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ORIGINAL ARTICLE

Type-specific human papillomavirus prevalence in women referred for colposcopy in Tehran

Rahim Soleimani-Jelodar[1], Arash Arashkia[2], Zabihollah Shoja[2], Kimia Sharifian[1], Setareh Akhavan[3], Fariba Yarandi[4], Somayeh Jalilvand[1*]

[1]Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Virology, Pasteur Institute of Iran, Tehran, Iran
[3]Department of Gynecology Oncology, Imam Khomeini Hospital Complex, Valiasr Hospital Tehran, Iran
[4]Department of Obstetrics and Gynecology, Yas Hospital, Tehran, Iran

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ABSTRACT

Background and Objectives: Although several studies have been achieved on the frequency of the HPV types among women with cervical cancer in Iran, HPV-positive samples were in some cases directed to specific-primer genotyping of HPV 16 and 18. Therefore, the other HPV types are underestimated. Several studies have also reported a greater prevalence of HPV 16 in cervical cancer in Iran than in the world. To clarify these subjects, the distribution of HPV types in women referred for colposcopy in Tehran was investigated.

Materials and Methods: In this cross-sectional study, a total of 148 cervical samples from women with normal, atypical squamous cells of undetermined significance, cervical intraepithelial neoplasia I-III, and invasive cervical cancer histopathology were included. HPV was detected by PCR assay and all HPV-positive specimens were subjected to direct nucleotide sequencing.

Results: Our results demonstrated that the total prevalence of HPV was 92.5%. The five most common HPV types were HPV 16 (49.3%), 18 (14.8%), 6 (7.4%), 31 (4.1%), and 11 (2.7%). About the histopathological stage, HPV 16 and 18 were dominant in all studied groups. In cervical cancer, HPV 16 and 18 were detected in 60% and 20% of cases, respectively.

Conclusion: HPV 16 and 18 were the most common in cervical cancer in Iran.

Keywords: Human papillomavirus; Uterine cervical neoplasms; Cervical intraepithelial neoplasia; Atypical squamous cells of the cervix; Human papilloma virus vaccines

INTRODUCTION

Cervical cancer is considered the fourth most prevalent cancer among women in the world. Globally 604000 and 342000 new cases and deaths were reported in 2020, respectively (1). This cancer is the seventh most frequent cancer with an age-standardized rate of 6.5 among Iranian women. Human papillomavirus (HPV) is responsible for almost all cases of cervical cancer (2, 3).

HPV infection is the most frequent viral sexually transmitted infection worldwide (2, 4). Although in most cases the immune system alerts within 6 to 24 months post-infection which leads to clearance of the virus, in a minority of cases, it can persist and progress toward cervical cancer (5, 6). Persistent in-

*Corresponding author: Somayeh Jalilvand, Ph.D, Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98-21-42933195 Fax: +98-21-88962343 Email: sjalilvand@tums.ac.ir

fection with 14 high-risk HPV types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 is almost responsible for cervical cancer development. Other HPV types (intermediate or low-risk types) are slightly detected in cervical cancer specimens (7-10).

Currently, two approaches have been established for cervical cancer prevention in the world, including HPV vaccination (primary prevention) and comprehensive screening programs (secondary prevention) (11-13). Three prophylactic HPV vaccines are currently available for cervical cancer prevention as follows: 1- Cervarix; against HPV 16 and 18, GARDASIL®: against HPV 6, 11, 16, and 18, and GARDASIL®9 against HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 (13-16). The WHO defined a global strategy to accelerate the elimination of cervical cancer, by 2030. One of which is the vaccination of 90% of girls against HPV (17). However, Iran has not integrated HPV vaccination into the national vaccination program.

It is well-documented that the frequency of HPV types may be different in diverse geographical regions; consequently, it can impact the potential influence of HPV vaccines in the prevention of infection and cancer (18). Therefore, regional data on the distribution of HPV types in women is crucial to predicting the potential impact of HPV vaccines.

While several studies on the frequency of HPV types had been achieved in Iranian women with cervical precancer or cancer (19), HPV-positive samples were in some cases directed to specific-primer genotyping (particularly HPV 16 and 18) (20). Consequently, other HPV genotypes are underestimated. Also, several studies have reported a greater prevalence of HPV 16 in Iranian cervical cancer than in the world (21-24). To clarify these subjects, the distribution of HPV types in women referred for colposcopy in Tehran was investigated. As HPV vaccination is not integrated into the Iranian national vaccination program, these data would be of interest to health policymakers to decide which HPV vaccines can be beneficially effective in Iran.

