

# Retrospective Analysis of Prevalence of High-risk and Low-risk Human Papillomavirus (HPV) Genotypes in Iranian Women During 2013-2016

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## Abstract

**Background and objective:** Considering the importance of HPV in cancer etiology, awareness of HPV prevalence and frequency of high-risk genotypes could help improve health care system management. We analyzed HPV prevalence in women from different provinces of Iran that is the largest sample till now. **Material and Methods:** In this retrospective cross-sectional study, 2969 outpatient and suspicious women aged 17- 78 referred to Noor pathobiology laboratory from 24 provinces of Iran were studied. DNA extraction and PCR were performed on samples and then HPV genotypes were also determined using hybridization kit. **Results:** HPV DNA was detected in 29.3% of valid liquid-based samples. Among HPV positive cases, 67.2% and 52.0% accounted for high-risk and low-risk HPV subtypes, respectively. In patients with high-risk HPV types, HPV16 was confirmed as predominant type (30.5%) followed by HPV53 (17.3%) and HPV39 (13.3 %). HPV6 was found as the most common low-risk HPV type with 60.6% frequency rate followed by HPV11 (17.9%) and HPV81 (8.6%) in this age group. It was apparent that age group >25 years accounted the highest frequency of HPV positivity. The prevalence of HPV was significantly different among different age groups ( $p < 0.0001$ ). **Conclusion:** It can be concluded that HPV infection is currently at a considerable high level in Iran. Looking at the high risk and oncogenic HPV subtypes frequency especially in younger age groups, a concern could be noticed about HPV relevant cancers which can be prevented by commercial and approved HPV vaccines.

**Keywords:** Genotypes- high risk- human papillomavirus- low risk- prevalence

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## Introduction

Human papillomaviruses (HPVs) family consists of more than 200 different types of non enveloped and double-stranded DNA viruses that can exclusively infect stratified squamous cutaneous cells or the mucosa of upper-respiratory and genitals tracts [1-2]. These viruses have worldwide dispersal that can be recognized based on their oncogenic potential, biological niche, and phylogenetic state. In the genital tract, about 40 HPV genotypes are generally found [3, 4]. HPV is one the most

common sexually transmitted agent that affects young and sexually active people of equal sexes [5-6]. Genital HPV types have been subdivided into high risk and low-risk types on the basis of their potential to cause malignant progression [7]. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68 have been involved in cervical carcinogenesis and categorized as high risk types, while other types, such as HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and HPV, 89/Cp 6108 are classified as low risk and related to genital warts [8-9].

The most predominant types of high risk HPV

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genotypes in the worldwide are HPV-16 and HPV 18 [10]. Genital wart is the most common benign tumor that is naturally presented on the outside of genitalia, including the penis, scrotum, vulva, perineum, and perianal skin [9, 11, 12]. Spreading of HPV viruses occur through skin or surface contact and the spreading can be horizontal (by genital-genital, manual-genital, or oral-genital contact) or vertical (from mother to fetus) [13, 14].

Cytological and molecular methods have been currently applied for detection and screening of HPV viral infection. Pap smear, as a cytological test, has been generally used as the principal approach for cervical lesion screening; however, it presented considerable false negative results [15-17]. Therefore, molecular assays such as PCR hybridizations, PCR, and Real-time PCR have been introduced for easier and more accurate HPV detection and genotyping [9].

Prevalence of HPV in developing countries (42.4%) is more public in comparison to the developed countries (22.6%) [18]. Out of 233.9 million women at reproductive age worldwide, nearly 32.03 million and 3.71 million were Asian and Iranian women, respectively [19]. In addition, South Africa (58.1%), Singapore (25.6%), France (15%), Russia (13%), China (12.27%), Chile (11.1%), Brazil (10%), and Iran (9.4%) have been recognized as HPV high prevalence areas based on the epidemiological studies [20-27]. Based on WHO report in 2010, the number of Iranian women aged 15 years and older is more than 25 million who are at risk of cervical carcinoma. The incidence rate of cervical cancer with an age normalization is 2.2 per 100,000 women each year, that is considered as the second most public threat of female reproductive system in Iran [28].

Thus, due to the importance of HPV prevalence and especially high risk genotypes, we persuaded to determine the prevalence of HPV infection and the related high-risk and low-risk genotypes in women from different provinces of Iran. Our findings can be very informative for decision making on HPV screening and prevention programs by prophylactic vaccines. For achieving this aim, we calculated HPV prevalence rates in a large number of patients referred to Noor pathobiology laboratory.

