

ORIGINAL ARTICLE

The Frequency and Typing of HPV Virus Among Suspected Women Referred to HPV Genotyping Test in Mazandaran, Northern Iran

Elaheh Hosseini¹, S. Sahar Yaghoubian¹, S. M. Bagher Hashemi-Soteh²

¹Novin Genetic Diagnostic Laboratory, Farah Abad Boulevard, Sari, Mazandaran, Iran

²Immunogenetic Research Center, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

SUMMARY

Background: Human papilloma viruses are a group of the Papillomaviridae family (ds DNA viruses), which infect basal epithelial cells. So far, 228 types of HPV have been identified, in which about 40 types infect the genital mucosa. In Iran, cervical cancer has been reported as the second most common malignancy in women which is approximately 8.8% of all cancers in women. HPV genotypes are classified as high-risk and low-risk according to cervical cancer. According to previous reports, nearly 70% of cervical cancers occur by HPV genotypes 16 and 18, of which genotype 16 is known as the most prevalent type. The main goal of this study was determining the frequency of HPV virus and its genotypes in the female population of Mazandaran.

Methods: This study was performed on 91 pathological samples. DNA was extracted from 500 µL of liquid-based cytology samples and PCR was performed for all of the samples. Genotyping step was performed based on strip assay method.

Results: HPV 39 (6.55%), 56 (3.27%), 51 (3.27%), and 68 (3.27%) were the most frequent types respectively. Also, HPV 11 (8.19%) and 6 (3.27%) show the most frequency among LR-HPV genotypes. HPV type 6 (16.39%), 56 (14.75%), 11 (14.75%), 16 (13.11%), and 66 (11.47%) were the four most common types seen in mixed infection samples.

Conclusions: Differences among the types of HPV can be due to various geographical distributions of HPV. Our results revealed HPV 39 (6.55%) is the most common type among of HR-HPV followed by HPV 56, 51, 68 (3.27%); however, HPV 16 and HPV 18 were seen in just one case. HPV 11 (8.19%) and HPV 6 (3.27%) were the most common type among of LR-HPV.

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Correspondence:

Mohammad Bagher Hashemi-Soteh, PhD
Immunogenetic Research Center
Molecular and Cell Biology Research Center
Medical Faculty
Mazandaran University of Medical Sciences
Sari, Mazandaran
Iran

Phone: +98 151-3543081-3

Fax: +98 151-3543087

Email: Hashemisoteh@mazums.ac.ir

KEY WORDS

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INTRODUCTION

According to the WHO, cervical cancer is the fourth most common cancer in women and second cause of cancer related deaths among women worldwide. In 2018, an estimated 570,000 women were diagnosed with cervical cancer worldwide and about 311,000 women died from the disease (https://www.who.int/health-topics/cervical-cancer#tab=tab_1).

Approximately 85% of deaths from cervical cancer occur in developing countries [1]. Cervical cancer shows

differences in distribution around the world and highest incidence rates of cervical cancer is estimated in sub-Saharan Africa with about 90% [2].

Human papilloma viruses are a group of the papillomaviridae family (ds DNA viruses), which infect basal epithelial cells [3]. So far, 228 types of HPV have been identified, of which about 40 types infect the genital mucosa (https://www.hpvcenter.se/human_reference_clones/). In Iran, cervical cancer has been reported as the second most common malignancy in women which is about 8.8% of all cancers in women [4]. HPV genotypes are classified as high-risk and low-risk according to cervical cancer [5,6]. Types of HPV 6 and HPV 11 cause benign warts [4], while high-risk types including 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 cause cervical cancer [7,5]. According to previous reports nearly 70% of cervical cancers occur by HPV genotypes 16 and 18, of which genotype 16 is known as the most prevalent type [8,9].

