International Journal of Infectious Diseases 111 (2021) 295–302

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Prevalence and Genotype Distribution of Human Papillomavirus Infection among 12 076 Iranian Women ☆

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ARTICLE INFO

Article history:
Received 9 June 2021
Revised 27 July 2021
Accepted 28 July 2021

Keywords:
Cervical cancer
Human papillomavirus
Iranian population
Genotype distribution

ABSTRACT

Introduction: Human papillomavirus (HPV) infection is one of the major health concerns of women in developing countries. This study gives an insight into the prevalence and genotype distribution of HPV infection and compares it with Pap smear results among Iranian women.

Methods: In this study, 12 076 Iranian women underwent routine examination from November 2016 to November 2018 using HPV Direct Flow CHIP System for HPV DNA typing. Cytology was undertaken for 5138 samples.

Results: Overall HPV prevalence was calculated at 38.68%. The most frequent HPV types were HPV 6, 16, 11, 62/81, 52 and 54. The most high-risk HPV (HR-HPV) types were HPV 16, 52, 18, 39, 31 and 51. These 2 groups represent approximately half of all HPV types detected, 47% and 55%, respectively. Among individuals who underwent cytological tests, 135 individuals (2.63%) were cytologically positive. In this group, 81 individuals (60%) were HPV positive, 62 (76%) of whom were HR-HPV positive, most frequently with HPV 16 (34%).

Conclusion: This study highlights the urgent need for public education and early diagnosis using HPV screening tests to prevent cervical cancer.

Introduction

Cervical cancer (CC) is the third leading cause of cancer among women worldwide and the 16th lead cause of female cancer among Iranian people (Taebi et al., 2019). Approximately 570 000 new cases and 310 000 deaths of CC are estimated annually worldwide (Wang et al., 2019). Viral infections, especially human papillomavirus infection (HPV), which is recognized as one of the most common sexually transmitted infections worldwide, are now one of the known risk factors for CC (Nishimura et al., 2021), i.e., CC is by far the most frequent HPV-related disease (Xing et al., 2021). More than 200 genotypes of HPV have been identified so far; approximately 40 may infect genital areas (Wang et al., 2015). They can be classified into either oncogenic, also called high-risk HPV (HR-HPV), or non-oncogenic—low-risk HPV (LR-HPV)—groups based on their associations with malignant or benign proliferative lesions (Al-Shabanah et al., 2013, Shafaghi et al., 2013). Although most HPV infections are transient and do not cause serious dis-

☆ Running title: Genotype distribution of HPV among Iranian women

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https://doi.org/10.1016/j.ijid.2021.07.071

ease, some persist for several years and may have great potential to cause tumor malignancy (Jalilian et al., 2017, Kamal et al., 2021).

Chronic infection with HR-HPV subtypes, found in more than 99% of all cervical squamous cell carcinomas, is the major risk factor for the disease and is associated with cervical intraepithelial neoplasia (CIN) (Hasanzadeh et al., 2019, Xue et al., 2021). The oncogenic activity of HR-HPV types is imputed to the random introduction of the virus genomic DNA into the host genome (Al-Shabanah et al., 2013, Liu et al., 2021). HR-HPVs are associated with high-grade lesions and CC invasiveness (Mchome et al., 2021). HPV-16 and HPV-18 are the most important types, explaining the cause of ~70% of CC cases (Herraez-Hernandez et al., 2013b). The most frequent LR-HPV types are HPV-6 and HPV-11, commonly giving rise to benign warts (Jalilian et al., 2017). It has been identified that primary prevention can be achieved by injecting HPV vaccines that prevent precancerous lesions and CC.

A growing body of research has investigated the prevalence and genotype distribution of HPV in different geographical regions, including Iran. It has been shown that the incidence and mortality rate of CC varies, depending on geographical location, which in turn can be chalked up to differences in healthcare education, prevention, and screening methods (Yeo-Teh et al., 2018). These underscore the importance of such studies in public healthcare systems.

Evaluation of HPV genotype prevalence is necessary for healthcare and vaccination programs. Pap smear, screening for HR-HPV, and colposcopy-based testing can effectively decrease HPV-associated CC development (Olusola et al., 2019). Unfortunately, HPV prevalence data are not yet clearly available for the total population of Iranian women (Jalilian et al., 2017). To help fill this gap, we determine the prevalence of HPV subtypes in 12 076 Iranian women.