## Materials and Methods

### *Patients and sample collection*

In this retrospective cross-sectional study, 2969 outpatient and suspicious women aged 17-78 years old (mean  $\pm$  SD 33.4  $\pm$  8.4) were studied who were referred from 24 out of 31 provinces of Iran to Noor pathobiology laboratory during November 2013 to September 2016. Patients were subdivided into <25 years, 25-34 years, 35-45 years, and >46 years age groups accounted for 11.5%, 51.9%, 26.4%, and 10.2% of cases, respectively. Brush vaginal swaps (n=2913) and genital warts (n=56) were checked for HPV infection according to prescribed order. Samples were collected by pastoCYTOprep (Pasture Institute of Iran, Tehran, Iran) transport medium.

### *HPV DNA extraction*

Viral DNA extraction from different samples was carried out by High pure PCR Template preparation kit according to manufacturer's instruction (Roche, Germany). Briefly, genital warts were initially digested using tissue lysis buffer and proteinase K (Roche, Germany) for 72 hr at 37 °C. Two hundred microliters of each sample was mixed with equal volume of binding buffer and 40  $\mu$ l proteinase K and incubated for 10 min at 70 °C. Then, 100  $\mu$ l isopropanol was added and the mixture was applied to a highly pure filter tube and centrifuged for 1 min at 8000  $\times$ g. The filter was washed with 500  $\mu$ l inhibitor removal buffer and consequently by wash buffer using centrifugation. Flow-through was discarded and DNA was eluted with 200  $\mu$ l elution buffer using centrifugation for 1 min at 8000  $\times$ g.

### *PCR amplification*

HPV DNA amplification was performed by PCR technique using the approved commercial kit (HybriBio 21 HPV GenoArray diagnostic kit, HybriBio, China). For each reaction, 24  $\mu$ l of PCR ready to use master mix, 1  $\mu$ l of DNA Taq polymerase, and 1  $\mu$ l of extracted DNA were transferred into appropriately labeled tubes. Hot start DNA polymerase was activated by holding at 95 °C for 9 min and sequentially cycled for 40 times at 95 °C for 20 sec, 55 °C for 30 sec and 72 °C for 30 sec and followed by final extension at 72 °C for 5 min. Non-infectious plasmid DNA supplemented in the master mix was applied as an internal control in all reactions. Positive and negative controls were used appropriately according to the kit procedure.

### *Hybridization*

HPV genotypes of amplified DNAs were detected using HybriBio 21 HPV GenoArray diagnostic kit (HybriBio, China). The GenoArray kit detected 21 HPV genotypes, including 13 high risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), 6 low risk types (HPV 6, 11, 42, 43, 44, and CP8304 (81)). The tests were executed according to the manufacturer's instructions. Briefly, all amplified DNA samples were denatured at 95 °C for 5 min and were promptly put into an ice block. Hybridization process was carried out in DNA HybriBioMax (Guang gong Kaipu Biotech Co, China) device in 3 different temperature conditions. At first step, HybriBioMax was set at 45 °C and then 0.8 ml of pre-warmed hybridization solution was added on hybridization probed membrane (HybriMem) located in the well and incubated for 3 min. Then, the buffer was replaced with 0.5 ml fresh hybridization solution mixed with denatured DNA. HybriMem and DNA were incubated for 20 min at 45 °C. At the second step, HybriBioMax was set at 25 °C and 0.5 ml of blocking buffer was added into the well when the temperature reached around 30 °C. Then, 0.5 ml of enzyme conjugate was added and incubated for 5 min. Next, the well was washed 3 times with wash buffer A. In the last step, HybriBioMax was set at 36 °C and NBT/BCIP solution was added and incubated for 5 min. Finally, the well was washed 3 times with wash

buffer B and distilled water. The results were interpreted by color visualization observed on the membrane using the provided guide. The average detection limit of the kit, as reported by the manufacturer, was around 300 copies per  $\mu$ l of target HPV DNA. No cross-reactivity was shown among all the 21 studied HPV genotypes.

#### Statistical analysis

Data were analyzed by SPSS 16 (Chicago, IL, USA). The difference in HPV prevalence in different age groups was compared by Chi-square test. Statistical significance was defined as  $p < 0.05$ .

## Results

Our results indicated that HPV DNA was detected in 29.3% (871 out of 2969) of valid liquid-based samples of which 848/2913 (29.1%) and 23/56 (41.1%) were HPV positive in brush vaginal swaps and genital warts, respectively. HPV prevalence was significantly higher in genital warts compared to brush vaginal swaps ( $p = 0.05$ ). Among HPV positive cases, 67.2% and 52.0% accounted for high-risk and low-risk HPV subtypes, respectively (Table 1). Following further data analysis, it was found that

HPV16, HPV18, and other HPV genotypes were in 20.5%, 7.0%, and 88.4% of HPV positive samples, respectively. In addition, 563/871 (64.6%) and 308/871 (35.4%) of infected subjects were single and multiple HPV types, respectively. In patients with high-risk HPV types, HPV16 was confirmed as the predominate type with 179/585 (30.5%) rate followed by HPV53 (17.3%) and HPV39 (13.3 %) in this category (Table 2). On the other hand, HPV6 was found as the most common low-risk HPV type with 60.6% frequency rate followed by HPV11 (17.9%) and HPV81 (8.6%) in this group (Table 2). Interestingly, HPV6 was the most detected genotype in wart samples (28.6%) (Table 2).