MATERIALS AND METHODS

Study population. The present study is a cross-sectional study from 2020 to 2022 in Tehran. One-hundred and forty-eight fresh biopsies of the uterine cervix from women who were admitted for colposcopy in two referral hospitals in Tehran (Yas and Immam-Khomeini Hospitals) were investigated in this study. The specimens were obtained following the ethical committee approval of the Tehran University of Medical Sciences (TUMS) (IR.TUMS.SPH.REC.1399.235). All individuals in this study signed the informed consent. A questionnaire was also filled out for all studied subjects regarding several variables including age, history of STD, multiple sexual partners, history of abortion, history of cancer, and smoking.

DNA extraction. Each of the tissues was cut into small pieces about 1-2 mm using a surgical blade. For every 50-100 mg of tissue, 1 ml of Trizol solution was added. Using a surgical blade and a homogenizer (Heidolph; Germany), the tissue was divided into smaller pieces and then homogenized. Chloroform was added to each sample (0.2 ml of chloroform per 1 ml of Trizol solution). After centrifugation, a three-phase solution was formed from top to bottom: colorless supernatant phase, white interstitial phase, and red organic phase. The middle layer containing DNA was transferred to a new microtube. Then chloroform was added to the volume of the transferred sample. After centrifugation, phenol was collected at the bottom of the tube and DNA was in the aqueous phase. Finally, the DNA precipitation was carried out by adding ethanol and sodium acetate salt. The integrity of the extracted DNA was assessed by PCR to amplify a 110 bp amplicon of the human β-globin gene by PC03/PC04 primers (25).

HPV DNA detection. All samples were tested by PCR using a GP5+/6+ primer pair to get a 150 bp amplicon of the L1 gene. The PCR reactions and amplification cycles were performed according to previously published procedures (22).

HPV genotyping. The PCR amplicons were sequenced and nucleotide sequences were edited with Bioedit software and aligned to reference sequences obtained from GenBank as follow: X00203 (HPV6), M14119 (HPV11), K02718 (HPV16), AY262282 (HPV18), J04353 (HPV31), M12732 (HPV33), X74477 (HPV35), M62849 (HPV39), X74479 (HPV45), M62877 (HPV51), X74481 (HPV52), X74482 (HPV53), X74483 (HPV56), D90400 (HPV58), X77858 (HPV59), U31794 (HPV66), D21208 (HPV67), X67161 (HPV68), X94165 (HPV73). The phylogenetic tree was constructed by the maximum likelihood method using Mega software 11 and the

reliability of the phylogenetic tree was calculated by the measurement of a bootstrap with 1000 replicates. All nucleotide sequences of the present study were available at GenBank with the following accession numbers: OR667393-OR667527.

RESULTS

A total of 148 samples were included in this study as follows: 40 cervical cancer (CC), 19 cervical intraepithelial neoplasia I (CIN I), 30 CIN II-III, 15 atypical squamous cells of undetermined significance (ASCUS), and 44 normal samples. Among 40 CC specimens, squamous cell carcinoma and adenocarcinoma were diagnosed in 32 and 8 samples, respectively. The mean age ± SD of studied women was as follows: 36.5 ± 9.3 (normal), 32 ± 6.5 (ASCUS), 37.3 ± 8.5 (CIN I), 37.2 ± 10.6 (CIN II-III), and 49.1 ± 14.1 (CC).

As shown in Table 1, several risk factors were investigated in our study. In total, 23.7% of women had a history of sexually transmitted diseases (STD), 18.9% of women had multiple sexual partners, and 6.8 % had sex with HPV-positive men. A history of cancer was present in 19.6% of women. History of abortion and smoking were seen in 7.5% and 10.8% of cases, respectively. Almost all variables, except for the history of cancer, were more prevalent among HPV-infected compared to HPV-uninfected samples.

The total prevalence of HPV was 92.5%. Concerning histopathology, HPV was detected in 95.4% of normal, 100% of ASCUS, 78.9% of CINI, 90% of CIN II-III, and 95% of CC specimens. The five most common HPV types regardless of histopathology were HPV 16 (49.3%), 18 (14.8%), 6 (7.4%), 31 (4.1%), and 11 (2.7%) (Table 2). High-risk HPV types were detected in 81.11%, 66.7%, 100%, 93.4%, and 87.5% of normal, ASCUS, CINI, CIN II-III, and CC samples, respectively. The remaining samples were infected with low- or intermediate-risk HPVs.

