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Prevalence, genotypes and phylogenetic analysis of human papillomaviruses (HPV) in northeast Iran

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ABSTRACT

Objectives: Human papillomaviruses (HPV) are the main etiology of invasive cervical cancer. Together HPV and viral hepatitis account for the cause of 25% of cancers in developing countries. To evaluate the association between population movements and the spread of HPV, this study looked at prevalence, genotypes, and phylogenetic assessment of HPV in Great Khorasan, a pilgrimage-tourism province in northeast Iran.

Methods: From March 2013 to July 2018, 567 samples were collected from three groups in Khorasan: Razavi and North Khorasan provinces (highly mobile population); South Khorasan province (conservative and desert); and diverse group (tourists).

Results: HPV prevalence was 48.4% in Razavi and North Khorasan (first group); 19.9% in South Khorasan (second group); and 33.6% in the diverse group. The four most common HPV genotypes were HPV-6, 11, 51 and 16, in the first group; HPV-6, 11, 16 and 58 in the second group; and HPV-6, 11, 16 and 53/89 in the diverse group. The most frequent genotypes that are known as high risk for cervical cancer were HPV-51 in the first group, HPV-16 in the second group and the diverse group. Among low-risk genotypes, HPV-6, and HPV-11 were more frequent in all groups. DNA sequencing and phylogenetic analysis of 20 HPV-positive samples showed that the distributions of the HPV genotypes were HPV-6 (50%), 11 (10%), 67 (5%), 16 (15%), 31 (10%), 54 (5%), and 89 (5%).

Conclusions: The findings show that areas associated with population movement should be frequently monitored for infectious diseases, while conservative and less populated areas have less risk for virus spread and endemicity. Health authorities should focus more on the establishment of HPV diagnostic facilities, screening, vaccination, and enhancement of public knowledge in these regions.

Introduction

Human papillomaviruses (HPVs) are a diverse group of dsDNA viruses, responsible for epithelial warts. HPV is a member of the *Papillomaviridae* family which has more than 200 genotypes (de Villiers, 2013). To date 228 types of HPV (HPV-1 to HPV-228) have been identified, including approximately 40 that preferentially infect the genital mucosa (https://www.hpvcenter.se/human_reference_clones/).

Infection with oncogenic genital HPV is the principal etiologic agent of invasive cervical cancer, the second most frequently

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occurring malignancy in women of middle- and low-income countries (Bray et al., 2018; Human, 1995). It is the most common neoplasia of women and the main cause of female death from cancer in developing countries (Cutts et al., 2007). Cervical HPV can induce cervical cancer over time, as documented by many experimental and epidemiologic studies (Schiffman et al., 2007).

Although most infected subjects eliminate the virus without any symptomatic manifestations, and only a few infected persons go on to develop HPV-associated cancers, approximately 570,000 new cervical cancers occurred worldwide in 2018 (Canfell et al., 2020).

It is well known that some HPV genotypes are higher risk for cervical cancer (zur Hausen, 1996). Phylogenetic studies of more than 200 HPV genotypes have classified them into low-risk (LR), probably high-risk (PHR), and high-risk (HR) types (Arbyn et al., 2014; Grahovac et al., 2007; Kocjan et al., 2015; Muñoz et al., 2003). HR HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 have been associated with cervical carcinogenesis, and with anogenital, neck and head cancers (Bouvard et al., 2009; Kocjan et al., 2015). LR HPV genotypes 6 and 11 have been regularly detected in benign or low-grade cervical tissue morphology changes, and genital warts such as condylomata acuminata (Cutts et al., 2007; Muñoz et al., 2003). The main common tumorigenic HPV genotypes are 16 and 18, causing nearly 70% of cervical cancers (Cutts et al., 2007); on the other hand, approximately 90% of genital warts are caused by HPV genotypes 6 or 11 (Greer et al., 1995).

In Iran, a developing country, it is estimated that around 947 women annually are diagnosed with cervical cancer, and 370 die from the disease. More than 70% of women with cervical cancer in Iran are assumed to have HPV-16/18 infection (Arbyn et al., 2014).

