Evaluation of the prevalent genotypes of human papillomavirus in the population of women in Golestan province in the northeastern of Iran

Kamran Voso1, Abdolazim Sarli2*, Yousef Yousefi1, Sareh Khavand1, Farasat Veisi1

ABSTRACT

Human papillomavirus (HPV) is a viral infection that usually causes growths on mucous membranes or skin (warts). It has been confirmed that there are more than 100 types of human papillomavirus (HPV). Some types of HPV infections cause warts, and some of them may cause different types of cancer. One of the most common ways of transmitting the HPV virus is unprotected sex, and this virus is the leading cause of cancers of the urinary and genital tracts. Cervical cancer is the fourth cause of cancer death in women, and more than 90% are associated with persistent infection by one of the most dangerous types of the virus. This research aimed to determine the genotypes of the HPV virus in DNA extracted from people suspected of being infected with HPV. In this cross-sectional epidemiological study, 225 cytology samples have been obtained from people suspected of being infected with the HPV virus, who had been referred to the Pardis laboratory of medical genetics in the Gonbad for diagnosis from 2022 to 2023. DNA extraction was done by CEDBIO kit and the determination of HPV virus genotypes was done by a GENOVA kit. The analysis of the data was done by Excel software. In this research, 225 samples have been studied. 106 women were positive while 118 women were negative. Furthermore, the most common high-risk genotypes were (16, 31, 33, 35, 39, 45, 52, 58, 59, and 67) and (18) while the most common low-risk genotypes were (40, 42, 43, 44, 54, 61, 62, 81, 83, and 89) and (6 and 11). Moreover, the maximum age range of positive women is between 28 and 35. This study has demonstrated that the determining of dangerous genotypes is considered an essential measure in managing people infected with HPV, especially persistent infection and prevention of cervical cancer.

1. Introduction

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted diseases worldwide, infecting numerous people every year, and is closely related to cervical cancer. This virus generally affects the epithelial tissue of the genital area, especially in women, and its infection is resolved by the individual’s immune system [1, 2]. Furthermore, sexually transmitted infections (STIs) are common around the world and pose a dramatic burden on individual health and public health systems. Missed diagnosis and late cure of STIs can lead to severe problems, for instance, infertility and cervical cancer [2, 3].

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The virus is transmitted through the first contact with genital skin and infects both men and women. Some but not complete protection against HPV can be achieved by using condoms as they do not cover all the genital skin. The HPV virus can be transmitted from an HPV-positive partner to a negative partner during the first sexual activity. According to the last research of the International Agency for Research on Cancer (IARC), HPV genotypes are classified into three groups: high-risk HPV (HR-HPV) genotypes (group 1: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), putative high-risk HPV(pHR-HPV) genotypes (group 2: types 26, 53, and 66), and the last group is called low-risk HPV (LR-HPV) types which includes (group3: types 6, 11, 40, 42, 43, 44, and 70) and dramatic evidence show that they cannot cause cancer (Group 4). It has been confirmed that persistent HR-HPV infection is the primary cause of cervical cancer so that the HPV16 and HPV18 are associated to more than 70% of the cases [4, 5].

The HPV virus belongs to the family of papillomavirus, and its DNA is double-stranded and 8 kilo-bases long. All members of this virus family have 8 open reading frames, which can be classified into three regions. The first region expresses E1 E2 E4 E5 E6 E7 proteins, among which the E1 gene plays a vital role in neutralizing antiviral immune responses. E5, E6, and E7 genes are involved in cell transformation and carcinogenicity. The next region is the delayed region, which includes the L1 and L2 genes that encode the virus’s surface proteins third region is the LCR region or control locus that plays a role in transcription [6].

It is worth mentioning that, there are more than 100 types of this virus, and they are divided into three categories based on whether they are benign or malignant, high-risk groups, almost high-risk groups, and low-risk groups. Low-risk types rarely cause cancer and, eventually, cause warts in the genital area of the head and neck, but the types that cause cancer especially genotypes 16 and 18, are very important and cause 70% of uterine wall cancer [7].