As indicated in Table 2 and Fig. 1, HPV 16 and 18 were dominant in all five studied groups. How-

Table 1. Human papillomavirus (HPV) prevalence among Iranian women regards to several variables

Variables		HPV infection		
		Samples N (%)	Positive N (%)	Negative N (%)
Age (yr.)				
	<=30	34 (23.0)	32 (23.3)	2 (18.2)
	> 30	99 (66.9)	90 (65.7)	9 (81.8)
	Unknown	15 (10.1)	15 (11.0)	0 (0.0)
History of STD				
	Yes	35 (23.7)	35 (24.8)	1 (9.1)
	No	113 (76.3)	103 (75.2)	10 (90.9)
Multiple sexual partners				
	Yes	28 (18.9)	28 (20.5)	0 (0.0)
	No	120 (81.1)	109 (79.5)	11 (100)
Partner infected with HPV				
	Yes	10 (6.8)	10 (7.3)	0 (0.0)
	No	138 (93.2)	127 (92.7)	11 (100)
History of cancer				
	Yes	29 (19.6)	27 (19.7)	2 (18.2)
	No	119 (80.4)	110 (80.3)	9 (81.8)
History of abortion				
	Yes	11 (7.5)	11 (8.0)	0 (0.0)
	No	137 (92.5)	126 (92.0)	11 (100)
Smoking				
	Yes	16 (10.8)	16 (11.7)	0 (0.0)
	No	132 (89.2)	121 (88.3)	11 (100)
Total		148 (100)	137 (100)	11 (100)

STD: sexually transmitted diseases

Table 2. Human papillomavirus (HPV) type-specific prevalence among Iranian women, stratified by histopathology and cervical cancer types

HPV genotype	Histopathology					Cervical cancer types		Total
	Normal N (%)	ASCUS N (%)	CIN I N (%)	CIN II-III N (%)	ICC N (%)	SCC N (%)	ADC N (%)	N (%)
Any	42 (95.4)	15 (100)	15 (78.9)	27 (90.0)	38 (95.0)	30 (93.7)	8 (100)	137 (92.5)
6	6 (13.6)	2 (13.3)	-	1 (3.3)	2 (5.0)	1 (3.2)	1 (12.5)	11 (7.4)
11	1 (2.3)	2 (13.3)	-	-	1 (2.5)	1 (3.2)	-	4 (2.7)
16	19 (43.1)	5 (33.3)	8 (42.2)	17 (56.7)	24 (60.0)	19 (59.4)	5 (62.5)	73 (49.3)
18	6 (13.7)	2 (13.3)	2 (10.7)	4 (13.4)	8 (20.0)	8 (25.0)	-	22 (14.8)
31	2 (4.5)	1 (6.7)	1 (5.2)	2 (6.7)	-	-	-	6 (4.1)
35	-	-	-	1 (3.3)	-	-	-	1 (0.7)
39	1 (2.3)	-	1 (5.2)	-	-	-		2 (1.3)
45	-	-	-	-	1 (2.5)	-	1 (12.5)	1 (0.7)
51	3 (6.8)	-	-	-	-	-	-	3 (2.0)
52	-	-	1 (5.2)	-	-	-	-	1 (0.7)
53	1 (2.3)	-	-	-	-	-	-	1 (0.7)
56	-	1 (6.7)	1 (5.2)	-	-	-	-	2 (1.3)
58	2 (4.5)	1 (6.7)	-	-	1 (2.5)	-	1 (12.5)	4 (2.7)
59	-	-	1 (5.2)	1 (3.3)	-	-	-	2 (1.3)
62	-	1 (6.7)	-	-	-	-	-	1 (0.7)
66	1 (2.3)	-	-	-	-	-	-	1 (0.7)
67	-	-	-	-	1 (2.5)	1 (3.2)	-	1 (0.7)
73	-	-	-	1 (3.3)	-	-	-	1 (0.7)
Total	44 (100)	15 (100)	19 (100)	30 (100)	40 (100)	32 (100)	8 (100)	148 (100)