Because of high mortality from cervical cancer in both developed and developing countries, and the close relation with HPV infection, the World Health Organization (WHO) planned a global strategy for elimination of cervical cancer through the Cervical Cancer Elimination Modelling Consortium. This program includes a triple-intervention of 90% HPV vaccination, 70% twice-cervical screening, and 90% treatment of pre-invasive lesions and invasive cancers by 2030 (https://www.who.int/news/item/04-02-2020-to-eliminate-cervical-cancer-in-the-next-100-years) (Brisson et al., 2020).

To the best of the authors' knowledge, no data exist about HPV prevalence and genotypes in the Khorasan population (8 million residents and around 20 million tourists annually), the main tourist and pilgrimage center in northeast Iran, (https://www.amar.org.ir/Iran National Census 2016). In the present study, HPV prevalence and genotype was evaluated among suspected and at-risk populations in this area who tested for HPV. Twenty HPV-positive samples were selected randomly for phylogenetic sequencing.

Material and methods

Study population

A cross-sectional study between March 2013 and July 2018 was performed for evaluation of prevalence and type of HPV genotypes in the Khorasan provinces (North, Razavi and South Khorasan). A map of the Khorasan provinces is provided in Figure 1. All subjects

Figure 1. Location of Khorasan provinces (North, Razavi, and South Khorasan).

were of Iranian descent. The suspected subjects were referred by gynecologists, urologists, and dermatologists from teaching hospitals to the two molecular medical diagnostic laboratories: Navid Medical Lab (http://www.navidmedlabs.ir) and the ACECR-Central Medical Lab (Academic Center for Education, Culture and Research, Razavi Khorasan, Mashhad, Iran; http://jdmlabs.ir). The screening of persons suspected and at-risk for HPV infection was brought to the attention of health authorities in Iran, however, there are no particular public health screening regulations or HPV monitoring programs in the country. Therefore, the specialists who dealt with these persons directed them to the referral laboratories. To achieve a reliable estimation of different forms of the sampling in the teaching hospitals for HPV infection, the vaginal and urethral discharges, paraffin-embedded tissue, and genital wart samples (fresh or fixed) were included in the study.

Five hundred and sixty-seven samples were collected from 527 females and 40 males in this study. The subjects were classified into three groups based on their residency in the previous ten years: Razavi and North Khorasan; South Khorasan; and diverse groups (outside of these areas).

For phylogenetic analysis, molecular polymerase chain reaction (PCR) detection and sequencing of L1 open reading frame (ORF), were performed for 20 randomly selected HPV-positive samples, using universal PCR primers.

Human papillomavirus DNA extraction

DNA extraction was performed on vaginal and urethral discharges, paraffin-embedded tissue, and genital wart samples, using the High Pure Viral Nucleic Kit (Roche, Germany), according to the manufacturer's instructions. The DNA extractions were then dissolved in TE buffer and kept at −20 °C, until further use.

HPV detection and genotyping

The INNO-LiPA HPV Genotyping Extra II kit (Fujirebio Europe N.V., Belgium) was used for the detection and identification of HPV genotypes, according to the manufacturer's protocol. The primer set used in the INNO-LiPA HPV Genotyping Extra had the potential to amplify at least 32 HPV genotypes (Else et al., 2011).

PCR performance and HPV DNA-sequencing

Positive PCR products were purified, using a High Pure PCR product purification kit (Roche, Germany), according to the manufacturer's protocols. The PCR-based sequencing was performed for each HPV DNA extraction. The provided HPV-positive and -negative controls were used to assess the performance of the amplification.

To confirm the presence of HPV DNA fragments, 10 μL of each PCR product was separated on a 2% agarose gel electrophoresis and visualized. PCR products were then sent for DNA-sequencing by universal PCR primers, to the Virology Department of Tehran University of Medical Sciences (WHO Collaborating Center, Tehran, Iran); the most conserved gene of L1 ORF was used to identify the new HPV types. Finally, sequences obtained were compared with virus sequences available on the National Center for Biotechnology Information (NCBI), using the online standard Nucleotide BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=-BlastSearch).