Methods

Study design and participants

This cross-sectional study was conducted for 24 months (from November 2016 to November 2018) using a total of 12 076 genital samples from Iranian women (aged from 18 to 71) who were referred to DeNA laboratory, Tehran, Iran, for routine CC screening. The women were referred by gynecologists, urologists, and dermatologists for various reasons, including physical examination, suspicious infertility, vaginitis, cervicitis, undiagnosed abdominal pain, genital warts, and cervical intraepithelial neoplasia. Although the screening of people suspected and/or at–risk of HPV infection was brought to the attention of health authorities in Iran, no particular public health screening has been initiated. Hence, the specialists who dealt with these patients usually refer them based on their symptoms to molecular medical diagnostic laboratories that usually use 'co–testing' or 'reflex testing'; in the former test, the Pap results are obtained regardless of HPV test, the latter testing is performed sequentially.

For our study, the following inclusion criteria were used: (i) the participant should have been sexually active; (ii) they should be >18 and <71 years; (iii) from the Iranian population; and (iv) interested in participating in the screening program. The exclusion criteria were: (i) pregnancy, (ii) a positive history of hysterectomy, trachelectomy, cervical intraepithelial neoplasia grade 2 or higher, malignant tumors, and cervical lesion treatment. Only participants who met these criteria and grasped sufficient verbal information about the significance of HPV testing to provide informed consent were included in the study. All participants were of Iranian descent and all were told to refrain from sexual activity and avoid washing their genitals for 48 hours before sample collection.

Data collection

To facilitate collecting adequate information about sociodemographics and sexual habits, and other germane risk factors of HPV infection, all participants filled in a standardized questionnaire during a face-to-face interview. Quality control of HPV genotyping included the following issues: sample quality requirements, test quality requirements, test report entry and issuance, and molecular diagnostic item analysis performance standards. The sample container was a disposable HPV collection tube.

After the cervix uteri were fully exposed by a vaginal speculum, a cytobrush was used to collect the cells at the junction between the endocervix and the ectocervix. The cells were stored in a preservative solution according to the manufacturer's instructions. Cervical cells were transported and stored at ambient temperature (10–30°C) for a maximum of 5 days until further manipulations. Cervical cytology smears were analyzed by a cytopathologist who was unaware of participants' HPV DNA test results. According to the relevant guidelines (Saslow et al., 2007, Sawaya and Smith-McCune, 2007), after providing HPV DNA test and cytology results, participants with atypical squamous cells of undetermined significance (ASCUS) or positive HR-HPV were referred to gynecologists for further examinations, e.g., colposcopy.

Blatt *et al.* have proposed that routine HPV tests performed with Pap test—also known as "co-testing"—are better protection than HPV-only, Pap-only, and reflex testing in women aged 30 to 65 years (Blatt et al., 2015). Herein, 5138 patients had been referred us for co-testing or reflex testing whereby they had been identified with HR-HPV and referred for further examination, i.e., cytology testing.

HPV DNA extraction, PCR amplification, and genotyping

After obtaining smeared cell slides for ThinPrep, samples were subjected to automated DNA extraction by ZP02003 MagPurix Viral Nucleic Acid Extraction Kit (Zinexts Life Science Corp., New Taipei, Taiwan) according to the manufacturer's instruction. The PCR reaction was performed at a final volume of 40 µL, including PCR mix, Hot Start DNA Polymerase, Uracil-DNA Glycosylate, and DNA in PCR tubes of 0.2 mL. Amplification conditions and reverse hybridization procedures were performed according to the HPV Direct Flow CHIP for hybriSpot 12 manufacturer's guidelines. The assay included the amplification of a 268 bp fragment of the human beta-globin gene (internal control) and a 150 bp fragment of the HPV L1 region (GP5+/GP6+). The reverse dot blot hybridization and read-out of the results were then performed in the e-BRID System™ (Master Diagnóstica, Granada, Spain). This PCR-based method can identify 36 HPV genotypes including 18 HR-HPV or putative high–risk 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).