ASCUS: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; ICC: invasive cervical cancer; SCC: squamous cell carcinoma; and ADC: adenocarcinoma

ever, the distribution of other HPV types was diverse among them. The distribution of HPV types in five studied groups was as follow: 16 (43.1%), 18 (13.7%), 6 (13.6%), 51 (6.8%), 31 (4.5%), 58 (4.5%), 11 (2.3%), 39 (2.3%), 53 (2.3%), and 66 (2.3%) in normal group; 16 (33.3%), 18 (13.3%), 6 (13.3%), 11 (13.3%), 31 (6.7%), 56 (6.7%), 58 (6.7%), and 62 (6.7%) in ASCUS group; 16 (42.2%), 18 (10.7%), 31 (5.2%), 39 (5.2%), 52 (5.2%), 56, and 59 (5.2%) in CIN I group; 16 (56.7%), 18 (13.4%), 31 (6.7%), 35 (3.3%), 59 (3.3%), 73 (3.3%), and 6 (3.3%) in CIN II-III group; and 16 (60%), 18 (20%), 6 (5%), 45 (2.5%), 58 (2.5%), 11 (2.5%), and 67 (2.5%) in CC group.

HPV genotype distribution among CC cases was different regarding the histopathological type of cervical cancer. In squamous cell carcinoma, HPV 16, 18, 6, 11, and 67 were detected in 59.4%, 25%, 3.2%, 3.2%, and 3.2% of samples, respectively. In adenocarcinoma, HPV 16 was identified in 62.5% of cases followed by HPV 45 (12.5%), HPV 58 (2.5%), and HPV 6 (12.5%).

DISCUSSION

It is well-documented that several risk factors such as the use of oral contraceptive pills, multiple sexual partners, STDs, smoking, and vaginal microbiota can promote a higher chance of HPV persistency and lead to the progression of cervical pre-cancer or cancer (26-29). In this study, a history of STDs was observed in 24.8% of women infected with HPV. It is shown that STDs particularly *Chlamydia trachomatis* infection can consider as a risk factor to accelerate the higher chance of cervical intraepithelial neoplasia or CC (27, 30-32). Almost 20% of HPV-positive women had more than one sexual partner and 11.7% were cigar smokers.

Knowing the distribution of HPV types in a region is essential for policy-makers to decide on proper