Phylogenetic analysis of HPV

Sequencing results for each patient were collected and aligned with each other, using BioEdit software (BioEdit, v.7, CA). Genotyping was carried out by phylogenetic analysis and compared to reference sequences in the NCBI GenBank database. Phylogenetic tree reconstructions were depicted, using Geneious software (v.8.1.9), with the maximum likelihood method (ML) and the Kimura-two parameter substitution model with 1000 bootstrapping replicates.

Statistical analysis

All data were analyzed, using SPSS Version 16 (SPSS, Chicago, IL, USA). The descriptive variables were statistically analyzed by *Chi*–square non-parametric test; $P < 0.05$ was considered statistically significant.

Results

Population characteristics

In this study, the prevalence of HPV genotypes in the 567 samples was determined. The participants belonged to three groups: Razavi and North Khorasan; South Khorasan; and diverse group. Forty of the study participants were male (7.1%) and 527 (92.9%) female; 54% of males and 35% of females tested positive for HPV. The gender difference in prevalence was statistically significant ($P = 0.01$).

The age range of participants was 15–56, participants were classified into five age groups. HPV was the most prevalent in the 26–35 age group (Table 1) where the gender ratio of HPV prevalence was 13:1 (female to male).

The type of referred samples such as paraffin-embedded tissue, vaginal discharge, fresh solid tissue, and urethral discharge, and their distributions are provided in Table 2.

Prevalence of HPV infection

The total HPV prevalence in referred samples, using conventional PCR, by the published GP5/GP6 PCR protocol, was 35.3%; therefore among 567 individuals, 35.3% ($n = 200$) tested positive for HPV and 64.7% ($n = 367$) tested negative. Table 3 presents HPV prevalence, based on geographical distribution. Razavi and North Khorasan 107 (49%) female and 14 (58%) male; South Khorasan 40 in total (20%); and diverse group 33 (34.7%) female and 6 (54%) male.

Distribution of HPV genotypes

The four most common HPV genotypes were: Razavi and North Khorasan HPV-6, 11, 16 and 51; South Khorasan HPV-6, 11, 16 and 58; diverse group HPV-6, 11, 16 and 53. Among HR-HPV genotypes, HPV-51 in Razavi and North Khorasan and HPV-16 in South Khorasan and the diverse group were the most frequent. Among PHR genotypes, HPV-66 in the Khorasan provinces and HPV-53 among the diverse group were the most frequent genotypes. HPV-6 and HPV-11 had the most frequent prevalence among LR HPV

Table 1
The number of study participants by age group.

Age (years)	Frequency	Percent (%)
15–25	83	14.6
26–35	285	50.3
36–45	132	23.3
46–55	53	9.3
>56	14	2.5

There was a significant positive correlation between the age groups ($P = 0.004$).

Table 2
Geographical distribution of samples based on their type.

Sample type	Razavi and North Khorasan Provinces	South Khorasan Province	[a]Diverse group
Paraffin Embedded Tissue	15	136	0
Vaginal discharge	193	59	78
Fresh solid tissue	8	2	0
Urethral discharge	15	0	11
Missing	19	4	27
Total (Frequency)	250	201	116

[a] Diverse group: Outside of Razavi, North, and South Khorasan provinces.

Table 3
HPV prevalence and frequency distribution according to the geographic region.

District		Frequency	Percent
Razavi and North Khorasan	Negative	129	51.6
	Positive	121	48.4
	Total	250	100
South Khorasan	Negative	161	80.1
	Positive	40	19.9
	Total	201	100
[a]Diverse group	Negative	77	66.4
	Positive	39	33.6
	Total	116	100

[a] Diverse group: Outside of Razavi, North, and South Khorasan provinces.

genotypes in all three groups; these genotypes were found most frequently in all screening groups.