To carry out the HPV test, specimens were analyzed by the HPV Direct Flow CHIP System for HPV DNA typing. The 5138 samples were screened using liquid-based cytology. By obtaining HPV DNA test and cytology results, women with ASCUS or worse, or positive HR-HPV DNA were referred to a gynecologist for further follow-up or colposcopy examination.

Statistical analysis

Chi-squared, Fisher's exact test, and Mann–Whitney U test were used to compare categorical variables. Proportions and odds ratio estimations were reported with their 95% CIs. Sample sizes, HPV prevalence and genotypes, and single and multiple HPV infection profiles were also described. Statistical analysis was performed by SPSS version 26.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism

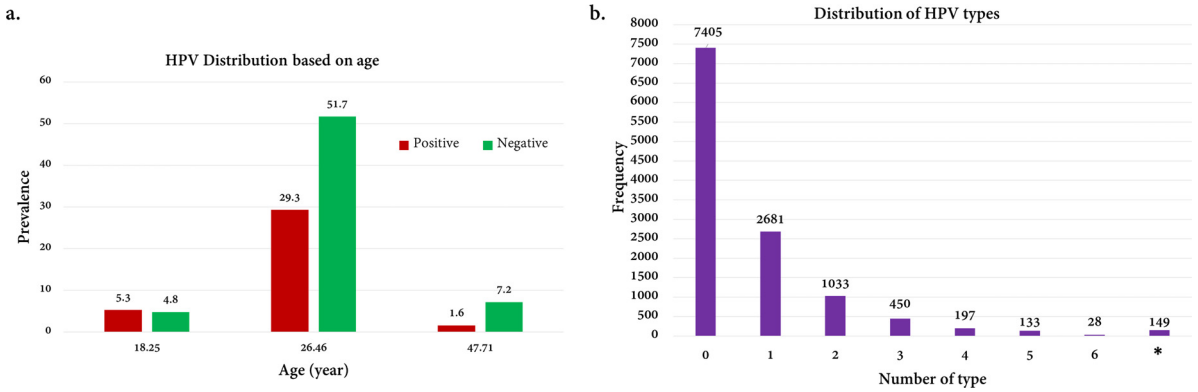

Figure 1. a) Distribution of human papillomavirus (HPV)-negative and HPV-positive participants based on their age. A substantial number of patients were determined in the second group (26–46 years old women). **b)** The number of negative cases and individuals with single and multiple HPV infections. An asterisk (*) indicates individuals were detected with other types of HPVs which include types other than types detected by this method.

Table 1
Frequency and prevalence of each type of human papillomavirus (HPV) infection—single and multiple types—in participants. Seven groups were considered to show the exact type of HPV infection.

Genotype	Frequency	Prevalence
Single	2681	57.4%
Double	1033	22.11%
Triple	450	9.63%
Quadruple	197	4.22%
Quintet	133	2.85%
Sextuple	28	0.6%
Other types*	149	3.2%

* Participants who were detected with other types of HPVs (other than types detected by this method).

v.7.0 (GraphPad, San Diego, USA). *P*-values <0.05 were considered to be statistically significant.

Results

Prevalence of HPV according to age parameter

A total of 12 076 samples were collected and screened for HPV genotypes. The samples were divided into 3 groups based on the age parameter (18–25, 26–46 and 47–71 years). While the majority of referred individuals (81.1%) were in the 26–46 years old group, the highest HPV prevalence was determined in the first group (18–25 years old women) (**Figure 1a**).

Prevalence of single and multiple HPV infection

As shown in **Table 1**, single infection (2681 cases, 57.4%) was the most common form among the participants. Furthermore, among multiple HPV infections, the prevalence of coincided HPV infections decreased significantly as the number of infected genotypes increased, with 1033 (22.11%) patients with double-infection, 450 (9.63%) with triple-infection, 197 (4.22%) with quadruple-infection, 133 (2.85%) with quintet-infection, and 28 (0.6%) patients with sextuple-infection. In total, 149 (3.2%) individuals were detected with other types of HPV (**Figure 1b, 2a, 2b**).