The prevalence of HPV varies worldwide and is reported from 2% to 44% [10]. Previous studies reported of HPV prevalence among women with normal cytology with 22.9%, 18.6%, 8.3%, and 6.6% frequency in Sub-Saharan Africa, Latin America, Asia, and Eastern Europe, respectively [11]. Frequency of HPV in Iran is reported from 4.5% in the center provinces to 11.7% in northern provinces of the country [12]. Determination of HPV genotype in tissue samples could be useful to identify people at risk. Due to the lack of sufficient information in prevalence and frequency of HPV virus in Mazandaran province, this study was performed to determine the frequency of HPV virus and its genotypes in the female population of Mazandaran.

MATERIALS AND METHODS

Subjects

This study was performed on 91 pathological samples from women referred to Novin genetic diagnosis laboratory in Sari, Mazandaran during 2019 and 2020. All ethical issues, including confidentiality and informed consent were observed in this study. All subjects were informed and a consent form was signed by patients.

DNA extraction and PCR

DNA was extracted from 500 μ L of liquid-based cytology samples using PZP Total DNA extraction kit (Cat. No.: 1149-100T) according to the manufacturer's protocol. The quality and quantity of DNA samples were measured by Nanodrop spectrophotometer (Thermo Fisher Scientific, Newington, NH, USA). Genomic DNA was amplified by PCR using Operon High + Low PapillomaStrip kit (REF.: 3.148.048.53.000). Briefly, PCR were performed in a total volume of 50 μ L containing 38 μ L working master mix, 5 μ L primers, and 100 ng of DNA. The following amplification conditions were used: initially denatured at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, an-

nealing at 58°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

Genotyping

Genotyping step was performed based on strip assay method using Operon High + Low PapillomaStrip kit (REF.: 3.148.048.53.000) according to reverse-hybridization method.

RESULTS

A total of 91 specimens without cytological manifests were investigated for HPV. Human papilloma virus was detected in 61 samples (67.03%) and 30 samples (32.96%) tested negative for HPV (Figure 1). Among positive samples, 23 cases (37.70%) show high-risk HPV types, 9 cases (14.75%) show low-risk HPV types, and 29 cases (47.54%) show mixed HPV infections (Figure 2).

Among HR-HPV genotypes, HPV 39 (6.55%), 56 (3.27%), 51 (3.27%), and 68 (3.27%) were the most frequent types. Also, HPV 11 (8.19%) and 6 (3.27%) were the most frequent among LR-HPV genotypes (Table 1). The HPV type 6 (16.39%), 56 (14.75%), 11 (14.75%), 16 (13.11%), and 66 (11.47%) were the four most common types seen in mixed infection samples (Table 2).

DISCUSSION

Human papilloma virus, the main cause of cervical cancer, shows different distribution in different countries, populations, and various parts of a country. Unfortunately, cervical cancer due to HPV has a high incidence in developing countries [13]. Hence, an epidemiological study to determine the frequency of HPV can be helpful.

In Iran, the frequency of HPV among women with normal cytology is estimated approximately 7.2% [12] (ranging from 0.6% in the south to 32.5% in the west, 4.5% in the center, and 11.7% in the north of the country) [14-18]. A previous study in 2002 from Mazandaran estimated the prevalence of HPV at 78.6% in cervical carcinoma, 64.3% in dys/metaplasia, and 9% of normal cases [19]. In the present study, total frequency of HPV is estimated 67.03%. This noncompliance can be explained by the fact that all participants in this study were suspected of having cervical cancer. In other words, in our study, the subjects do not represent the normal population.

Although the prevalence of HPV itself shows a geographical difference in different areas of the country, HPV-serotype 16 is reported as the most prevalent type among different areas [20]. In Asia, HPV 52 and HPV 58 are reported as more prevalent types after HPV 16 and HPV 18 [21]. In Iran, based on a previous study, HPV 16 and HPV 18 are reported as two of the most prevalent types with frequencies of 2.03% and 1.7%, re-

Table 1. Prevalence of HPV genotypes as a single infection (n = 61).

High/Low risk	Type	Number	Frequency (%)
H	39	4	6.55
H	66	1	1.63
H	56	2	3.27
H	16	1	1.63
H	51	2	3.27
H	68	2	3.27
H	18	1	1.63
H	53	1	1.63
L	11	5	8.19
L	6	2	3.27
L	91	1	1.63
L	84	1	1.63
	Mixed mutation	38	62.29

Table 2. Prevalence of HPV genotypes in mixed infections (n = 61).