In Razavi and North Khorasan 13.9% (66/108) had a single infection, 11.2% (28/108) had co-infection with two genotypes, 4.8% (12/108) with three genotypes, 0.4% (1/108) with four, and 0.4% (1/108) with four or more genotypes. In South Khorasan 26.4% (28/37) had a single infection, 3% (6/37) had co-infection with two genotypes, 1% (2/37) with three, and 0.5% (1) with four or more genotypes. In the diverse group 20.7% (24/37) had a single infection, 8.6% (10/37) had co-infection with two genotypes, 0.9% (1/37) with three, and 1.7% (2/37) with four or more genotypes.

HPV DNA-sequencing and phylogenetic analysis

The results of DNA sequencing were processed, using ClustalX 2.1. (BioEdit, CA) and Geneious software. The molecular genotyping demonstrated the prevalence of HPV DNA in 4.2% of cases (20/567) with a predominance of HPV-6 (Figure 2). The distribution of the HPV genotypes 6, 11, 67, 16, 31, 54 and 89 were 50% (10/20), 10% (2/20), 5% (1/20), 15% (3/20), 10% (2/20), 5% (1/20) and 5% (1/20) of the selected sequences, respectively.

For genotypic confirmation and phylogenetic evaluation, 20 HPV-positive DNA samples were chosen randomly and sequenced. After analysis of the sequencing results, data were submitted to the NCBI GenBank, of which 11 were registered in the NCBI Nucleotide database as new HPV isolates of Khorasan A–K (GenBank accession numbers: MG734703.1; MG734702.1; MG734701.1; MG734700.1; MG734699.1; MG734698.1; MG734697.1; MG734696.1; MG734695.1; MG734694.1; and MG734693.1).

The sequencing findings and phylogenetic analysis (Figure 2) demonstrated that the distribution of the HPV genotypes, and particularly phylogenetic relatedness was very divergent. This may be due to the mobile population in the Great Khorasan; this province, particularly, Razavi Khorasan, hosts very large populations of pilgrims and tourists from the Middle East.

Discussion

According to the National Comprehensive Cancer Network guideline (NCCN Guidelines®), HPV tests play an important role in cervical cancer prevention. In addition, HPV genotyping is required to select individuals eligible to receive vaccination (Bissa et al., 2015; Choi and Park, 2015; Kim et al., 2014).

The Great Khorasan province in Iran is divided into the North, Razavi, and South Khorasan provinces (Figure 1) with a population of more than 8 million; it borders with Afghanistan in the east and Turkmenistan in the north. Until 2006, Mashhad, the capital of Khorasan, situated in northeast Iran, was a holy pilgrimage city with a population of 3 million people (Ghezeldasht et al., 2013), attracting more than 20 million tourists and pilgrims every year. Pilgrimage, immigration and tourism in these provinces therefore has a serious impact on the epidemiology of infectious diseases (Ghezeldasht et al., 2013) such as HPV, relative to the rest of the country.

In the present study, both LR and HR HPV genotypes were assessed in each sample using a conventional PCR test. In many studies, only HR genotypes, or only HPV-18 or 16, in the vaginal sample or cancerous paraffin-embedded tissues from females, are evaluated using primer-specific PCR tests. In this study, the results for HPV infection, using the INNO-LiPA® kit, demonstrated an overall HPV prevalence of 54% in males and 35% in females, in which HPV-6 was the most frequent genotype, and HPV-11 the second most commonly detected genotype. The differences among the three regional groups were significant. In Razavi and North Khorasan provinces 48.4% tested HPV-positive; 19.9% in South Khorasan; and 33.6% in the diverse group. These findings are predictable because the Razavi and North Khorasan provinces are tourist and pilgrimage regions and our studies show the prevalence of other blood-borne viruses such as hepatitis E, hepatitis C, and particularly, human T-lymphotropic virus-1, is higher here than in other provinces in Iran (Ghezeldasht et al., 2017, 2013; Rafatpanah et al., 2011).

On the other hand, South Khorasan is a conservative province with low population densities in a desert area, in which the prevalence of many infections due to sexually-transmitted disease (STD) and blood-borne viruses are low. Differences in the prevalence and distribution of HPV genotypes worldwide have been shown in numerous studies (Capra et al., 2008; Kocjan et al., 2015; Martins et al., 2016), and can be related to the complex interaction between distinct HPV genotypes with host immunogenic factors, behavioral attitudes, age and geographic location of the patients, or even the variation in the methodology of the studies.