HPV prevalence and genotype distribution

The details of genotype distribution of HR-HPV and LR-HPV are shown in the **Table 2** and **Figure 3**. Ten common HR-HPV genotypes were as follows: HPV 16 (552, 16.98%), HPV 52 (286, 8.8%), HPV 18 (250, 7.69%), HPV 39 (248, 7.63%), HPV 31 (242, 7.45%), HPV 51 (213, 6.55%), HPV 45 (183, 5.63 %), HPV 68 (180, 5.54%), HPV 66 (171, 5.26%), and HPV 58 (157, 4.83%). HPV 16 was the most common high-risk genotype among all participants, while HPV 26 was the least frequent. Furthermore, HPV 6, 11 and 62/81 were the top 3 most prevalent genotypes among LR-HPVs.

HPV infection rate and prevalence

The HPV infection rate was 38.68%, of which high-risk, low-risk and high- and low-risk multiple infection rates were estimated at 17.41%, 32.03%, and 50.56%, respectively. Of the 36 detected HR- and LR-HPV subtypes, the most common infections were HPV 6, 16, 11, 62/81, 52, and 54. These explained approximately 47% of all detected HPV types.

The analytical sensitivity and specificity of this method were certified after enrolment in the 2011 WHO Proficiency Panel, i.e., the analytical sensitivity of the test determined 100% agreement with reference values in 43 samples evaluated at different concentrations (from 5 to 500 GE) in the setting of single and multiple infections. Furthermore, no cross-reactivity among genotypes was observed; therefore, the analytical specificity for these samples was 100% (Herraez-Hernandez et al., 2013a, Herraez-Hernandez et al., 2013b). Additionally, according to the manufacture's recommendation, the detection limit was determined between 1 to 10 copies of viral genome equivalents. Regarding analytical specificity, no cross-reactivity was found between HPV genotypes included in the test, and reactivity with genital pathogens Herpes was also excluded. Since sensitivity, specificity, and reproducibility of HPV Direct Flow CHIP were determined as stated earlier we did not recalculate these values.

HPV distribution based on cytological features

The overall prevalence of HR-HPV infection was 38.68% (single or multiple HR-HPVs or multiple LR-HPVs). HPV 16 and 18 infections (alone or with other HPVs) were detected in 552 (16.98%) and 250 (7.69%) cases, respectively. HPV genotype distributions are summarized in **Table 2**. A total of 5138 samples were assessed by cytology, and 97.37% of women had normal cytology. It was determined that 3672 individuals (71.46%) were negative for both cytology and HPV tests and 1331 individuals (25.90%) were cytology-negative and HPV-positive. Among individuals who underwent cytological tests, 135 (2.63%) were positive. ASCUS, atypical squamous cells of undetermined significance, the low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) were found in 99 (73%), 4 (3%), 29 (21%), and 3 (2%) cases, respectively (**Table 3**). Of the 135 cytology-positive peo-

