C, and lastly, 1 day after sampling, relevant laboratory tests were performed.

DNA extraction and PCR

All steps before amplification, DNA extraction, and identification of HPV genotypes were executed in the molecular genetics department of the laboratory, in accordance with the protocols specified by the quality control manager and with the administration of RCCV (Research Center of Clinical Virology). HPV-DNA was extracted with a CedExtra purification kit (cedbio, Iran) according to the manufacturer’s guidelines. In the next step, the HPV genotype was detected and determined by using the High + Low PapillomaStrip kit (Operon, Spain). This kit can detect 19 high-risk genotypes including HPV-16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82 as well as detect 18 low-risk genotypes including HPV-6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 70, 71, 72, 74, 81, 83, 84, and 91.

Statistical analysis

Frequency, median, average and standard deviation were used to describe the data in this study. Mann Whitney U test was performed to test the equality of age and the Chi-square test was performed to test the equality of gender in HPV-positive and HPV-negative groups. A significant level was considered as 0.05. The statistical package IBM SPSS-27 was applied for analysis.

Result

A total of 428 subjects were analyzed, among them 382 (89.3%) were female and 46 (10.7%) were male. The mean and standard deviation of age were 34.9 and 8.83, respectively. Out of 428 participants, the HPV test result was positive for 129 (30.1%) and negative for 299 (69.9%). Wald’s 95% confidence interval for the portion with positive test result was (25.4%-34.8%). Among female participants, 115 (30.1%) were identified positive for HPV infection (Wald’s 95% CI: 25.4% to 34.8%) and 267 (69.9%) were reported negative, while 14 (30.4%) were reported positive (Wald’s 95% CI: 17.1% to 43.7%), and 32 (69.6%) were negative for HPV infection among male participants. Among the people who referred to the laboratories, the prevalence of HPV infection was about 30%. HPV infection was not related to gender, statistically (Pearson Chi-Square statistic=0.002, p-value=0.963) Table 1. The mean, standard deviation, and median age

Table 1 The frequency and distribution of HPV in total and by gender

HPV result Gender	Positive	Negative	Total
Female	115 (30.1%)	267 (69.9%)	382 (89.3%)
Male	14 (30.4%)	32 (69.6%)	46 (10.7%)
Total	129 (30.1%)	299 (69.9%)	428 (100%)

were reported in HPV positive and negative participants, followed by the mean age in the HPV negative group was slightly higher, and this difference was significant (Mann–Whitney Test Z score= -1.98, p-value=0.047) (Table 2).

Distribution of HPV genotypes

Consistent with the genotypic groups among HPV-positive individuals, 81 patients (49.4%) had at least one high-risk genotype, 48 patients (29.2%) had at least one low-risk genotype, and 35 patients (21.3%) had at least one potential high-risk genotype. The results showed that the males examined in this study only had low-risk genotypes. Among the participants, 101 (78.3%) had only one group of genotypes (low-risk, high-risk, or potential high-risk) (Fig. 1). 21 patients (16.3%) had a combination of two genotype group, while only 7 patients (5.4%) had three genotype group.

Among 89 people (69%) with one genotype, 75 (84.3%) were female and 14 (15.7%) were male. Also, 40 patients (31%) with several HPV genotypes were identified, none of them were male. Among females with infection, 25 were identified as having two genotypes, and the highest number of genotypes identified among patients was 5 genotypes, which were identified in 3 females (2.3%). The most frequent genotype among the identified genotypes is HPV-6 (10.30%), followed by HPV-16 (8.7%), HPV-51 (7.7%), and HPV-39 (6.7%) among the four genotypes. On

Table 2 Age in HPV infection groups

Age groups	Mean (SD)	Median	Min	Max
HPV positive	33.7 (8.71)	33	17	63
HPV negative	35.4 (8.85)	35	17	70