In a study in Quebec, Canada, the overall prevalence of HPV and HR-HPV was 28.9% and 20.4%, respectively (Hamlin-Douglas et al., 2010). In a study in the west region of Germany, 36% of the HPV screened subjects were positive for HPV, from which 14% harbored more than one HPV genotype. The main regularly HPV genotypes were 16 and 31. This group was followed by HPV-6 (5.7%), 18 (5.3%), 58 (4.5%), 61 (4.5%), 53 (4.4%), 42 (4.3%) and 51 (4.0%) (Speich et al., 2004). In the present study, in Razavi and North Khorasan provinces, the HPV-6, -11, 16 and 51 genotypes were the most frequent; HPV-6, 16, 11 and 58 in South Khorasan; and HPV-6, 16, 11 and 53 in the diverse group.

In any region, the highest cases of cancer morbidity and mortality are brought to the attention of health authorities. For example, in Eastern Asia, around 1.7 million deaths occur among females. Outside Eastern Asia, the highest numbers of cancer and

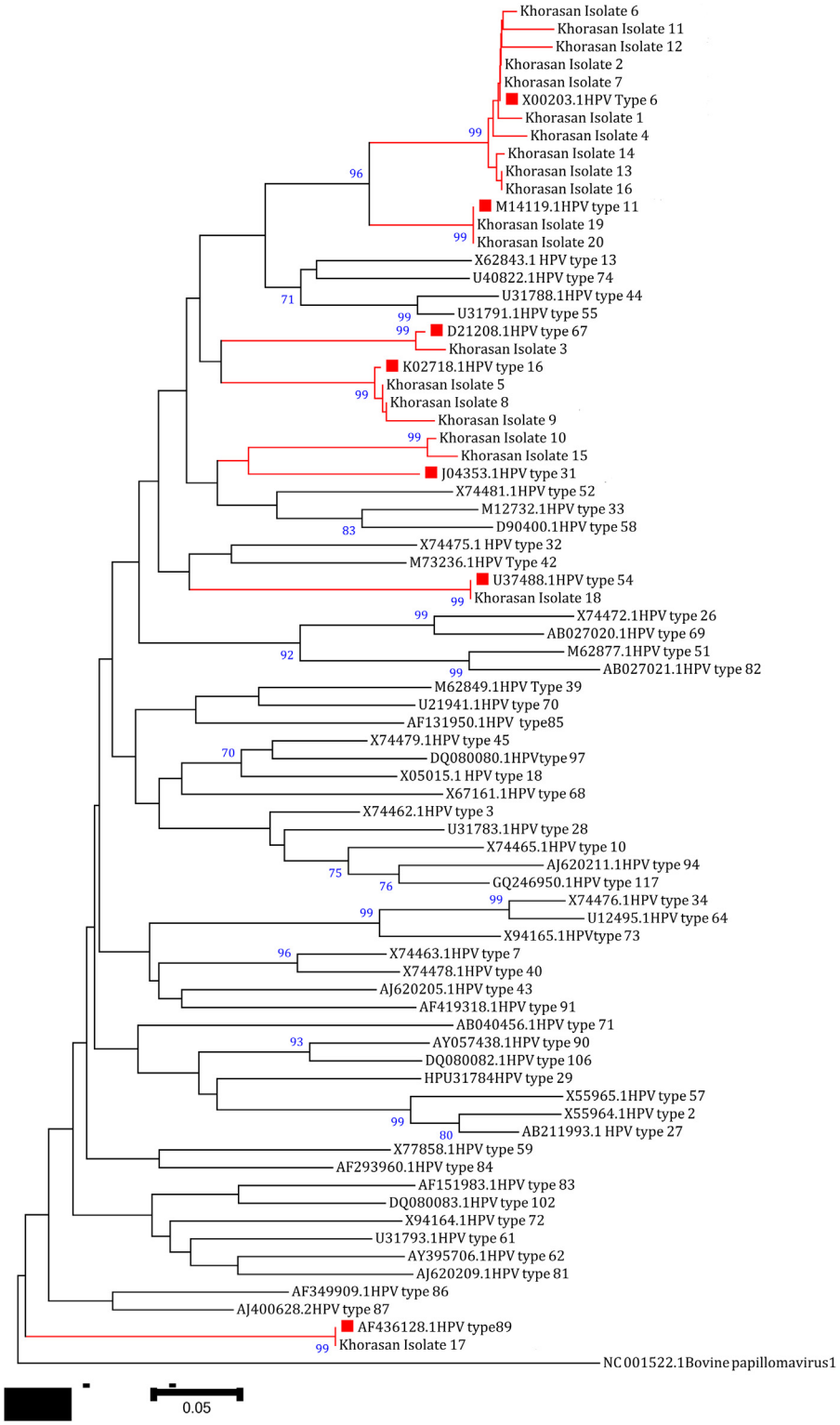

Figure 2. Phylogenetic tree constructed by the maximum likelihood method (ML), based on the genotype sequences of 20 Khorasan HPV isolates.

deaths happen in North America and then South-Central Asia (Richman et al., 2017). In the Austrian series, in distinct from global findings, no case of HPV-18 or 45 was reported (Pils et al., 2017).

In Qatar, one study showed that HPV prevalence is lower than global levels, and present in women with abnormal cytology with the most prevalent HR-HPV genotypes being 16 and 59, while HPV-35, 33, 39 and 59 were most prevalent in normal cytology samples. The most frequent LR genotype was HPV-81 (Bansal et al., 2014). One survey of Iraqi women revealed that in 16 cervical samples, 5 had HPV-16 and 5 HPV-18 (Abdul-Samad and Kandala, 2018). In

Turkish and Albanian women, a study showed that HPV-16 and HPV-6 are the most common HPV genotypes. The other common types are HPV-18 and 39 for Albanian and Turkish women, respectively (Hancer et al., 2018).