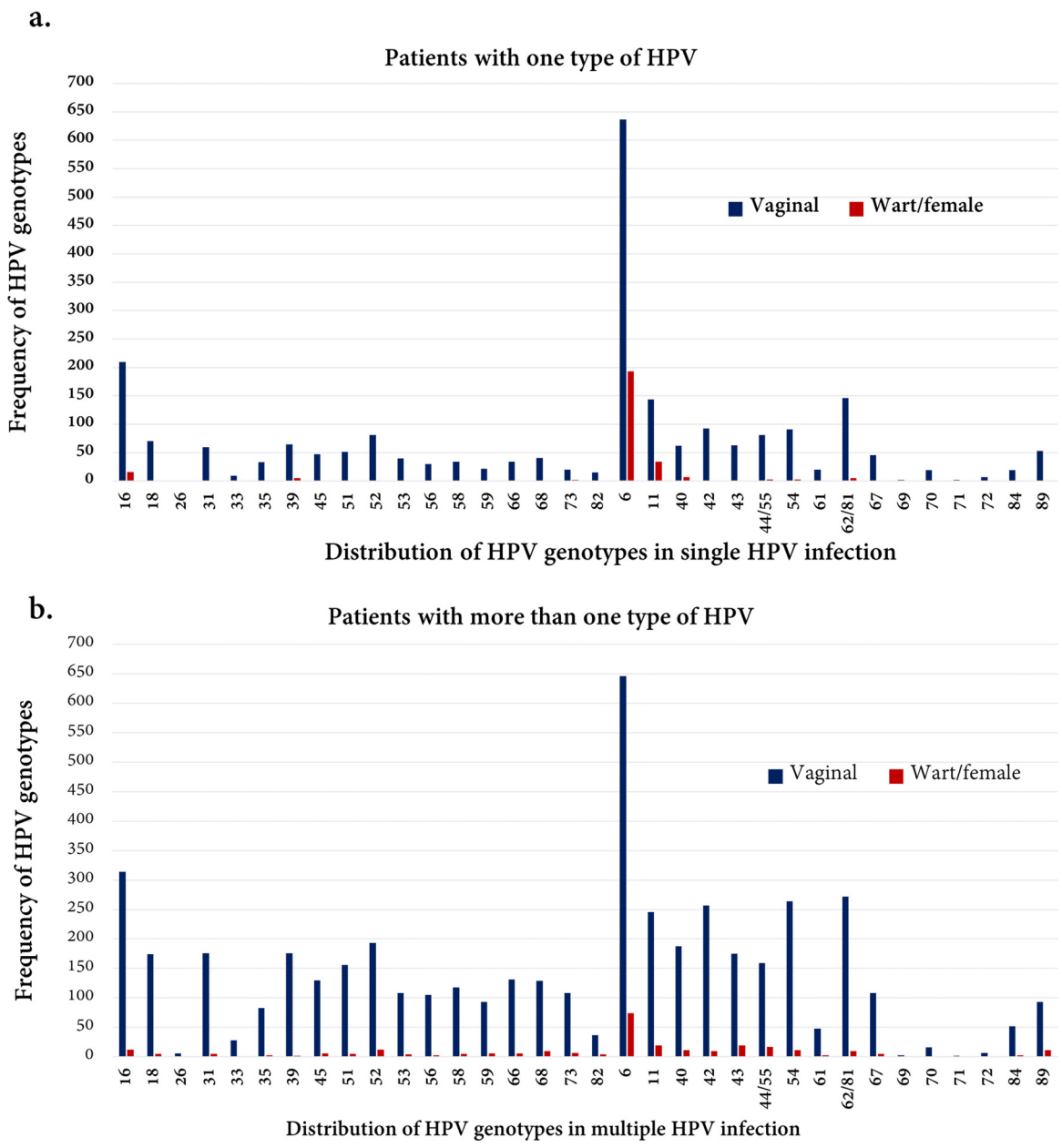

Figure 2. a) and b) Prevalence of each HPV genotype in single and multiple infections, respectively. HPV 6 was detected as the most common type in both groups.

ple, 81 individuals (60%) were also HPV-positive; 62 (76%) were HR-HPV-positive, and the most frequent type was HPV 16 (34%) (**Table 4**).

Discussion

In 2018, 467 CC deaths in the Iranian population were reported compared with 311 365 deaths worldwide (Bray et al., 2018). CC is the 12th leading cause of female cancer deaths and the 10th in women aged 15 to 44 years in Iran (Bray et al., 2018). Persistent infection with HR-HPV types can put the cells in danger of malignancy and causes CC (Tranberg et al., 2020). HPV prevalence has been reported as 22.1% in Africa, 11.3% to 20.4% in America, 8.1% in Europe, and 8.0% in Asia (Chen et al., 2013). Although severe life-threatening malignancies do not arise from LR–HPV subtypes, benign lesions cause stress and discomfort in patients. However, relatively few studies have investigated HR- and LR-HPV genotyping in Iranian women (Jamdar et al., 2018, Kesheh and Keyvani, 2019, Sabet et al., 2021). In this study, we determined the HR-HPV and LR-HPV prevalence among 12 076 Iranian women who had been subjected to routine CC screening and HPV DNA typing using the 'HPV Direct Flow CHIP System'.