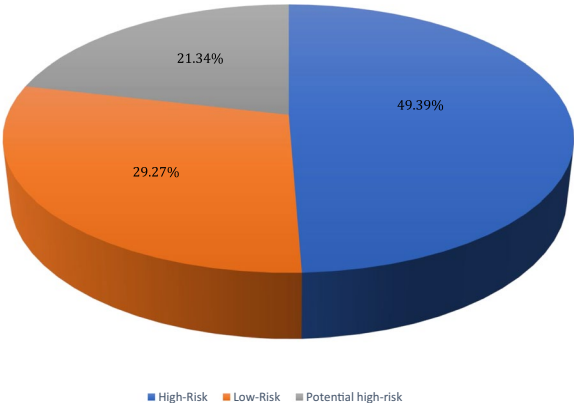


Fig. 1 Schematic frequency and distribution in terms of percentage of genotypic groups (High-risk, Low-risk, or potentially high-risk) identified in infected people

the other hand, HPV-11, HPV-61, HPV-91, and HPV-44 with a frequency of 1%, and HPV-18, HPV-40, HPV-84, HPV-83, and HPV-67 with a frequency of 0.5%, were among the lowest identified genotypes (Fig. 2).

Discussion

Our study reveals several significant findings regarding HPV infection and genotype distribution among the participants in Mashhad. We found that 30.1% of the 428 subjects tested positive for HPV, with no significant gender differences in infection rates (p -value=0.963). The mean age of participants was 34.9 years, with HPV-positive individuals having a slightly lower mean age compared to those who were HPV-negative, and this difference was statistically significant (p -value=0.047). The distribution of HPV genotypes among the positive cases showed that HPV-6 was the most prevalent (10.3%), followed by HPV-16 (8.7%) and HPV-51 (7.7%). Notably, HPV-39 was present in 6.7% of the cases. Our analysis also revealed that among HPV-positive individuals, 81 patients (49.4%) had at least one high-risk genotype, 48 patients (29.2%) had at least one low-risk genotype, and 35 patients (21.3%) had at least one potential high-risk genotype. Males in this study exclusively had low-risk genotypes, while females had a broader range, including high-risk types.

Regardless of age, marital status, or socioeconomic status, unpublished data from Iran shows that during the past ten years, the number of sexually active Iranians

with genital tract HPV infection has rapidly increased [22]. Lack of sex education for youth and restricted availability to the HPV vaccine in the nation are the two main causes of this notable increase. Likewise, an early onset of sexual activity, a shift in sexual behavior, having numerous sexual partners, and insufficient preventive measures have all been linked to the markedly elevated incidence of HPV infections in many countries [23].

In line with the results of our study, which reported the prevalence of HPV in infected females was 30.1%, another study conducted in Tehran on 12,076 females reported an overall HPV infection prevalence of 38.68% [24]. Similarly, a study from northeastern Iran found that 35% of females were infected with HPV [25]. These data highlight the significant prevalence of HPV among Iranian females and underscore the need for more preventive measures effective vaccination in this population. In contrast to the 30.4% prevalence of HPV in males reported in our study, other studies have found higher prevalence rates in males, at 54% and 55.7%, respectively [25, 26]. In this study, 78.3% of the participants tested positive for one of the HPV genotype groups (high-risk, potentially high-risk, or low-risk), of which 49.4% had the HR-HPV genotype. Meanwhile, a study performed in the west of Iran reported a 69% prevalence of high-risk HPV [21]. Additionally, previous research in Mashhad found that 6.2% of patients ($n=75$) had high-risk HPV genotypes [27]. Moreover, a study conducted in China in 2020, showed that infection with high-risk HPV