Numerous doses of the HPV vaccine have been made and prescribed worldwide. In 2006, two types of vaccines were developed, a bivalent vaccine (2vHPV, CervarixTM) and a quadrivalent vaccine (4vHPV, Gardasil®) are available against this virus. These two types of vaccines are recombinant and contain virus-like particles (VLP) that induce an immune response from a natural infection. The bivalent type is effective against genotypes 16 and 18, while the second type is effective against genotypes 6 and 11. Other studies are also aimed at investigating the distribution of HPV types to determine the common genotypes in the populations of a region which can be useful in providing vaccines [8, 9].

2. Materials and Methods

In this epidemiological study, uterine cytology samples were obtained from people suspected of having this disease who had been referred to the Pardis Laboratory of Medical Genetics in the Gonbad Kavus City between 1400 and 2023. In this study, 225 samples were examined. Sampling in women was done by Pap smear method. Considering that the purpose of this study was to determine the HPV genotype in people infected with this virus, sampling was done from people who had been referred to the medical diagnostic laboratory by a gynecologist due to the occurrence of lesions (warts) or the possibility of infection.

Viral DNA extraction was done by CEDBIO kit according to the manufacturer’s instructions, and a GENOVA kit (GA 32Plex HPV Typing RT-PCR) was used to determine virus genotypes. To determine the genotype of each sample, there are 3 master mixes. Each master mix contains three channels named FAM (blue color), ROX (orange color), and HEX (green color). In the second master, the ROX channel is considered as an internal control. The temperature and time program of the reaction is as follows: The first step includes a cycle for two minutes at a temperature of 37 degrees Celsius. The second stage also includes a cycle at 95°C for 3 minutes. The third stage includes denaturation, Annealing, Extension, and fluorescence measurement involve 46 cycles of 30 seconds each at 60°C.
The kit can identify 32 common types of papillomavirus, including high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68) and possibly high-risk types (26, 53, 70, 73, and 82) and low-risk types of papillomavirus (6, 11, 40, 42, 43, 44, 54, 61, 62, 81, 83, and 89) and based on the identification of E6 and E7 genes, according to Taqman Real-Time PCR, which can be used in more accurate diagnosis of the cause of complications and diseases be helpful. This kit amplifies DNA fragments by polymerase chain reaction (PCR) and identifies the E6 and E7 genes of 32 various genotypes of the HPV virus in three separate mixtures, which are then amplified by the reaction mixture in chitin regions and the release of the fluorescence signal created by the Real-Time PCR device is measured.

The primer and probes of this kit are designed based on the sequence of the protected E6 and E7 genes specific to the HPV virus and have shown a high ability to identify this sequence. Statistical analysis of the results was done by using Excel software.

3. Results

It can be seen from the bar chart that the age of suspected women of the HPV virus is from 17 to 55 yr. According to the bar chart, most ages are between 30 and 34, and they are 66 women and after that, they are from 35 to 39 and it is 49 women. Furthermore, the lowest ages are between 47 and 51. What is more, the age group of 2 to 30 compared with the age group of 34 to 38 is almost equal (Figure 1).

![Fig. 1. The age-suspected women from 2022 to 2023](image)

It has been demonstrated that the most and lowest reasons why women have been referred to the laboratory. By way of example, the most common reasons for referring are checkups, to ensure, warts, bleeding, infection, irregular periods, and so on. On the other hand, the lowest reasons are secretions, pimples, vaccines, suspicions, skin appendages, and others (Table 1).

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The number of negatives (118 women (53 percent)) is more than the number of positives (106 women (47 percent)).