Herein, the overall HPV prevalence (HR-HPVs and LR-HPVs) was determined at 38.68%, in line with previous findings (Yousefzadeh et al., 2014). The prevalence of HR-HPV—single and/or multiple infections—was approximately 15%, consistent with the previous report by Jamdar *et al.*, suggesting that the prevalence of HR-HPV infection undergoing routine Pap smears was 10.3% if 4 types of HPV (HPV 26, 53, 73, 82) that are not assessed in the Cobas method were excluded (Jamdar et al., 2018). The high positivity of HPV may arise from using different inclusion criteria and population profiles with a high rate of cases with abnormal cervical cytology (Costa et al., 2021). Besides, Hamkar *et al.* showed that 20 HR-HPV and LR-HPV types were detected in 7.2% of 1218 women with normal cervical cytology (Hamkar et al., 2002). The differences between our findings and previous data could be ex-

Table 2
The prevalence of human papillomavirus (HPV) genotype distribution among participants. HPV 16 and HPV 6 were identified as the common types of high-risk (HR) and low-risk (LR) HPVs, respectively.

Number	HR-HPV type	No. (%)	Number	LR-HPV type	No. (%)
1	16	552 (16.98%)	1	6	1550 (34.69%)
2	52	286 (8.8%)	2	11	443(9.91%)
3	18	250 (7.69%)	3	62	433(9.69%)
4	39	248 (7.63%)		81	
5	31	242 (7.45%)	4	54	369(8.26%)
6	51	213 (6.55%)	5	42	360(8.06%)
7	45	183 (5.63%)	6	40	268(6%)
8	68	180 (5.54%)	7	44	260(5.82%)
9	66	171 (5.26%)		55	
10	58	157 (4.83%)	8	43	257(5.75%)
11	53	152 (4.68%)	9	67	160(3.58%)
12	56	138 (4.25%)	10	89	157(3.51%)
13	73	137 (4.21%)	11	61	75(1.68%)
14	59	121 (3.72%)	12	69	75(1.68%)
15	35	119 (3.66%)	13	70	36(0.81%)
16	82	56 (1.72%)	14	72	16(0.36%)
17	33	38 (1.17%)	15	71	5(0.11%)
18	26	7 (0.21%)	16	84	4(0.09%)
Total	-	3250	-	-	4468

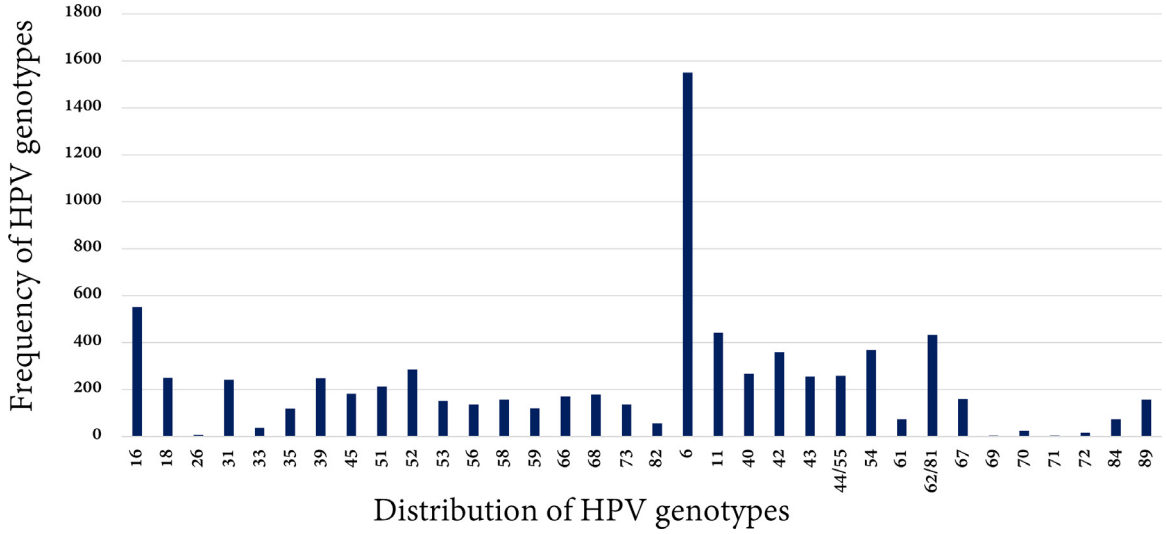

Figure 3. Prevalence of each HPV genotype in the participants. HPV 6 was the most common type of HPV detected.

plained by factors such as using different laboratory techniques and differences in the age of participants. Thus, comparison of prevalence between studies should be made with caution.