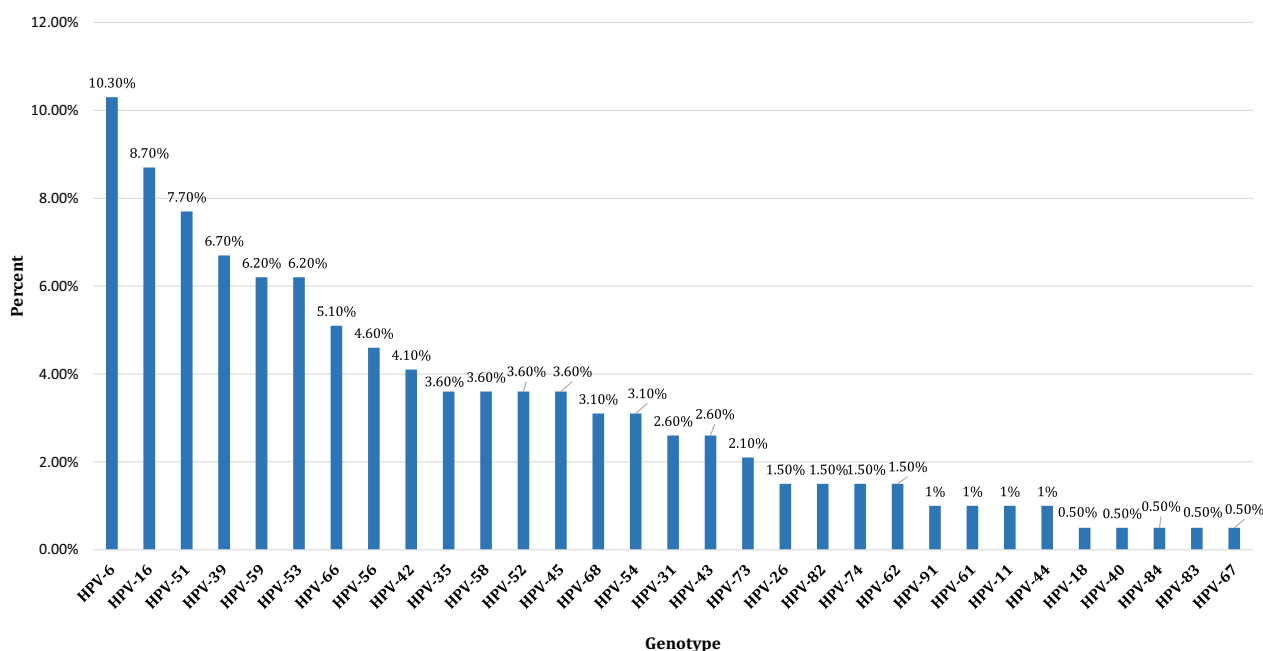


Fig. 2 Distribution and frequency by genotypes observed in HPV-positive patients in both genders

genotypes (single or multiple genotypes) was 8.80% [28]. In addition, another study in Turkey in 2021 showed a significant prevalence of multiple infections with high-risk HPV genotypes with a prevalence of 40.7% [29]. Our analysis found that HPV-6 (10.30%), HPV-16 (8.70%), HPV-51 (7.70%), and HPV-39 (6.70%) had the highest frequency, whereas HPV-40, HPV-84, HPV-83, HPV-67, and HPV-18 (0.5%) had the lowest frequency. Consistent with our findings, in a study performed between 2011 and 2016 in Iran, HPV-6 prevalence was 66.5% and 26.1% in males and females, respectively [30]. Similarly, another study in Iran recognized HPV-6 as the most common genotype among participants, with a prevalence of 9.3% [31]. Consistent with the findings from our study, where HPV-16 was the second most common genotype identified (8.70%), another study conducted in Iran between 2022 and 2024 reported HPV-16 as the most prevalent genotype, accounting for 11.7% of cases [32]. Although HPV-18 was identified as a genotype with a low prevalence (0.5%) in this study, it was among the most common high-risk HPV genotypes in the study conducted between 2015 and 2020 in Mashhad and the study performed in 2019–2020 in Tehran, with a prevalence of 2% and 17.4%, respectively [27, 33].