In Iran, Jalilvand et al. (2014) showed that HPV genotypes 16 and 18, in addition to inducing cervical cancer, are associated with head and neck malignancies (Jalilvand et al., 2014; Jamdar et al., 2018). In line with our study, Eftekhaar et al. reported the high prevalence of HPV-6 (75%) (9/12) and HPV-11 (16.7%), and co-infection with HPV-6 and 11 (Eftekhaar et al., 2017). Salehpour et al. studying paraffin-embedded tissue samples of invasive breast carcinoma patients via PCR method describe the distribution of HPV genotypes in northern Iran as 25.7% for HPV-11, 6.8% for 6, and 2.7% for 6 and 11 together. Although in that study, the association between HPV and breast cancer was confirmed, none of the samples were positive for HR HPV-16, 18 or 31 (Salehpour et al., 2015). The study of Ahmadi et al. in Zanjan, Iran showed that the most frequent HR-HPV genotype was 18 and for LR 6 was most dominant (Ahmadi et al., 2017). The prevalence of HPV infection detected in different Iranian studies is represented in Table 4.

The present study demonstrates prevalence and phylogenic analysis of HPV infection among people at-risk and suspected for HPV in the largest provinces in Iran and indicates the genotype variation in different parts of the Khorasan provinces. In this study, the most frequent HR genotype was HPV-51 in Razavi and North Khorasan and HPV-16 in South Khorasan and the diverse group; among the PHR genotypes HPV-66 in Razavi, North and South Khorasan and HPV-53 in the diverse group was frequent. Additionally, HPV-6 and HPV-11 had the most frequent prevalence among LR HPV genotypes in all three groups (Table 5).

The method for detection of HPV and population study among the Iranian studies may be the reason for variations in HPV prevalence in the different studies. In many of these studies, only HR/a minimum number of HPV genotypes are screened. Other factors could be attributed, such as sampling methods, the study population, geographical variations, race, ethnicity, and the method of HPV detection. As mentioned, the Khorasan provinces have a diverse population of different ethnic residents, immigrants and travelers with close contact. Therefore, the mobile population from different parts could quickly spread communicable infections as can be observed in the tourist area in Razavi and North Khorasan provinces (Ghezeldasht et al., 2013).