In the present study, we determined that the candidate population (aged 18–25 years) were vulnerable to HPV infection, consistent with previous findings (Castellsagué et al., 2009, Harper, 2008, Hu et al., 2011). We also showed that the prevalence of single infections (57.4%) remained higher than for double or multiple infections. Double infections accounted for 56.11% of multiple HPV infections. There were more single HPV infections than multiple in this study. Infection with multiple HPV genotypes is associated with an increased risk of HPV persistence (Oyervides-Muñoz et al., 2020). Our low detection rate of multiple HPV infections could be because most participants in this study were healthy or without severe conditions.

We showed that the most prevalent HR genotype was HPV 16. Previous data demonstrated that the prevalence of HPV 16 and/or HPV 18 among Iranian women with normal cytology, low-grade cervical lesions (LSIL/CIN-1), high-grade cervical lesions (HSIL/CIN-2/CIN-3/CIS), and CC were 2.8%, 42.9%, 67.6% and 58.6%, respectively (Ahmadi et al., 2017, Esmaeili et al., 2008, Jalilvand et al., 2015). Besides, Sudenga *et al.* reported that HPV 6 and 11 are often seen in genital warts in patients (Sudenga et al., 2017), and our findings were consistent with these findings.

In this study, the most frequent LR-HPV types were HPV 6, 11, 62/81, 54, 42, 40 and 44/55 and the HR-HPV types were HPV 16, 52, 18, 39, 31 and 51. Consistent with these findings, Mobini *et al.* reported that among 10 226 participants assessed, 16.6% of females showed HPV 16 and 9.6% HPV 52. Therefore, in the Iranian population, the most frequent HR-HPV types are HPV 16 and 52. A 9-valent vaccine including virus-like particles of HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 has been introduced, which could potentially prevent 70%–90% of HPV-related cancers (Joura et al., 2015), although it is not yet available for the whole population in Iran.

Our findings revealed that approximately 2.6% of available cervical cytology results were abnormal (ASCUS or worse). In total, 46.26% were HR-HPV positive, and 33.87% were positive with HPV 16 alone. Together HPV 18, 31 and 53 were the second most common HR types, found in 11.29% of abnormal samples. The diagnostic frequency of ASCUS has been reported as 1.6% to 9% (Joura et al., 2015); in this study, we determined it at approximately 1.9%. In total, HPV distribution in Iran is consistent with

Table 3
Frequency of high-risk human papillomavirus genotypes—including single and multiple infections—of patients with positive cytological results.

HPV type	ASC-US	LSIL	ASC-H	HSIL
16	13	5	2	1
18	3	3	1	-
26	-	-	-	-
31	6	1	-	-
33	-	-	-	-
35	4	1	-	-
39	2	1	-	-
45	4	-	-	2
51	2	2	-	-
52	3	2	-	-
53	4	3	-	-
56	3	2	-	-
58	3	1	-	-
59	1	-	-	-
66	3	-	-	-
68	3	1	-	-
73	1	2	-	-
82	4	-	-	-

Abbreviations: ASC-US; atypical squamous cells of undetermined significance, LSIL; low-grade intraepithelial lesion, ASC-H; atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, HSIL; high-grade intraepithelial lesion

the previous findings in the general population with early diagnosis, e.g., HPV 16 is the most common reported genotype in cancer or precancerous lesions (Li et al., 2021, Oyervides-Muñoz et al., 2020). HPV vaccination is currently part (though not thoroughly) of the national vaccination program in Iran (Taebi et al., 2019) and the quadrivalent vaccine that prevents HPV 16 and 18 infections is now available and can reduce the incidence of CC; however, insurance companies do not cover the vaccine cost.

There are several limitations of this study. Firstly, we recruited participants in a clinical setting, and our findings may not be generalizable to all Iranian women. Secondly, in many cases, cytological analyses of cervical swabs were not available to evaluate the possible association between HPV infection and clinical lesions. Lastly, some factors had not been documented when this study commenced, e.g., the age at first intercourse, sexual partners, homosexual or heterosexual intercourse, prolonged oral contraception, smoking, poor socio-economic conditions, and hormonal contraceptive use. Hence, these limited us to a broad set of factors for the analysis of risk.

