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Molecular Detection of Human Papilloma Virus (Type 16,18) Using PCR and Its Frequency in Patients with Cervical Cancer in Iranian Women

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ABSTRACT

Background & Objective: Cervical cancer is the fourth most common cancer in women worldwide. HPV is a heterogeneous virus, and a persistent high-risk HPV infection contributes to cancer development. This study aims to determine the relative frequency of HPV genotypes in Kerman, Iran.

Materials & Methods: This cross-sectional study was conducted on 50 women with Pap smear samples, referring to a general laboratory in Kerman, Iran. Detecting two HPV genotypes was carried out using Multiplex Polymerase Chain Reaction (PCR) technique. The sociodemographic survey was conducted for each HPV-positive woman.

Results: Out of 50 cervical cancer patients, 33 women (66%) were HPV 16, 18-positive. HPV 16 (38%) was the most rampant type, followed by HPV 18 (14%) and HPV 16, 18 (14%).

Conclusion: Studying the prevalence of HPV genotypes and their respective risk factors can lead to efficient vaccine development against the virus in each geographical area. It can also be beneficial for illness management and high sensitivity diagnosis of cervical intraepithelial neoplasia.

Keywords: Cervical Cancer, HPV, Kerman, PCR

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Introduction

Cancer is the second most common cause of death in developed countries and the third most common cause in developing countries, including Iran **(1)**.

According to the latest epidemiological studies in Iran, cancer is the third leading reason of death following cardiovascular illness and unintentional events. More than 30,000 people die of this disease in Iran annually and it is estimated that more than 70,000 new cases of cancer occur annually in the country **(1)**.

Cancer prevention, early diagnosis and timely treatment are effective in reducing the mortality rate. Infectious agents cause 15-20% of cancers worldwide and 70% of cancers in Eastern Mediterranean countries **(2)**.

With more than 100 genotypes and worldwide contagion, human papillomaviruses (HPVs) are in the *papillomaviridae* family with a circular protein structure and an uncoated ds DNA genome. Papillomaviruses cause a variety of malignant and benign tumors in the human skin and mucous membranes (3). HPV-related diseases and cancers in humans include cancers of the cervix, skin, larynx, head and neck, breast, genital warts, and conjunctivitis of the eyes and mucous membranes **(4, 5)**.

HPVs are found in 99% of cervical cancer cases with HPV16 and HPV18 being the most prevalent genotypes observed in 70% of cervical carcinomas **(6)**.

Cervical cancer is a malignant illness among women globallyMore than 80% of cervical cancer deaths occur in developing countries **(7, 8)**. This study aims to detect the frequency of HPV16, 18 in women with cervical cancer in the Kerman, Iran.

Materials and Methods

Sample separation

In this experimental study, 50 tissue samples related to malignant cervical lesions of different patients were collected from the pathology laboratory of Kerman province (Pasargad laboratory, Iran).

DNA extraction

Virus DNA extraction from 50 clinical specimens was performed using a commercial nucleic acid extraction kit (intron, Korea).

The extracted samples were examined at 260 nm and their purity was evaluated on A260-A280 basis. In this study, specific primers (HPV16 and HPV18) were used to identify HPV. The band size of the products on gel electrophoresis is mentioned in Table 1.

Performing PCR to Identify HPV16 and HPV18 Genes

To perform multiplex polymerase chain reaction (PCR) in the final volume of 25 µl of master mix, 3 µl of DNA with a 50 µl concentration, 10 µl of primer, and sterile distilled water were used according to the following protocol:

Primary denaturation at 95°C for 15 minutes followed by 40 seconds of secondary denaturation, annealing (primer bonding) at 58°C for 1 minute, initial expansion at 72°C for 2 minutes, and final expansion at 72°C for 7 minutes. Multiplex PCR products were electrophoresed on 1% agarose gel in 90 TBE XT buffer (Cinagen, Iran) for 90 minutes and analyzed using Gel documentation **(9, 10)**.

Table 1. Primers used to propagate HPV 16, 18 (FR: forward primer, RP: reverse primer) 5

Primers	Primer sequence (5′-)	Target location	Product size
HPV-16	FP- 5′-TCA AAA GCC ACT GTG TCC TGA-3′	421-440	119 bp
	RP- 5′-CGT GTT CTT GAT GAT CTG CAA-3′	521-540	
HPV-18	FP- 5′-CCG AGC ACG ACA GGA ACG ACT-3′	533-553	172 bp
	RP- 5′-TCG TTT TCT TCC TCT GAG TCG CTT-3′	682-705	

FR: forward primer, RP: reverse primer

Results

This study aimed to determine the presence of HPVs in uterine cancer and the relationship between cancer morbidity and the the virus in proportion to the virus type. Accordingly, samples were examined by molecular methods. Table 2 shows the characteristics of the collected samples, along with specific PCR results of the two common types. Out of 50 patients with cervical cancer, 100% were HPV-positive. The distribution of HPV types were as follows: 7 patients (14%) had HPV18, 19 (38%) had HPV16, 7 (14%) had HPV16 and HPV18, and 17 (34%) did not have any of the two types. In other words, the prevalence of HPV infection in this population equaled 66% (Figure 1). The positive control group had both types 16 and 18 while the negative control group had none.