These particular observations in Mashhad may be attributed to several reasons. As the capital of Khorasan Razavi province in northeastern Iran, Mashhad stands as the country's second most populous city after Tehran. This prominence results in patients from other cities being referred to Mashhad's medical facilities. Additionally, the city attracts millions of pilgrims annually from across Iran and other Muslim nations, which may also contribute to the findings observed in this study. Another significant contributing factor is the extremely low rate of HPV vaccination. In Iran, HPV vaccines are distributed by private entities, with no coverage or financial support from insurance companies. As a result, many individuals cannot afford the vaccine, making it inaccessible to a large portion of the population [34, 35]. The high cost of commercially available HPV vaccines, combined with a lack of awareness among women about the risks of HPV infection, has led to this low vaccination coverage. Furthermore, the vaccine is not part of Iran's national immunization schedule [36]. The challenge is compounded by the presence of HPV genotypes like HPV 51, one of the most common HR types identified in this study, against which the currently available vaccines, such as Gardasil®, may not offer adequate protection. To address these issues, it is crucial to implement cervical cancer screening programs for early detection of HR-HPV types in Mashhad and to include HPV vaccination in national prevention programs, with the government covering the vaccination costs.

It's important to highlight that our study has several limitations. The limitation of our study is that since the HPV typing test is not available in all parts of the country and is expensive, it is impossible to properly compare the genotypes in our study divided by cities. In addition, larger-scale studies with more variables and larger populations are needed to acquire more precise results regarding HPV prevalence. The results of our study can be generalized to low- and middle-income countries, where screening and vaccination strategies should be more sensitive, and proper management should be done according to the genotypes in the rotation. Another limitation of our study is the potential for selection bias, as the data were collected from individuals referred to private laboratories. This specific population may not be fully representative of the general population, as individuals visiting private laboratories might differ in terms of socioeconomic status, health-seeking behavior, or other demographic factors. These differences could influence the findings and limit the generalizability of our results. Therefore, the outcomes of this study should be interpreted with caution, and further research is recommended to validate these findings in a more diverse and representative population. As well, a potential issue in our study is the inconsistency in HPV detection rates due to the different sample collection methods employed for females and males. Cervical swabs were used for females, whereas urine samples were collected from males. These differing methods may not be equally effective in detecting HPV, which could result in discrepancies in detection rates between genders. It is crucial to note that the laboratories involved in the study followed specific protocols that we could not alter. As a result, our analysis was based solely on the data provided by these laboratories, and this methodological difference should be considered when interpreting the findings.

Conclusion

In conclusion, the findings of our research show that the prevalence of HPV genotypes is 30% in both sexes. For example, although a significant prevalence of the HPV-6 genotype was seen in both sexes, another low-risk genotype (HPV-11) was not extensively detected among the participants. On the other hand, although the HPV-18 genotype was among the 5 genotypes that had the lowest prevalence, HPV-16, HPV-51, and HPV-39 genotypes were among the most common high-risk genotypes identified among the participants. These results probably show the difference in the prevalence of high-risk genotypes that are less common in this city. The findings of this study still emphasize the continuation of further investigations regarding the prevalence of HPV. Determining the prevalence of high-risk genotypes is critical

for cancer prevention and improving screening strategies. Furthermore, the high prevalence of HR-HPV genotypes, especially the genotypes not covered by the vaccine, highlights the need to create and improve targeted educational programs to use safer sex methods and encourage the general population to promote vaccination.

Author contributions

A.L, I.R, M.P: Supervision, Review and editing, Investigation. A.F,T,F, M.MB, H.P, Z.S, O.A, M.N, K.H, S.N: Methodology, Writing original draft. S.N, M.A, S.S: Investigation.

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Availability of data and materials

Data will be made available on reasonable request from corresponding author.

Declarations

Ethics approval and consent to participate

Ethics approval has been obtained from Tehran University of Medical Sciences, and written consent to participate has been received from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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