Despite these limitations, to the best of our knowledge, this study was the first report investigating the association between cytology results and the prevalence of HPV infection and HPV genotype distribution among a large group of Iranian women. However, we believe that further studies should be carried out among ado-

Table 4
Distribution of human papillomavirus genotype in individuals with positive cytology results.

	ASC-US	ASC-H	LSIL	HSIL
HR-HPV positive (single, multiple: coinfection with other HR and/or LR HPVs) individuals	HR: HPV 16	HR-HPV 16	HR-HPV 16	HR-HPV 16
	HR-HPV 16	HR-HPV 16	HR-HPV 16	HR-HPV 45
	HR-HPV 16	HR-HPV 18	HR-HPV 16	HR-HPV 45; LR-HPV 6
	HR-HPV 16		HR-HPV 53	
	HR-HPV 16		HR-HPV 56, 68	
	HR-HPV 16		HR-HPV 39,51	
	HR-HPV 16		HR-HPV 18; LR-HPV 42	
	HR-HPV 16		HR-HPV 18; LR-HPV 6	
	HR-HPV 16		HR-HPV 35, 53, 73	
	HR-HPV 16		HR-HPV 31; LR-HPV 42	
	HR-HPV 16, 52, 53; LR-HPV 54, 62/81		HR-HPV58, 73; LR-HPV 42	
	HR: HPV 16, 45, 51, 66; LR-HPV 42		HR-HPV 52	
	HR-HPV 16, 35; LR-HPV 11		HR-HPV 51; LR-HPV 6, 40	
	HR-HPV 31		HR-HPV 52	
	HR-HPV 31		HR-HPV 56	
	HR-HPV 31		HR-HPV 53; LR-HPV 6	
	HR-HPV 31, 68, 82		HR-HPV 16; LR-HPV 62/81	
	HR: 31, 39, 45, 52, 66; LR-HPV 43, 67		HR-HPV 16,18	
	HR-HPV 31; LR-HPV 43			
	HR-HPV 82; LR-HPV 54, 62/81			
	HR-HPV 53			
	HR-HPV 51, 52, 56; LR-HPV 11, 54, 61, 44/55			
	HR-HPV 53			
	HR-HPV 58; LR-HPV 44/55, 62/81			
	HR-HPV 18, 73; LR-HPV 11, 54			
	HR-HPV 45, 58, 68, 8; LR-HPV 62			
	HR: 18, 58; LR-HPV 40			
	HR-HPV 35; LR-HPV 11			
	HR-HPV 82			
	HR-HPV 53, 58, 68			
	HR-HPV 18			
	HR-HPV 56			
	HR: 58; LR-HPV 40			
	HR: 58; LR-HPV 44/55			
	HR-HPV 51			
LR:11				
	HR-HPV 66			
LR-HPV 54				
	HR-HPV 35			
	HR-HPV 35, 39, 45, 56, 59			
Single or multiple LR HPV positive	10	6	1	-
HPV negative	49	5	-	-
Undetected	2	-	-	-

lescents to better understand HPV distribution in Iran by considering various factors to stratify the case population, e.g., cultural events, lifestyle.

Conclusions

It has been suggested that HPV can become a dynamic threat. In the context of protective and preventive methods for HPV infection, the present study highlights the genotype distribution of this infection in the Iranian population. Determining the HPV prevalence and the distribution of specific genotypes in a large population of Iranian people can improve health policies implemented by government and health agencies. The results obtained from the present study may be useful for policymakers to specify cost-effective interventions and recommendations to improve national immunization against HPV and CC.

Statement of ethics

The authors declare that they have conducted the project ethically in accordance with the World Medical Association Declaration of Helsinki. All study participants were informed about the study and signed the written consent form

Disclosure statement

The authors declare that they have no competing interests.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

MR.H., M.G. and F.B. Designed the project, T.A., M.MG., M.M., S.A., and F.Ch. Collect the samples, F.B., and M.Kh. Conducted the experiments, S.S. Analysis of the cytological test, F.B., M.Kh., E.R., R.Kh., and P.R. Data collection and analysis, F.B., M.Kh., E.R., and M.G. wrote the paper; all authors read and approved the final manuscript.

Acknowledgments

We sincerely appreciate the staff of the DeNA laboratory for helping us in this research.

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