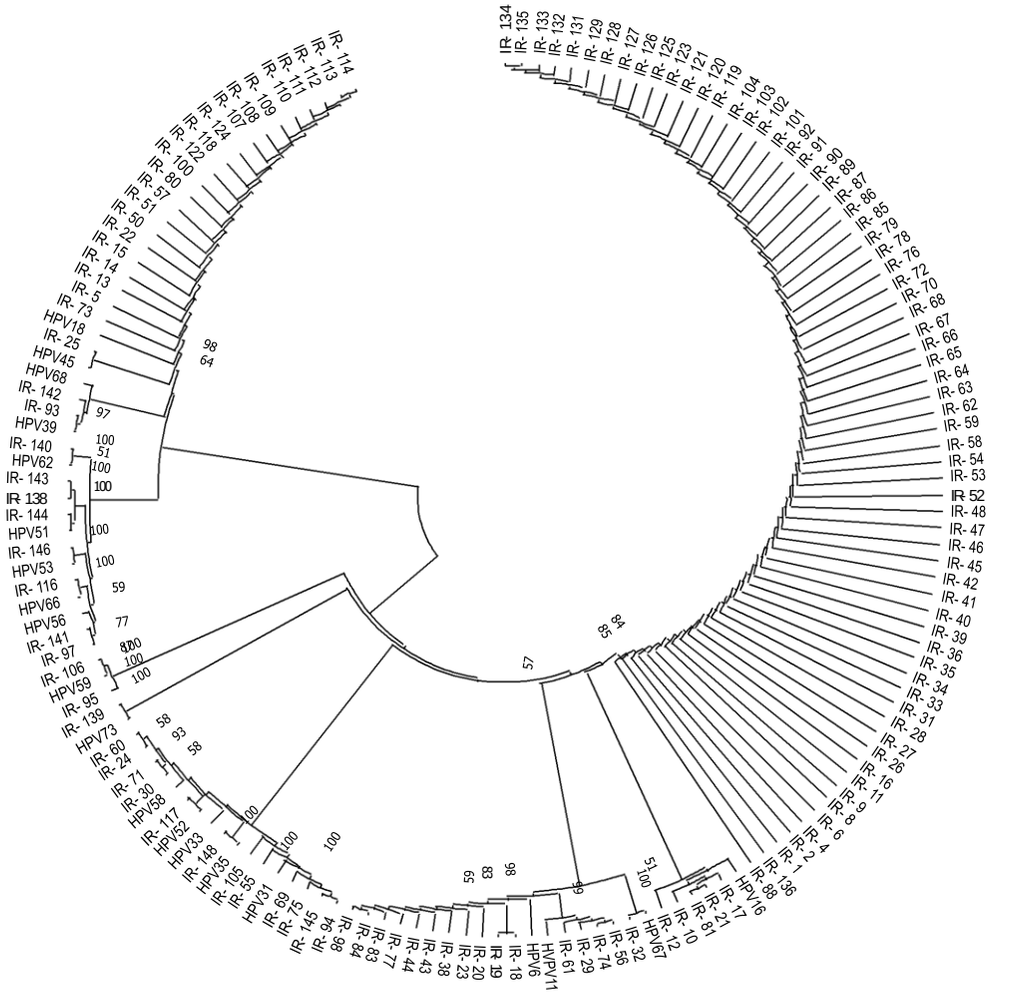

Fig. 1. Phylogenetic analysis of the HPV L1 gene was conducted in MEGA11 by using the Maximum Likelihood method based on the Kimura 2-parameter model. All reference sequences were indicated by a black circle. Numbers above the branches indicate the bootstrap values.

prevention strategies for cervical cancer, particularly by vaccination. This study makes an estimation of HPV-type frequency among women with different histopathological stages. In total, HPV was detected in the most of samples (92.5%). By histopathology, HPV is present in 95.4%, 100%, 78.9%, 90%, and 95% of normal, ASCUS, CINI, CIN II-III, and CC samples, respectively. The prevalence of HPV in normal and ASCUS groups was significantly higher than what had been previously reported in Iran (19). This can be due to the fact these women were referred for colposcopy as a triage or treatment of a preceding abnormal cytology or even a positive-HPV DNA testing report.

HPV 16 and 18 altogether were detected in 56.8% of normal, 46.3% of ASCUS, 52.2% of CINI, 70.1% of CIN II-III, and 80% of CC samples. This finding is consistent with most studies in the world that have shown HPV 16 is the dominant type in normal to cervical cancer samples (18). It is well-known that HPV 16 and 18 have a higher chance of persisting and finally progressing to cervical premalignant or malignant lesions. Indeed, these two types are detected in 70% of cervical cancer samples globally (33). This finding revealed that two present HPV vaccines named Cervarix and Gardasil® could be very effective in Iran. Regard to our data, five HPV types 31, 33, 45, 52, and 58 that were present in Gardasil® 9 (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) were not very common in Iran as they were detected in 9% of normal, 13.4% of ASCUS, 10.4% of CIN I, 6.7% of CIN II-III, and 2.5% of cervical cancer samples.

In this study, an important increase in HPV detection among cervical cancer samples (95%) was shown in comparison to former studies from Iran (73.4%-80.4%) (20). It is suggested that the sensitivity of the HPV DNA detection method, the quality of samples, and the type of analyzed samples can affect the results (34). In this study, fresh cervical biopsies were investigated while almost all previous studies in Iran used formalin-fixed paraffin-embedded tissue samples.

About the cancer type of CC, HPV 16 was almost equally prevalent in squamous cell carcinoma and adenocarcinoma. This result is incompatible with most studies in the world that reported the higher prevalence of HPV 16 in squamous cell carcinoma compared to adenocarcinoma cases (33-36). However, regard to the prevalence of HPV 18, our finding is inconsistent with the most aforementioned studies in the world that had shown the increased prevalence of HPV 18 in adenocarcinoma in comparison to squamous cell carcinoma. It is possible that lack of HPV 18 in adenocarcinoma samples can be due to the low sample size in this group.

Unusually, HPV 6/11 was found in 7.5% of CC samples and this prevalence is higher than most studies in the world (37). Several explanations could be assumed. First of all, it might be due to variants of HPV 6 and 11 circulating in Iran. Some host genetic mutations might also prone women to cancer development by low-risk HPV types. Generally, direct sequencing can detect all identified and even unidentified HPV types it is not suitable for the detection of multiple infections (38). Another explanation is that these samples would be infected with more than one type and the ones with higher viral load were identified by PCR-sequencing. The limitation of this study is no data regarding HPV multiple infections.

In conclusion, the results of this study reveal that HPV types 16 and 18 were the most frequent in Iranian women with cervical cancer. Therefore, both Cervarix and Gardasil® could be beneficial to the vaccination program in Iran.

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