It is clear that the percentage of high-risk is more than others, and it is 60%, then the probably high risk is 14% and the low-risk genotypes are 26%. The most common low-risk genotypes (26 percent) belonged to virus types (6, 11, 40, 42, 43, 44, 54, 61, 62, 81, 83, and 89) while the most common high-risk genotypes (60 percent) belonged to virus
It has been illustrated (Figure 2), the percentage of prevalent types of HPV genotypes. It can be seen that the highest percentage of genotype is related to type 16, which is situated in the high-risk group. The percentage of type 18 is 7 and this type is located in a high-risk group and lower than type 16. What is more, these two types (16 and 18) are important and cause 70 percent of uterine wall cancer. Furthermore, the percentage types of (31, 33, 35, 39, 45, 52, 58, 59, and 67) which are located in high risk is 18% and equals a group of (40, 42, 43, 44, 54, 61, 62, 81, 83, and 89) which is situated in a low-risk group and percentage is 18%. The percentages of other genotypes are 8% (6 and 11), 9% (53), 13% (51, 56, 66, and 68) and 5% (26, 70, 73, and 82).

In this research, 225 female samples were studied, the results of HPV infection were positive in 106 people out of the total number of samples and the rest were negative (118 women). Based on this, the identified genotypes indicate that 15 people have low-risk types, 60 people have high-risk types, 6 people have almost high-risk types, 5 people have both high-risk and almost high risk and 20 people have both high-risk and low-risk types.

In the current study, 164 patients were in the age range of 25 to 45 years, and the most significant number of patients and HPV-positive people were in the age range of 20 to 39 years, considering HPV positive people, the maximum incidence of the virus in women is between The age of 26 to 39 years was observed (Figure 3).

**Discussion**

Every year, 630,000 women in the world are diagnosed with HPV-related cancers and including cervical cancer, other anogenital cancers and head and neck cancers. Contrary to widespread vaccination in advanced societies and the reduction of HPV infection in them the spread of this virus is observed in middle and low-income countries [10].
The prevalence of cervical cancer in Iran is 6 per 100,000 women, of which 42% lead to death. Identification of HPV types in populations, in addition to strengthening the vaccination approach, plays a significant role in the screening of infected and exposed to cervical cancer [11-13].

HPV prevalence studies in Iran have started in the last decade, but there is no comprehensive information on the exact prevalence of this virus, especially its genotypes. This is why plenty of studies have been done for instance, the common genotypes of HPV have been determined in Iran and more specifically in Tehran and finally, the prevalent genotypes of HPV in Golestan province were done. Therefore, determining the common genotypes of the HPV virus provincially in Iran for the design of native vaccines and common genotypes for diagnosis and studies is of particular importance [14-17].

Currently, Human papillomavirus (HPV) has been spread around the world mostly by unprotected sex and its infection and incidence depend on a lot of factors for instance geographical region, socioeconomic development, culture, and molecular variability of viral genome. Identification of HPV genotypes and related risk factors in each population can affect the policies of this virus. Also, it should play a role in the screening of genotypes with high sensitivity to identify people infected with the virus and to diagnose intraepithelial neoplasia in infected people [18-20].

Conclusion

It was determined, 47% of the HPV samples were positive, among which the majority belonged to the high-risk types of the virus. Genotypes 16, 18, 31, 33, 35, 37, and 39 are among the high-risk types, and types 6, 11, 40, 42, 43, 44, 54, 61, 62, 81, 83, and 89 are the most common genotypes in the low-risk category of the studied population were identified. The present research prepares basic information about the distribution, genotypes and diversities of HPV among the female population in Golestan province, which would assist in the formulation of preventive methods and the development of diagnostic probes and vaccines for HPV in this region.

Conflict of interest

The authors declare that they have no potential conflict of interest.

Ethics approval and consent to participate

This research effectively adhered to the tenets mentioned under the Declaration of Helsinki. We thank and appreciate all the people who cooperated with the researchers in the process of implementing this study. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publication

All authors read and approved the final manuscript for publication.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

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