As presented in Figure 1, HPV was detected in 133/341 (39.0%), 476/1542 (30.8%), 195/784 (24.8%) and 67/302 (22.1%) of age groups  $< 25$ , 25-34, 35-45, and  $> 45$  years, respectively. It was apparent that age group  $> 25$  accounted the highest frequency of HPV positivity. In addition, HPV infection decreased from 39.0% among women aged  $< 25$  years to 22.1% in women aged  $> 45$  years. The prevalence of HPV in the age groups was significantly different ( $p < 0.0001$ ). We did not access to Pap smear results to compare HPV prevalence in cytological normal

Table 1. Prevalence of High-Risk and Low-Risk HPV Types in Women from 21 Different Provinces of Iran

HPV	High-risk (%)	Low-risk (%)	Total (%)
Positive	584 (19.7)	453 (15.3)	871 (29.3)
Negative	-	-	2098 (70.7)

Table 2. Frequency of HPV Genotypes in 2969 Iranian Women Attended to Noor Pathobiology Lab

Category	HPV type	Frequency (%)	Brush swap samples	Genital warts
High-risk	HPV16	179 (6.0)	177	2
	HPV18	61 (2.1)	59	2
	HPV31	72 (2.4)	72	0
	HPV33	4 (0.1)	4	0
	HPV35	21 (0.7)	21	0
	HPV39	78 (2.6)	78	0
	HPV45	32 (1.1)	32	0
	HPV51	73 (2.5)	73	0
	HPV52	56 (1.9)	55	1
	HPV53	101 (3.4)	100	1
	HPV56	36 (1.2)	35	1
	HPV58	53 (1.8)	51	2
	HPV59	25 (0.8)	24	1
	HPV66	61 (2.1)	61	0
	HPV68	23 (0.8)	22	1
Low-risk	HPV6	329 (11.1)	313	16
	HPV11	81 (2.7)	78	3
	HPV42	10 (0.3)	9	1
	HPV43	5 (0.2)	5	0
	HPV44	11 (0.4)	11	0
	HPV81	39 (1.3)	38	1

Table 3. The Profile of HPV Frequency and HPV Genotypes Distribution in Iranian Women

Place	Time	Samples data		Prevalence (%)			High frequent type	Ref.
		Size	Subjects	HPV	HPV16	HPV18		
Shiraz	1999-2000	101	Cervical carcinoma	87.1	26.7	0	HPV16	[42]
Tehran	2005-2006	127	Outpatients	31.5	23.6	3.2	HPV16>HPV18	[43]
Tehran	2005-2006	7	Cervical carcinoma	100	85	28	HPV16>HPV18	[43]
Bushehr	2008-2009	200	Healthy women	5.5	3.5	1.5	HPV16>HPV18>HPV53	[34]
Zabol	2006-2007	265	Gynecology outpatient clinic	32.5	9	9.4	HPV18> HPV16	[40]
Shiraz	2010	402	Healthy women	5.5	2	0	HPV16	[44]
Tehran	2010-2012	851	Gynecology outpatient clinic	31.1	7.3	2.8	HPV16> HPV18	[41]
Gorgan,	2011	378	Gynecology outpatient clinic	20.1	5.8	4	HPV16> HPV18	[45]
Tehran	2011	825	Healthy women	6.6	1.8	0.3	HP16>HPV45>HPV31	[35]
Tehran	2011	45	Cervical carcinoma	35.3	8.8	0	HPV16=HPV58	[35]
Tehran	2011-2013	436	Cervical lesions	45.4	32.8	5	HPV16> HPV53>HPV31=HPV39	[46]

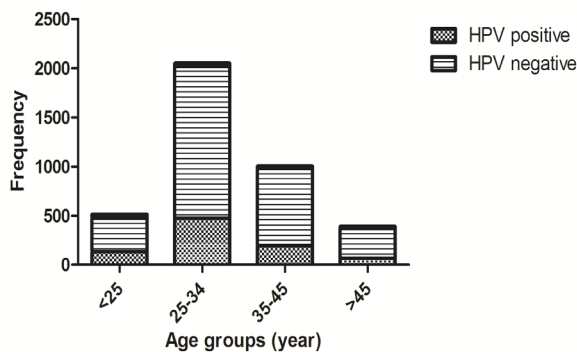


Figure 1. Age Specific Prevalence of HPV in Liquid Based Samples of Iranian Women

and abnormal groups.