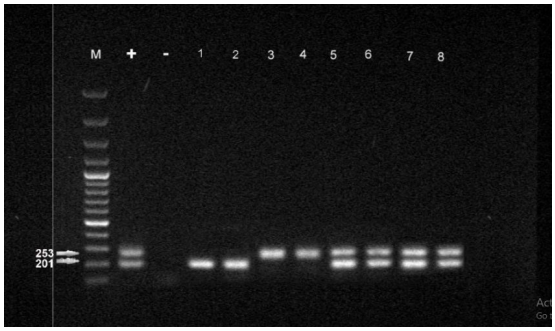


Figure 1. Resulting band samples from PCR run on the agarose gel belonging to the HPV positive, negative and control samples.

+: Positive control / -: Negative control / 1: Does not have HPV16 or HPV18. / 2: Only HPV16 / 6: Only HPV18. / 5: Affected by both HPV16, 18.

Table 2. The prevalence of two common types of HPV among cervical cancer samples.

HPV types							Pathological findings
Total	negative	positive	-16/18	+16/18	18	16	cervical cancer
50	17	33	17	7	7	19	Number of patients

Discussion

It has been proven that continuous HPV infection can lead to cervical cancer and intra epithelial neoplasia **(11)**. Each year, more than 500,000 women are diagnosed with cervical cancer, mostly in developing countries **(12,13)**. HPV has been found to be a necessary but not sufficient cause of cervical cancer **(14)**. Serological methods and viral culture are not completely sensitive and reliable in terms of detecting HPV. Thus, application of precise and fast tests is gaining more popularity **(15, 16)**.

In the study, the distribution of HPV types 16 and 18 was as follows: 7 patients (14%) had HPV18, 19 (38%) had HPV16, 7 (14%) had HPV16 and HPV18, and 17 patients (34%) did not have any of the two types.

More than 99% of uterine cancers are caused by HPV. European studies have mainly focused on HPV16 and HPV 18 variants **(17)**.

In 2006, Meshkat *et al.* conducted a study on detecting provincial papillomavirus types 16 and 18 by restriction fragment length polymorphism (RFLP) PCR in cervical cancer samples isolated with paraffin. More than 200 types of HPV are known based on genomic DNA sequence, of which 85 types are well known and 120 types have new genotypes **(18)**.

In 2016, Stamenkovic *et al.* examined the distribution of HPV in cervical cancer tissue. HPV genotype was identified in 19 to 22 positive sample. There were six high-risk genotypes, including 16, 18, 33, 45, 58, and 53 of which HPV16 was the most common **(19)**.

In a study by Kan *et al.*, 50 samples of endocervical cancer were examined by PCR. HPV16, HPV18, HPV33, and HPV18 genomes were observed in two uterine cancer samples in Australian women. Out of 50 samples, 24 (48%) were HPV-positive **(20)**.

In a study by Mahmoodi *et al.*, Which examined the prevalence of papilloma virus in uterine cancer, the results showed that 60 samples (43.3%) out of 72 samples were infected with HPV. The highest prevalence rate was observed in the age range of 48-63 years. HPV16 was the most prevalent papilloma virus (100%) **(21)**.

In a study by Cocuzza *et al.*, which examined the prevalence of papillomavirus in uterine cancer samples, the results showed that 41 samples (34.2%) out of 120 plasmid samples were positive for papillomavirus DNA, and the prevalence of HPV45, HPV51, and HPV16 equaled 46.3%, 29.6%, and 18.5%, respectively **(22)**.

Szostek *et al.* examined the physical state of HPV16 in cervical intraepithelial lesions and concluded that several factors contribute to the persistence and progression of HPV in cervical cancer, including suppression of the immune system due to infection with HPV or other microorganisms mentioned early onset of sexual life, long-term hormonal methods of contraception, smoking, and other sexually transmitted infections **(23)**. In cervical cancer, due to the availability of relevant tissue, prevention, early diagnosis and timely treatment are effective in reducing mortality **(24)**. In recent years, global scientific centers have increasingly focused on developing HPV vaccines **(25)**. Due to the relatively high prevalence of the virus in cancer tissues, antiviral vaccines may be used in high-risk population in the future. Interferon drugs are also used to treat lesions associated with HPV infection **(25)**.

Using this treatment is necessary to detect the presence of infection in the tissues. Therefore, it seems necessary to perform molecular tests along with other studies in precancerous and cervical cancer lesions and even suspicious cervicovaginal smears.

Conducting a cohort study in a larger population and in different provinces of Iran is suggested in order to accurately evaluate factors associated with acute leukemia, cancer risk factors and prevention.

Conclusion

Our study suggests that HPV16 was the most prevalent type (38%) in HPV-positive women. The study can also be beneficial for disease management and high sensitivity diagnosis of cervical intraepithelial neoplasia. Further studies should be conducted to check the HPV genotyping in other areas in Iran or other parts of the world.

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Conflict of Interest

The author declares that he has no conflicts of interest related to the subject matter or materials discussed in this article

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