Migration and population mobility can affect spread of infectious diseases such as HPV, which could effectively be

Table 4
Summary of selected studies conducted on human papilloma virus prevalence in Iran.

No.	study	Year	Region	Study population	Sample size (*n*)	Determined genotype	Methods	Gender	Prevalence (%)	References
1	*Aghakhani et al.*	2017	Tehran	outpatients who referred for routine checkup/mild diseases	540	16 & 18	Antibody detection	378 females 162 males	HPV-16: 29.2 HPV-18: 20.7	Aghakhani et al. (2017)
2	*Taghizadeh et al.*	2017	Mashhad	cervical cytology samples	143	18 HR-genotypes & 4 LR-genotypes	1.Nested PCR 2.Reverse dot-blot hybridization	Female	HR groups: 74.1% LR group:25.9%	Taghizadeh et al. (2017)
3	*Zandi et al.*	2010	Bushehr	voluntarily subjected	200	16,18 & 53	1.HPV PCR 2.Sequencing	Female	11 (5.5%) Positive HPV-16:7, HPV-18:3, HPV-53:1	Zandi et al. (2010)
4	*Moradi et al.*	2011	Gorgan	cervical Papanicolaou examination	308	16 & 18	HPV PCR	Female	20.1% Positive HPV-16 & HPV-18: 48.6%	Moradi et al. (2011)
5	*Shahramian et al.*	2011	Zabol	Cervical Disease Screening	265	16 & 18	HPV PCR	Married woman	66.9% Positive Monogamous & polygamous wives: 29% & 37.9%.	Shahramian et al. (2011)
6	*Mehran* et al.	2015	Rasht	Abnormal cells	103	Not Mentioned	HPV PCR	Female	Normal cell: 98 Positive:4 Abnormal cell:5 Positive:1	Mehran et al. (2015)
7	*Yaghoobi et al.*	2015	Ahvaz	Anogenital warts	54	16	HPV PCR	43.4 females 56.6 males		Yaghoobi et al. (2015)
8	*Salehi-Vaziri et al.*	2015	Tehran	Genital lesion	483	28 different genotypes		Male	Positive: 269 High Risk: 46 (9.5%) Probable high-risk: 4 (0.8) Low-risk : 268 (55.5)	Salehi-Vaziri et al. (2015)
9	*Nasseri et al.*	2015	Tehran	Oligospermic, azoospermic & normal male	90	28 different genotypes	Nested-PCR HPV Genotyping	Male	Positive:26 High Risk:6 Low risk:20	Nasseri et al. (2015)

Table 5
The distribution and frequency of HPV genotypes.

HPV genotype	Razavi and North Khorasan	South Khorasan	[a]Diverse group
HPV16 (HR)	10	5	6
HPV18 (HR)	5	1	1
HPV31 (HR)	7	3	0
HPV33 (HR)	1	1	1
HPV35 (HR)	2	1	1
HPV39 (HR)	6	1	2
HPV45 (HR)	1	1	0
HPV44 (LR)	4	2	1
HPV51 (HR)	11	0	1
HPV52 (HR)	4	3	3
HPV56 (HR)	3	1	2
HPV58 (HR)	4	4	3
HPV59 (HR)	1	1	2
HPV68 (HR)	4	2	2
HPV26 (PHR)	0	1	0
HPV53 (PHR)	7	1	4
HPV66 (PHR)	8	2	2
HPV70 (LR)	1	0	0
HPV73 (LR)	1	0	0
HPV82 (LR)	3	0	0
HPV06 (LR)	46	12	11
HPV11 (LR)	18	8	7
HPV40 (LR)	3	0	0
HPV42 (LR)	0	1	0
HPV43 (LR)	0	0	1
HPV54 (LR)	5	0	1
HPV61 (LR)	3	0	0
HPV62 (LR)	0	2	0
HPV67 (LR)	0	1	2
HPV81 (LR)	1	0	0
HPV84 (LR)	1	0	0
HPV89 (LR)	2	2	4