Type	High/Low risk	Number	Frequency (%)
6	L	10	16.39
56	H	9	14.75
11	L	9	14.75
16	H	8	13.11
91	L	8	13.11
66	H	7	11.47
39	H	6	9.83
51	H	6	9.83
67	L	6	9.83
52	H	5	8.19
42	L	5	8.19
31	H	4	6.55
68	H	4	6.55
58	H	4	6.55
43	L	4	6.55
53	H	3	4.91
54	L	3	4.91
18	H	2	3.27
59	H	2	3.27
45	H	2	3.27
61	L	2	3.27
84	L	2	3.27

* The percentages were computed using the formula: frequency/total number.

Table 3. Comparison of the frequency of 4 common types of HPV.

HPV types	Tehran	West	Northwest	East	Northeast	This Study
6	3%	32%	5.1%	64%	50%	3.27%
11	2%	2%	0	24%	10%	8.19%
16	19.1%	6%	79.6%	4%	15%	1.63%
18	8.9%	12%	6.5%	4%	4%	1.6%

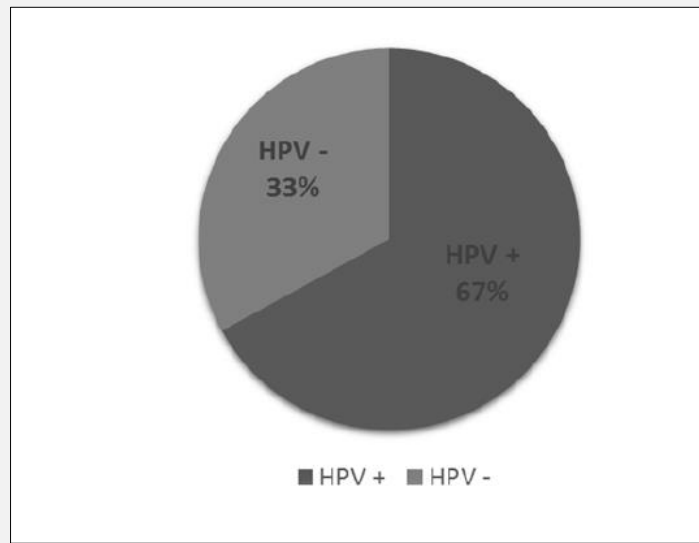


Figure 1. Total HPV prevalence in 91 samples.

spectively [22]. While another study performed on 436 Iranian women between 2011 to 2013 by Saleh-Vaziri et al. [23] showed HPV 16 (32.8%) as the most common HR-HPV genotype, followed by HPV 53 (9.1%). Also, HPV 6 (22.2%) and HPV 44 (6.1%) were the most prevalent low-risk genotypes.

In one study in Tehran, Iran, HPV 16 was reported as the most prevalent type, followed by HPV 39 and HPV 18 [6] (Table 3). In other studies performed in the west of Iran, HPV 6 was the major genotype (32%), followed by the types 18 (12%), 16 (6%), and 11 (2%) [24,4] (Table 3).

In northeast of the Iran, distributions of the HPV genotypes is reported as HPV-subtype 6 (50%), 11 (10%), 67 (5%), 16 (15%), 31 (10%), 54 (5%), and 89 (5%) [25]. In Khorasan , Eastern province of Iran, HPV 6 and HPV 11 showed the most frequency while HPV 16 and HPV 18 were seen in just one case [26]. Regarding the place and organ involved in cancer, the HPV frequency

was reported as 20% in atypical, 44.5% in cervical intraepithelial neoplasia I, 92.3% in cervical intraepithelial neoplasia II - III, and 98.2% in invasive cervical cancer in northwest Iran. In which, the most frequent HPV type was HPV 16 (79.2%), followed by types 18, 6, and 33 at frequencies of 6.5%, 5.1%, and 2.7%, respectively [27].