## Discussion

Some HPV types are well-accepted as the main etiological factors of cervical carcinoma which have been detected in almost all cervical cancer cases [29]. Besides cervical cancer, HPV virus has also been recognized as a definite cause of penis, vulva, vagina, anus, and oropharynx cancers [30-31]. In this context, HPV genomes integration into human genomes is a critical point of carcinogenesis process that leads to the viral proteins expression and induction of normal cell transformation [32].

HPV infection was associated with 4.8% of estimated 12.7 million new cancers in 2008 worldwide which considerably had higher incidence and mortality rates in developing countries compared to developed ones [33].

Bearing in mind the importance of HPV in cancer etiology, awareness of HPV prevalence as well as frequency of high-risk genotypes could help improve healthcare system management in order to prevent the related cancers. Thus, we retrospectively analyzed HPV prevalence in 2969 liquid-based samples (swap and genital wart) of women from different provinces of Iran that is the largest sample till now to the best of our knowledge.

In this regard, our results signified that HPV virus was detected in 29.3% of women referred by physicians to Noor pathobiology lab. In other studies, HPV prevalence was reported in 20.1-32.5% of Iranian women attended to gynecology clinics (Table 3). Our finding is almost compatible with these reports; however, it is noteworthy to be noticed that this rate is higher than normal population of Iranian women with HPV infections [34-35]. In a meta-analysis study of HPV prevalence in healthy women from different provinces of Iran, it was reported that total prevalence of HPV was 9.4% with 2.0%, 1.7%, and 5.3% accounted for HPV16, HPV 18, and other genotypes, respectively [27-36]. In an elegant study, the prevalence of HPV infection was globally calculated between 11%-12% among women without cervical abnormalities. The highest rates of HPV infection were reported for sub-Saharan Africa, Eastern Europe, Latin America, and South-Eastern Asia with incidence of 24.0%, 21.4%, 16.1%, and 14.0%, respectively [30-36-37].

Analysis of HPV positive cases showed that high risk and low-risk HPV subtypes were categorized into 67.2% and 52.0% of samples, respectively. Totally, HPV16, HPV18, and other HPV genotypes were found in 6.0%, 2.1%, and 25.9% of samples, respectively (Table 2). Among high-risk HPV types, HPV16 (6.0%) was confirmed as predominate type followed by HPV53 (3.4%) and HPV39 (2.6 %) (Table 2). In comparison with our findings, Pouryasini (2014) showed that HPV53 followed by HPV16, HPV66, HPV18, HPV52, and HPV39 as the most prevalent genotypes in liquid-based samples of women from Tehran [38]. In other study in Tehran, prevalent HPV genotypes belonged to HPV16 (1.8%), HPV45 (0.8%), and HPV31 (0.6%) in normal population [35]. In a Meta-analysis, HPV 16 (54%), HPV18 (14%), and HPV31 (6%) were shown as the most frequently detected types in Iranian patients with cervical cancer [39]. Comparing to the worldwide HPV distribution, HPV16 (3.2%), HPV18 (1.4%), HPV52 (0.9%), HPV31 (0.8%), and HPV58 (0.7%) were the most widespread HPV types [30, 36]. These findings demonstrated that the pattern of HPV subtypes distribution could be varied among different regions.



Regarding HPV prevalence in age groups, compatible with our finding, Shahramian (2011) reported that the most HPV infection was detected in married women at 15 -25 years age group at Zaboul located at South -Eastern of Iran. They also observed a decreasing trend of HPV frequency in older subgroups of 26- 35, 36-45, and >45 years [40]. Shafaghi (2013) indicated that 19 HPV types were detected in 31.1% of Iranian women attended gynecology outpatient clinic [41]. Their finding demonstrated the highest prevalence of HPV in young women in 18-25 years old subgroups which encountered a decrease in the HPV frequency in older subgroups [41]. Furthermore, they showed that the prevalence of HPV16 and HPV18 were 7.3% and 2.8%, respectively [41]. Their findings have overall compatibility with ours. In addition, the HPV distribution in different age groups has diverse patterns in the world with a higher prevalence in the younger ages (<25 years) and a return at older ages ( $\geq 45$  years) in the Africa and Americas [36].

In conclusion, this study generated valid data regarding HPV prevalence in Iranian women from 24 different provinces. The large sample size and applying a standard and approved commercial HPV diagnostic kit were two important encouraging points of our study which let compare our findings with others. In overall, it can be concluded that HPV infection is currently at a considerable high level in Iran. Looking at the high risk and oncogenic HPV subtypes frequency in studied samples especially in younger age groups, a concern could be notified about HPV relevant cancers which can be prevented by commercial and approved HPV vaccines.

#### Conflict of Interest

All authors declare no conflicts of interest.

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