HR, High-risk; PHR, Probable high-risk; LR, Low-risk.
[a] Diverse group: Out of Razavi, North, and South Khorasan provinces.

controlled if those factors were taken into consideration. In the present study, HPV infection in both genders was highest in the 26–35 age group, then 36–45, 15–25, 46–55, and lowest in the >56 age group. In Martins et al., when the results were stratified by age group, the highest HPV transient infections were observed in women under 31, while it seems that HPV positivity in women above 30 would be stable infections (Martins et al., 2016).

In the present study, in Razavi and North Khorasan, multiple HPV genotypes were seen: 11.2% (28/108) had co-infection with two genotypes, 4.8% (12/108) with three, 0.4% (1/108) with four, and 0.4% (1/108) with four or more types. In South Khorasan, 3% (6/37) had co-infection with another genotype; within the diverse group, 8.6% (10/37) had co-infection with two genotypes, 0.9% (1/37) with three, and 1.7% (2/37) with four or more types.

Martín et al. reported multiple HPV subtype infections in 35% of women, which were more frequent in those less than 31 years old. This finding is consistent with other studies (Martín et al., 2011; Mejlhede et al., 2009). Therefore, multiple infections could be associated with sexual activities. It is interesting that the multiple co-infections and HPV prevalence decreases with age (González-Bosquet et al., 2008; Martins et al., 2016; Sjoeborg et al., 2010).

In contrast to some guidelines, recommending the test for genotypes 16 and 18, some studies demonstrated that it would be useful to test for other HPV genotypes, including HPV 31, 33 and 33/58 (Halfon et al., 2013; Wheeler et al., 2013; Wright Jr et al., 2012).

From this study it appears that planning for frequent monitoring, screening, and controlling infectious diseases in general, and STDs in particular, is an urgent need, particularly in urban regions with mobile populations. Applying reliable molecular diagnostic techniques for HPV, HPV based screening, HPV vaccination, and public education programs for STD (and in our study HPV) are suggested for prevention and control of emerging, re-emerging, and particular health-threatening infectious agents (Ghezeldasht et al., 2013).

In Iran, there is no governmental HPV vaccination program (*Human Papillomavirus* and Related Diseases Report (on 17 June 2019), Iranian HPV information center (https://hpvcentre.net/statistics/reports/IRN.pdf)) and people can only access a commercial HPV vaccine. The Iranian health system appears to still be challenged by a lack of evidence for the cost effectiveness of GARDASIL® vaccine administration (Mohammad Pour et al., 2020).

However, as HPV infection rates rise, reassessment of the benefits of an HPV vaccination program should be taken into account. Without a public vaccination program, in a conservative country with particular rules, regulations and beliefs, it seems that encouraging people to self-sample is crucial for cervical cancer screening. It is more acceptable and feasible if people are educated by social media for home-based self-sampling which can increase the coverage of screening for HPV infection, particularly in women (Lam et al., 2017).

Conclusions

In conclusion, due to a concentrated population in the cities and a change in behavioral attitudes, it can be seen that HPV can become a dynamic threat. Therefore, given the existence of protective and preventive methods for HPV infection, the present study highlights the need for integrated action at the local, national, and regional levels, by implementing vaccination and educational programs.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and, or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was reviewed and approved by the Research Ethics Committee of Mashhad University of Medical Sciences **(IR.MUMS.MEDICAL.REC.1398.811)**. The inform consent was obtained and signed from all subjects for participation in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this paper and available from the corresponding author (S.A.R. Rezaee) upon reasonable request.

Conflict of interest

None.

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Authors' contributions

FS, AM, SAG, and SB handled the sampling from study subjects, performed the experiments and data collection and entered data into the software. SAS, and KS contributed as the main advisors of the study. SAR designed and supervised the study. SAR, FS, AM, SAG, and SA conducted the data analyses and wrote the manuscript. All authors have read and approved the final manuscript.

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