In the present study, HPV 11 (11.47%) and HPV 6 (9.83%) were the most frequent types in accordance with the results from the study by Hamkar and colleagues [19]. As mentioned, differences among the types of HPV can be due to various geographical distributions of HPV. Our results revealed HPV 39 (6.55%) is the most common type among of HR-HPV followed by HPV 56, 51, 68 (3.27%) but HPV 16 and HPV 18 were seen in just one case. HPV 11 (8.19%) and HPV 6 (3.27%) were the most common types among of LR-HPV.

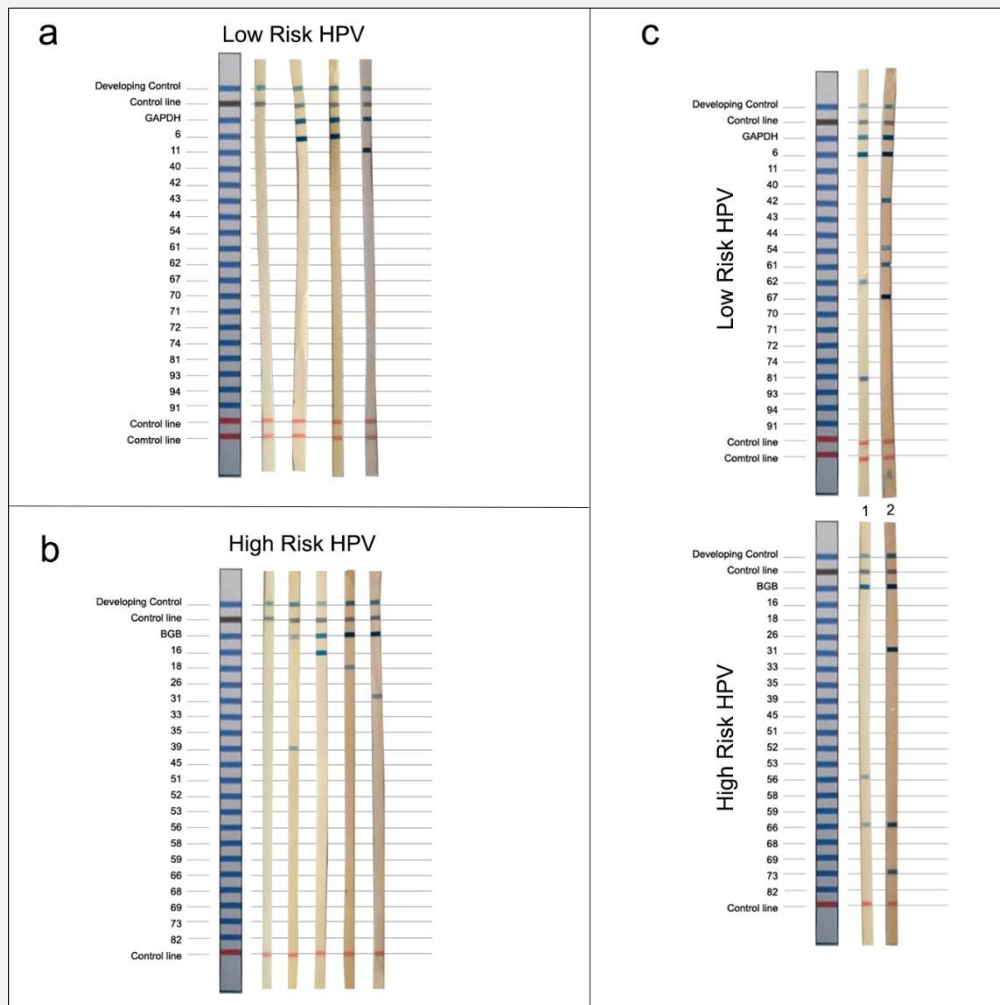


Figure 2. a: Samples for Low-Risk HPV. b: Samples for High-Risk HPV. c: Two samples of mixed HPV infection.

Declaration of Interest:

The authors have no conflicts of interest.

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